1. FACTOR V LEIDEN HETEROZYGOSITY, AN ETIOLOGY FOR IDIOPATHIC INTRACRANIAL HYPERTENSION

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Background: In adults with idiopathic intracranial hypertension (IIH), there are no uniformly agreed-upon pathogenetic, but familial and acquired thrombophilia-hypofibrinolysis as well as poly cystic ovary syndrome (PCOS) with insulin resistance have been implicated as etiologies. IIH is rare in childhood, compared to adulthood, and very little is known about IIH etiologies in childhood.

Specific Aim: In 30 children <= age 21 diagnosis, our hypothesis was that IIH might be associated with familial thrombophilia-hypofibrinolysis, and with PCOS-insulin resistance.

Methods: In a retrospective, case-control study, 30 Caucasian children, 19 females (63%) and 11 males (37%), mean ages 15.2 +/- 3.8, median 16 years, had PCR measures of the Factor V Leiden, Prothrombin II (G20210A, MTHFR C677T/A1298C, and PAI-1 4G4G mutations, as well as proteins C, S, antithrombin III, homocysteine, plasminogen activator inhibitor activity, Lp(a), and Factor VII:Cila mutations. Factor VIII, XI, ACLA IgG and IgM, and the lupus anticoagulant. Fasting plasma insulin was also measured and PCOS was diagnosed by the Consensus Conference criteria. The control group included 164 healthy Caucasian children who had come to the Cincinnati Children’s Medical Center for same day tonsillectomies, including 72 females (44%) and 92 males (56%) with mean +/- SD age 7.4 +/- 5.1 years and median 7 years.

Results: Of the 30 children with IIH, 4 (13%) were heterozygous for the V Leiden mutation vs 7 (4%) of the controls, p = 0.05. There were no other significant case-control differences in coagulation measures. Of the 30 patients, 10 (33%) had a fasting serum insulin greater than the laboratory 95th percentile (17 uU/ml), 6 times greater than the expected 5%, chi square = 50.7, p < 0.0001. Of the 19 female patients, 14 (74%) were diagnosed with PCOS, of whom 11 were successfully treated with Glucophage, ameliorating their endocrinopathy and promoting weight loss and improvement of IIH symptoms.

Conclusions: Children with IIH are characterized by an increased prevalence of the Factor V Leiden heterozygosity, are commonly hyperinsulinemic, and frequently have PCOS. The finding of an excess of the V Leiden heterozygosity, also reported in adults with IIH, suggests that this subset of “IIH” is not idiopathic. If IIH involves failure to re sorb CSF in the arachnoid sinus, which is associated with iron microthrombosis, then we speculate that in children with IIH, unresponsible to conventional medical and neurological therapy, consideration might be given to anticoagulation which has been shown to be safe and effective in adults with familial thrombophilia and IIH unresponsible to conventional therapy. The enrichment of pediatric IIH with PCOS and insulin resistance has implications for the pathogenesis of IIH. Further studies are necessary to establish the role of thrombophilia-hypofibrinolysis in IIH etiology.

2. METHYLALTREXONE INHIBITS EGF-INDUCED HUMAN BRONCHIOALVEOLAR CARCINOMA PROLIFERATION AND MIGRATION: ROLE OF THE MU OPIOID RECEPTOR, SRC, GAB1, PI3 KINASE AND STAT3

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Introduction: Non-small cell lung cancer (NSCLC) has a very poor prognosis and improved therapies are needed. Expression of the mu opioid receptor (MOR) is increased in metastatic sites of patients with NSCLC, a disease whose current therapies include inhibiting EGF receptor signaling. In this study, we investigated whether inhibition of MOR by the peripheral MOR antagonist, methylaltrexone (MNTX), attenuates EGF-mediated human bronchioloalveolar carcinoma (BAC) molecular pathways involved in NSCLC oncogenic potential.

Methods: Human H358 BAC cells were pretreated with various siRNAs targeting MOR, Src, Gab1 or STAT3 or pre treated with MNTX (1.0-250 pm), 6-Nitrobenzo[b]thiophene-1,1-dioxide (STAT3 inhibitor, 1 pm) or PP2 (Src inhibitor) prior to the addition of EGF (10 ng/ml). Functional (cell proliferation and migration) and biochemical studies (immunoprecipitation, immunoblotting) were then conducted.

Results: 14 NSCLC cell lines (7 adenocarcinomas, 3 SCC, 2 bronchioalveolar carcinoma, 1 large cell carcinoma, 1 adenosquamous carcinoma) were examined for MOR expression using immunoblotting techniques. MOR expression was increased in most NSCLC cell lines with BAC having the highest levels of MOR expression (7-9 fold higher that control primary lung epithelial or BEAS-2B cells). EGF treatment of BAC (10 ng/ml, 5-30 minutes) stimulated MOR association with the EGF receptor. MNTX inhibited EGF-mediated BAC, but not control BEAS-2B, proliferation and migration in a dose-dependent manner (IC50 = ~10 and 100 nM). On a mechanistic level, we observed that MNTX inhibited EGF-induced Src activation, Src-dependent tyrosine phosphorylation of the adapter protein, Gab1 (GRB2-associated binding protein 1) and complex formation of Gab1 with the EGF receptor. Silencing (siRNA) MOR, Src, Gab1 or MNTX treatment inhibited EGF-mediated PI3 kinase and STAT3 (Signal Transducer and Activator of Transcription protein) phosphorylation/activation, events which are involved in BAC oncogenic signaling. In addition, inhibiting (siRNA and/or chemical inhibitors) PI3 kinase and/or STAT3 attenuated both EGF-induced BAC growth and migration (50-90%).

Conclusion: We have shown that MNTX inhibits EGF-mediated oncogenic signaling through inhibition of MOR-mediated, Src-dependent, Gab1 recruitment to the plasma membrane and consequent attenuation of PI3 kinase and STAT3 activation leading to reduced BAC proliferation and migration. Further study of this molecule as a potential therapeutic agent is warranted.

3. C1INH INHIBITS HABP2-MEDIATED HUMAN PULMONARY ENDOTHELIAL CELL BARRIER DYSFUNCTION

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C1 inhibitor (C1NH), a potent protease inhibitor involved in complement and coagulation pathways, protects against vascular leukoaggregation through mechanisms that are not completely understood. We explored potential targets of C1NH including Hyaluronic Acid Binding Protein 2 (HABP2), a serine protease that mediates endothelial cell (EC) barrier disruption. Immunoprecipitation and Western Blot analysis of human pulmonary vascular endothelial cell (HPMVEC) media revealed that HABP2 forms in vitro complex with C1NH. This complex formation was inhibited by treatment of EC with the barrier disrupting agent, lipopolysaccharide (LPS). Furthermore, challenging HPMVEC with LPS and/or low molecular weight hyaluronan (LMW-HA, ~2,500 Da) reduced C1NH and increased HABP2 protein expression. In contrast, the EC barrier enhancing agent, high molecular weight hyaluronan (HMW-HA, ~1 million Da), had an opposite effect on C1NH and HABP2 expression in EC. Importantly, overexpression of C1NH attenuated HABP2, LPS and LMW-HA-induced EC barrier disruption, while augmenting HMW-HA-mediated EC barrier enhancement. In accordance with our in vitro results, LPS-induced acute lung injury (ALI)
in mice inhibited in vivo complex formation between C1INH and HABP2, allowing for activated HABP2 to be expressed. In addition, intratracheal LPS challenge downregulated C1INH and upregulated HABP2 protein expression in mouse lung homogenates. These results suggest that HABP2 is a target for C1INH both in vitro and in vivo. Further, C1INH inhibits agonist-mediated endothelial cell barrier disruption and may be a potential therapeutic agent for the vascular leakiness observed in ALI.

4 THE IMPACT OF LY2189265 (GLP-1 ANALOG) ON GLYCEMIC CONTROL IN HISPANIC AND NON-HISPANIC CAUCASIANS WITH UNCONTROLLED TYPE 2 DIABETES: AN EGO STUDY ANALYSIS

J Noriega, F Botros, R Threlkeld, J Shu, J Anderson, L Glass, E Bastyri Indianapolis, IN and Ann Arbor, MI. Indiana University School of Medicine. The US Hispanic population has a disproportionately increased incidence and severity of type 2 diabetes mellitus. Despite the emergence of tailored pharmaceuticals as an important drug development goal, little research has been conducted to compare drug effectiveness between ethnicities. Differences in drug efficacy among ethnicities have previously been reported. Some factors that contribute to this could include differences in baseline disease progression, drug metabolism, and compliance. In this retrospective analysis of data from the EGO study, we examine differences in metabolic outcomes comparing Hispanic (H) versus non-Hispanic (NH) Caucasian populations following treatment with the long-acting glucagon-like peptide-1(GLP-1) analog LY2189265. 262 patients were randomized to once-weekly subcutaneous injections of either placebo or 1 of 3 LY dose regimens: 1) 1.0 mg for 16 weeks; 2) 0.5 mg for 4 weeks then titrated to 1.0 mg for 12 weeks; or 3) 1.0 mg for 4 weeks then titrated to 2.0 mg for 12 weeks. The 172 patients randomized to LY treatment (115 NH and 62 H) had similar baseline characteristics compared to the entire randomized population. The primary metric for comparison for the 2 ethnic groups was glycemic control, as measured by HbA1c change from baseline at 16 weeks. Secondary measures were change in 1) fasting serum glucose (FSG), 2) glucose excursion (AUC) response to a solid mixed meal test, and 3) the homeostasis model assessment examining indices of beta cell function (HOMA2-%B), insulin resistance, and sensitivity (HOMA2-%IR and HOMA2-%S). Differences between groups were tested for clinically evaluable patients using two-sample T-test using a nominal significance level of 0.05 for comparisons. In all randomized patients, the H group had a statistically significantly higher baseline HbA1c compared to the NH group (8.4±0.97%, n=88 vs. 8.09±0.88%, n=150, p=.006). In LY randomized patients, basal postprandial AUC glucose excursion was significantly higher in the H group (12.18±3.88, n=60 vs. 10.25±5.28 [mmol/L]h, n=112, p=.007). In response to LY treatment, the H group experienced a larger reduction in HbA1c as compared to NH at endpoint (-1.47±0.99%, n=61 vs. -1.14±0.80%, n=111, p=.020). There was also a 6-fold larger decrease in postprandial AUC glucose excursion in the H group compared to the NH group (-2.82±3.78, n=56 vs. -0.46±5.69, n=89, p<.003). Controlling for baseline HbA1c (NH 8.55±0.84%, n=82 vs. H 8.45±1.00%, n=62), the larger decrease in postprandial AUC glucose excursion in the H group compared to the NH group was maintained (-2.82±3.78, n=56 vs. -0.05±6.17, n=66, p=.003). Changes in FSG, HOMA2-%B, HOMA2-%IR, or HOMA2-%S in response to LY treatment were not significantly different between groups. The H group had higher baseline HbA1c values compared with the NH group. Treatment with LY was associated with significantly greater reductions in HbA1c and postprandial glucose excursion in H compared to NH. Changes in FSG and the homeostasis model assessment were not significantly different between groups. The mechanism by which LY treatment may result in a larger decrease in HbA1c and postprandial AUC glucose excursion in the H group as compared to the NH group is currently unknown. In conclusion, LY treatment had differential effects in the H population compared to the NH population with type 2 diabetes mellitus. Further studies are warranted to prospectively evaluate differential effects of LY treatment in the Hispanic population with type 2 diabetes mellitus.

5 SPECIFICITY OF A SYMMETRIC BENZIMIDAZOLE HELICASE INHIBITOR

C Belon, YD High, T Lin, F Pauwels, DN Frick Valhalla, NY and Mechelen, Belgium. New York Medical College. I-N,4,4-bis[4-(1H-benzimidazol-2-yl)phenyl]benzen-1,4-dicarboxamide ((BIP)2B) is a symmetrical benzimidazole-based compound that has previously been shown to inhibit the NS3 helicase-catalyzed DNA unwinding derived from hepatitis C virus (HCV) genotype 1. Since current anti-HCV drugs and most compounds in clinical development show pronounced differences in efficacy against different HCV genotypes, we have examined (BIP)2B specificity. Using a molecular beacon based helicase assay, the ability of (BIP)2B to inhibit HCV NS3 helicase-catalyzed DNA unwinding was determined for various HCV genotypes using both full-length and truncated NS3 proteins. Results show that the IC50 for inhibition of HCV NS3-catalyzed DNA unwinding ranges between 1 and 2 µM for all genotypes tested, suggesting the compound could be effective against a variety of HCV genotypes. To rule out the possibility that (BIP)2B functions non-specifically, we also tested its ability to inhibit more distantly related proteins. HCV is a member of the Flaviviridae family which also contains Japanese encephalitis virus and Dengue virus (DV), both of which cause significant disease in humans. We expressed and purified NS3 helicases from JEV and DV as well as DDX3, a similar helicase of human origin. Since these proteins are poor DNA helicases compared to HCV NS3, they were instead used to determine the ability of (BIP)2B to block RNA from stimulating helicase-catalyzed ATP hydrolysis, which fuels helicase motion. All enzymes tested showed comparable low levels of ATPase activity in the absence of RNA, and their ATPase activity was stimulated to much higher levels with increasing concentrations of poly(U) RNA present in the reaction. The dissociation constants describing RNA binding to each enzyme (K_RNA) that were derived from ATPase stimulation were 2.7, 13, 56 and 51 µM for the JEV, DV, HCV and human proteins, respectively. Assuming no enzyme inhibitor interaction with RNA, the dissociation constants describing inhibitor binding (Ki) were 0.03, 0.49, 7.0 and 8.3 µM for JEV NS3, DV NS3, HCV NS3 and DDX3, respectively. The fact that (BIP)2B binds some proteins 275-times more tightly than others yet retains an ability to inhibit a wide variety of HCV genotypes suggests that (BIP)2B could potentially be useful as an anti-HCV agent. However, its potential effects on the JEV and DV helicases suggest that it might be more useful as a flavivirus inhibitor.

6 NEURONAL GUANYLYL CYCLASE C REGULATES APPETITE AND BODY WEIGHT

M Valentin, JE Lin, P Li, A Snook, G Marszalowicz, S Schulz, SA Waldman Philadelphia, PA. Thomas Jefferson University. We live amidst a global obesity pandemic with far-reaching health and economic consequences, underscored by the absence of FDA-approved therapies that produce safe, durable weight loss. Guanylyl cyclase C (GCC), an intestinal transmembrane receptor, is one of a family of homologous signaling proteins synthesizing cyclic GMP as their proximal effector. GCC is the receptor for the endogenous paracrine hormones guanylin and uroguanylin which regulate epithelial cell dynamics contributing to the spatiotemporal organization of the crypt-villus axis. Unexpectedly, GCC has emerged as an important intermediary in signaling programs controlling appetite and body weight. C57Bl6 mice in which GCC signaling was eliminated exhibited overeating, obesity, and glucose intolerance (p<0.001), which was amplified by a high-fat/high-calorie diet (143.6±16.2% of wild-type, p<0.001). This excess body weight was associated with adipocyte hypertrophy, an increase in subcutaneous and visceral adipose mass, and several obesity-related co-morbidities including cardiac hypertrophy, hepatic steatosis, hyperinsulinemia, and glucose intolerance. Interestingly, elimination of GCC signaling produced obesity in the absence of differences in intestinal lipid absorption efficiency, activity levels and calorie expenditure/basal metabolic rate. However, GCC-deficient mice were hyperphagic when fed standard (121.2±5.4% of wild-type, p<0.05), high-fat (118.2±3.9% of wild-type, p<0.01), or high-carbohydrate chow (114.0±2.3% of wild-type, p<0.001). The hyperphagia in GCC-deficient mice was exacerbated by fasting, and these mice showed deficient nutrient-induced satiety responses. Moreover, excess weight gain in GCC-deficient mice was eliminated by restricting their diet to wild-type levels. These data suggested a defect in mechanisms contributing to the gut-neural axis which regulates hunger. Beyond nutrient digestion and absorption, the intestine plays an important role in energy homeostasis by regulating appetite circuits within the hypothalamus, mediated by the intestinal secretion of anorexigenic hormones following food intake and luminal nutrient absorption. While guanylin and uroguanylin are endocrine hormones secreted by the intestine...
into the circulation in mice and humans, GCC expression outside intestine has not been demonstrated previously. Interestingly, there is an established role for neureginal guanylyl cyclase and activation of the downstream cGMP-activated protein kinase (PKG) in eliciting quiescence (satiation) in invertebrates. Here, GCC expression in the hypothalamus, the master regulator of mammalian appetite and satiety, was confirmed by qRT-PCR, immunohistochemistry, molecular cloning, and functional assays of GCC-ligand induced cGMP accumulation. Importantly, stimulation of this extraintestinal GCC by IV administration of peptide agonists produced an acute (2 hr) satiety response (35% decreased food intake), an effect which is absent in GCC-deficient mice. However, elimination of GCC expression did not affect the signaling of other established anorexigenic peptides, as wild-type and GCC-deficient mice had equivalent responses to the gut satiety hormones PYY and cholecystokinin. These observations demonstrate a novel gut-neural signaling pathway in the regulation of appetite and body weight, and highlight a therapeutic paradigm in which hormone supplementation with GCC ligands could enhance satiety responses, thereby restricting appetite and defending against obesity.

7 INTEGRATION OF PRENATAL CARE WITH THE TESTING AND TREATMENT OF HIV AND SYPHILIS IN PERU

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Background: In Peru, various barriers prevent women from receiving prenatal care, from being screened for HIV and syphilis during prenatal visits, and—when they do receive screening—from receiving test results and appropriate clinical intervention in a timely manner. These barriers contribute to a preventable increase in the morbidity and mortality associated with HIV and syphilis among women of childbearing age and in the transmission of these infections to their children and sexual partners.

Objectives: The principal aims of this report are (1) to identify strengths and weaknesses in the testing and treatment of HIV and syphilis during pregnancy in Peru and (2) to propose intervention areas for improving prenatal care as an entry point for diagnosis and management of HIV and syphilis in women in Peru.

Methods: From August to November of 2008, we conducted semi-structured qualitative interviews in twenty of Peru’s administrative regions focusing on nine key themes: (1) problems in planning due to incomplete data, (2) integration of prenatal care with the testing and treatment of HIV and syphilis, (3) data collection (4) referral to other health care providers, (5) lack of continuity during prenatal care, (6) prolonged laboratory turnaround times, (7) delays in initiation of prophylaxis and treatment, (8) gaps in postpartum follow-up of women diagnosed with HIV or syphilis and of their newborns, and (9) health care providers’ problematic attitudes towards women diagnosed with HIV; we examine how these factors impede provision of prenatal care, from being screened for HIV and syphilis during prenatal visits, and timely return of test results and initiation of appropriate clinical intervention. Third, we review some interventions successfully developed in Peru to better integrate prenatal care as an entry point for diagnosis and management of HIV and syphilis in women in Peru.

Results and Conclusions: First, we present a summary of the health care system in Peru; a review of the technical guidelines for prenatal care, HIV, and syphilis, and a description of the relevant epidemiological data by region. Second, we outline weaknesses in the health care system as they relate to the integration of prenatal care with the testing and treatment of HIV and syphilis, focusing on nine key themes: (1) problems in planning due to incomplete data, (2) difficulties in the procurement of diagnostic tests, (3) difficulties in the procurement of antiretroviral drugs, (4) late presentation for prenatal care, (5) lack of continuity during prenatal care, (6) prolonged laboratory turnaround times, (7) delays in initiation of prophylaxis and treatment, (8) gaps in postpartum follow-up of women diagnosed with HIV or syphilis and of their newborns, and (9) health care providers’ problematic attitudes towards women diagnosed with HIV; we examine how these factors impede provision of prenatal care, screening for HIV and syphilis during prenatal visits, and timely return of test results and initiation of appropriate clinical intervention. Third, we review some interventions successfully developed in Peru to better integrate prenatal care as an entry point for diagnosis and management of HIV and syphilis in women in Peru. [This situation report was written for the first phase of the Latin American and Caribbean Initiative for the Integration of Prenatal Care with the Testing and Treatment of HIV and Syphilis, a partnership among eight Latin American countries’ AIDS programs and several international public health agencies. Of Peru, the Initiative is a collaboration among the Ministry of Health, UNICEF, UNAIDS, Socios En Salud (SES, or Partners in Health), and Harvard Medical School. The full text of the report, published as a bilingual (Spanish and English) book in November 2009 by SES, is available online: http://www.unaids.org/en/resources/books/Integration_prenatal_care_in_HIV_testing_treatment.pdf].

8 CHILD MANIA RATING SCALE: A VALID PARENT-RATED PHARMACOTHERAPY OUTCOME MEASURE

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Pediatric bipolar disorder (PBD) is a serious psychiatric disorder characterized by a chronic and refractory course, low recovery, and high relapse rates. Even with the best pharmacotherapy integrated with psychosocial treatment, response to treatment is notoriously poor. Although severe symptoms and the episodic nature of PBD are partially responsible for poor outcomes, current standards for assessment and measurement also could be limited in evaluating accurate and sensitive measurement of change related to treatment. The development of valid and sensitive treatment outcome measures is imperative to evaluating the effectiveness of current treatment approaches. Pharmacotherapy for PBD often targets the alleviation of mania symptoms. Previous measures to assess mania symptoms were limited by their focus on clinician report, length, complexity, and inability to assess variability in symptom presentation. The Child Mania Rating Scale (CMRS) was developed to address the need for a short, easily administered, comprehensive, parent-report screening measure for pediatric mania. The CMRS is a 21-item scale for pediatric mania that exhibits excellent psychometric properties and accuracy in screening for mania symptoms and differentiating pediatric mania from ADHD. It is widely used as a diagnostic screening tool in clinical work and research. The utility of the CMRS would be further enhanced if it was a valid measure of longitudinal symptom change. The current study evaluated the sensitivity of the CMRS to symptom change over time as the result of pharmacotherapy treatment. The hypotheses tested were: (1) the CMRS would demonstrate sensitivity to symptom change from pre-to post-treatment assessment; and (2) change on the CMRS would correlate with change on the gold-standard clinician-rated YMRS. Data for this study was collected as part of a 6-week double-blind, placebo-controlled, randomized outpatient medication treatment trial of risperidone vs. divalproex for PBD (N=66). The YMRS and CMRS were administered weekly for 8 weeks of treatment to examine both the magnitude and trajectory of symptom change over time. Results suggest that the CMRS is a sensitive and valid measure of symptom changes resulting from pharmacological treatment for PBD. Similar to the YMRS, the CMRS demonstrated statistically and clinically significant change in symptom report from pre to post-test. The overall similarity to the YMRS in the magnitude and trajectory of change from pre to post-test suggests that the CMRS is sensitive to detect linear and nonlinear treatment change comparable to a clinician-rated gold standard measure. Estimating the correlation between the linear and quadratic slopes of the CMRS and the validity measures using the joint growth curve models provides a direct measure of the sensitivity of the CMRS to change. The correlation indexes the similarity between individual linear and quadratic slopes on two measures. The association between change as measured by the CMRS and the YMRS was strong and positive. There was a significant association between nonlinear change on the CMRS and the YMRS. These results suggest the CMRS is a valid and sensitive measure for assessing symptom change over the course of treatment. This finding represents a significant contribution to the field because the CMRS is shorter and easier to administer as compared to other measures. Additionally, as a parent-report measure, the CMRS likely captures a broader and more accurate representation of the child’s functioning across contexts than an in-session clinician rating. The CMRS may now be applied to both research and clinical practice as a sensitive and valid indicator of treatment-related symptom change in addition to use as a diagnostic screening tool.

9 HIGH FACTOR XI, RECURRENT PREGNANCY LOSS, ENOXAPARIN


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Midwestern Regional Program Abstracts
Objective: We hypothesized that thrombophilic high Factor XI (≥150%) was a significant, treatable cause of recurrent pregnancy loss (RPL, ≥3 pregnancy losses).

Design: Excluding women with Factor V Leiden, Prothrombin, and MTHFR mutations, we compared high Factor XI in 149 women with ≥1 live birth pregnancy and 0 pregnancy losses (324 pregnancies, 312 live births), 82 with 1 sporadic pregnancy loss (213 pregnancies, 120 live births, 82 pregnancy losses), and 32 with RPL (195 pregnancies, 51 live births, 139 pregnancy losses).

Setting: Research center, obstetrics office.

Patients: 263 women with consecutive Factor XI measures.

Interventions: Enoxaparin 40 mg twice/day RPL patients with high Factor XI.

Main Outcome Measures: Association of high Factor XI with RPL.

Results: Of the 149 women with 0 pregnancy losses, 7 (5%) had Factor XI ≥150%, vs 5/32 (16%) RPL. (p = 0.04), vs 4/82 (5%) with 1 sporadic pregnancy loss, p<0.5. Three of the 5 women with high Factor XI-RPL, with 19 previous pregnancy losses and 0 live births, were given Enoxaparin during 5 subsequent pregnancies, and had 6 term live births and 1 miscarriage (p <.0001).

Conclusion: High Factor XI is independently associated with RPL and should be measured in women with RPL. Enoxaparin facilitates term live births in women with high Factor XI and RPL.

10 COMPARATIVE STUDY ON THE PHARMACOKINETIC PROFILE OF SOTALOL FOLLOWING ORAL AND INTRAVENOUS ADMINISTRATION


Introduction: Sotalol (S) is an effective antiarrhythmic agent that is available for oral administration (O). We evaluated an intravenous formulation (IV) of S in healthy subjects to determine an optimum IV administration regimen to obtain similar serum concentration to those obtained following oral S administration.

Methods: In a two-treatment, two-period, crossover study, 15 healthy subjects (age 32±8 year) received IV S 75 mg over 2.5 hr at a constant infusion rate or 80 mg of S orally. Oral S is only 90% bioavailable so more oral S than IV was given. Blood samples were collected at 18 time points during the 48 hr study period. Serum concentrations were determined by LC-MS/MS assay. Individual serum concentration-time profiles were analyzed by a noncompartmental method.

Results: The 2.5 hr IV administration of S resulted in a significantly greater Cmax than that found following oral S administration (953±522 vs. 723±296 ng/ml), a 32% overshot in Cmax. After modeling the concentration time curves, simulation studies were performed to determine the optimum IV administration regimen resulting in similar Cmax and AUC to that obtained following oral S administration. With increasing the length of the IV infusion to 3.5 hr, the Cmax decreased to 772±359 ng/ml, a 7% overshot compared to O administration. With a 4 hr duration of infusion, the Cmax became practically identical between IV and oral administration (IV; 728±335 ng/ml, O;723±296 ng/ml), a 0.6% difference. The length of infusion did not affect the AUC (IV; 682±264 ng/ml*hr, O; 689±1964 ng/ml*hr).

Conclusions: From an IV and oral comparative study we were able to model an appropriate IV dosing regimen that would result in a Cmax and AUC that is similar for the O and IV routes of administration. IV sotalol at a dose of 75 mg administered over 3.5 to 4 hr produce a similar Cmax and AUC to an oral dose of sotalol 80 mg. The IV sotalol dose may be adjusted to administer 150 mg over 4 hr to match an oral sotalol dose of 160 mg.

11 INTRAVENOUS ADMINISTRATION OF SOTALOL RESULTS IN LESS VARIABILITY IN SERUM LEVELS THAN NASOGASTRIC DRUG DELIVERY


Introduction: Sotalol is an effective antiarrhythmic agent that is available for oral administration. In patients who are not able to take sotalol orally, administration via a nasogastric (NG) tube if often employed. There are reports on the effectiveness of NG drug administration and all indicate that NG administration results in significantly lower serum concentration with a larger inter individual variability when compared to regular oral administration. Studies with ciprofloxacin and amiodarone found the coefficient of variation (CV) in Cmax to be an average of 75% with the CV for AUC is 107%.

Methods: 15 healthy subjects (age 32±8 year) received 75 mg IV sotalol over 2.5 hr with a constant infusion rate. Blood samples were collected at 18 time points during the 48 hr study period. Serum concentrations were determined by LC-MS/MS assay. Individual serum concentration-time profiles were analyzed by noncompartmental method. The mean, SD, and coefficient of variation (CV) were calculated for the maximum serum concentration (Cmax), AUCO-48 hrs and AUCO -infinity.

Results: With intravenous sotalol administration, the Cmax was 739±405 ng/ml, the AUC0-48 hr was 5292±2048 ng/ml*hr and the AUC0 -infinity was 5419±2097 ng/ml*hr. The CV that measures inter-individual variability was: 55% for the Cmax, 39% for AUCO-48 hrs, and 39% for AUC0 -infinity. Results from studies with ciprofloxacin and amiodarone indicate that the Cmax with NG administration to be for 34–39% for oral administration. The CV of Cmax was 75% and the CV of AUC was 107% for drug levels following NG administration.

Conclusions: Drug administration via a NG tube results in highly variable serum concentrations and AUCs that may be due to the differences in drug absorption. Intravenous administration is a more reliable way of drug delivery as variable absorption does not occur following intravenous administration of drug. Parenteral administration may be preferred over NG administration for these reasons. Availability of intravenous sotalol represents a reliable formula for drug administration.

12 THROMBOPHILIC HYPERHOMOCYSTEINEMIA, RETINAL VEIN AND ARTERY OCCLUSION, AND NON-ARTERITIC ISCHEMIC OPTIC NEUROPATHY

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We recently examined whether, and to what degree familial and acquired thrombophilia and homocysteinemia were significantly associated with central retinal vein occlusion (CRVO) and central retinal artery occlusion (CRAO), and with non-arteritic ischemic optic neuropathy (NAION). Thirty-eight patients with CRVO were compared to 76 race-sex matched healthy controls, 17 with CRAO, 4 with NAION (poled with CRAO) were compared to 84 race-sex matched healthy controls. Cases were compared to controls for PCR measures of homocysteinemia (Factor V Leiden, G20210A Prothrombin, C677T and A 1298C MTHFR mutation) and serologic measures of thrombophilia (resistance to activated protein C, homocysteine, anti-cardiolipin antibodies [IgG and IgM], the lupus anticoagulant, Factors VIII and XI proteins C; S; and lipoprotein (a). The 38 patients with CRVO included 14 men and 24 women, 37 Caucasians and 1 African-American, with mean ± SD age 56 ± 16, with 25 (66%) age >50. The 21 patients with CRAO-NAION included 8 men and 13 women, 20 Caucasians and 1 African-American, with mean ± SD age 54 ± 16, with 14 (67%) age >50. Thrombophilic homocysteinemia was high (≥15 umol/L) in 5 of 38 (13.2%) of CRVO patients vs 1 of 76 (1.3%) controls, Fisher’s p = .014. Thrombophilic homocysteinemia was high (≥15 umol/L) in 5 of 38 (13.2%) of CRVO patients vs 1 of 76 (1.3%) controls, Fisher’s p = .014. Thrombophilic homocysteinemia was high (≥15 umol/L) in 5 of 38 (13.2%) of CRVO patients vs 1 of 76 (1.3%) controls, Fisher’s p = .014. Thrombophilic homocysteinemia was high (≥15 umol/L) in 5 of 38 (13.2%) of CRVO patients vs 1 of 76 (1.3%) controls, Fisher’s p = .014. Thrombophilic homocysteinemia was high (≥15 umol/L) in 5 of 38 (13.2%) of CRVO patients vs 1 of 76 (1.3%) controls, Fisher’s p = .014. Thrombophilic homocysteinemia was high (≥15 umol/L) in 5 of 38 (13.2%) of CRVO patients vs 1 of 76 (1.3%) controls, Fisher’s p = .014.
Efficacy and Safety of Increasing Lovaza to 6-8-12-16 G/day in Patients with Triglyceride >300 mg/dl Despite Initial Maximal Therapy with Diet-Fibrin Acid-Lovaza 4G/day; A 9 Month Prospective Study

AA Khan, P Raj, M Umar, LR Knoop, SS Ali, N Khan, M Maddipati, P Wang, CJ Glueck, Cincinnati, OH, Jewish Hospital of Cincinnati.

Background: In patients with severe hypertriglyceridemia (HTG), the most common treatment regimen often includes resolving factors that cause secondary high TG, and treatment with diet-fibrin-acid-Lovaza 4 g/day. However, many patients continue to have very high TG (>1000 mg/dl) despite this conventional treatment.

Objective: Our specific aim was to assess the efficacy and safety of titrating Lovaza up to 6-8-12-16g/day as needed in patients with severe HTG despite resolution of secondary factors raising TG, and despite therapy with diet-fibrin-acid-Lovaza 4g/day.

Methods: This was a 9 month prospective study. With diet and fibrin acid dose stable, we increased Lovaza up to 6-8-12-16g/day, with a minimal goal of reducing TG <500 mg/dl, and optimally to <200mg/dl. The main outcome measure was significant reduction in TG to <500 mg/dl and then to <200 mg/dl as we increased the Lovaza dose.

Results: We assessed 94 patients, 60 male, 34 female, 89 Caucasians, 4 African-Americans, and 1 other. Patients’ initial TG distributions on diet-fibrin-acid-Lovaza 4 g, and subsequent TG, duration of therapy, and Lovaza dose at first and last follow-up are displayed below. Initial mean TG was high (1889 mg/dl) despite diet-fibrin-acid-Lovaza 4 g/dl, and after initial 1.1 months and 9.1 months on diet-fibrin-acid-increased Lovaza, TG fell to 481 and to 453 mg/dl (<0.001 for both). In 93% of patients, TG was lowered to < 1000 mg/dl (a level where HTG-induced pancreatitis often occurs), and in 71% of patients with initial median TG 1726, final TG was <500, median 265 mg/dl. There were no bleeding, petechiae, or other serious adverse affects observed; dyspepsia was reported in a few patients beyond 8 g Lovaza/day.

Conclusion: In patients with persistent high TG (>1000 mg/dl) despite conventional maximal therapy with diet-fibrin-acid-Lovaza 4 g/day; titrating Lovaza up to 6-8-12-16g/day is effective and safe, leading to major further significant reductions in TG, lowering TG below levels (>1000 mg/dl) where HTG-induced pancreatitis may occur, and in 71% of cases, lowering TG below 500 mg/dl.

FEASIBILITY STUDY OF PARTNER-ASSISTED THERAPY (PAT) DEMONSTRATES SAFETY AND ACCEPTABILITY OF TREATMENT

AR Brandon, NL Ceccotti, RB Jarett, Dallas, TX, University of Texas Southwestern Medical Center.

Objective: Perinatal depression has adverse consequences for mother, baby, and spouse/partner. Although poor partner support is a key risk factor for depression in perinatal women, past research has not included partners in treatment beyond psychosocial education. In a project funded by the NIMH (K23MH085907-02), a theoretical approach to treatment, “Partner-Assisted Therapy (PAT),” is being developed and tested in a sample of depressed perinatal women at the Women’s Mental Health Center of the University of Texas Southwestern Medical Center. PAT blends two existing treatments for depression, Interpersonal Psychotherapy (IPT) and Emotionally Focused Couple Therapy (EFTC), including the partner as an active participant in early acute psychotherapy sessions and one follow-up session.

Method: Eight couples completed an open trial of the PAT protocol. Women ≥ 8 weeks estimated gestational age and ≤ 12 weeks postpartum referred to the Principal Investigator for psychiatric assessment who fulfilled DSM-IV criteria for Major Depressive Disorder (SCID), reported at least moderate symptom severity (Ham-D17 ≥ 16), and met study criteria attended eight weekly psychotherapy sessions along with their partners. Also assessed were relationship satisfaction (Dyadic Adjustment Scale), partner support (Antenatal and Postpartum Partner Support Scale), antenatal attachment (Maternal/Paternal Antenatal Attachment Scale), partner assessment of symptoms (Edinburgh Depression Scale, Partner Version), and parental assessment of infant temperament (Infant Behavior Questionnaire). Seven couples returned for a follow up visit six to eight weeks after childbirth or, in postpartum women, six to eight weeks after Session 8. In February 2010 a Focus Group was held to determine participant satisfaction with PAT process.

Results: No couples dropped out of treatment, but one couple was lost to follow up after the acute phase. Although sample size prevents meaningful interpretation of data, 7 of 8 women met criteria for response (Ham-D17 ≤ 7) at the conclusion of acute phase treatment and, to date, five have met criteria for recovery at the six-week follow-up assessment. There was a trend for improvement in relationship satisfaction and partner support.

Conclusion: Incorporating partners in the treatment of Major Depressive Disorder during the transition to parenthood is safe and feasible, but needs further testing to evaluate efficacy.
Hamilton Rating Scale for Depression (17 Item) Scores (HRSD)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>BILS Pre</td>
<td>57.7±18.5, 55.0</td>
<td>38.4±29.6, 40.0</td>
</tr>
<tr>
<td>BILS Post</td>
<td>32.7±22.3, 30.0</td>
<td>59.6±40.5, 73.0 (p &lt; 0.01)</td>
</tr>
<tr>
<td>BILS 8 wk</td>
<td>63.3±31.9, 55.0</td>
<td>57.6±36.6, 50.0 (p &lt; 0.08)</td>
</tr>
<tr>
<td>BILS 14 wk</td>
<td>61.6±31.2, 53.0</td>
<td>65.5±32.3, 50.0 (p &lt; 0.01)</td>
</tr>
<tr>
<td>BASS Pre</td>
<td>2.7±0.8, 2.3</td>
<td>2.3±0.7, 2.4</td>
</tr>
<tr>
<td>BASS Post</td>
<td>2.5±0.6, 2.4</td>
<td>2.6±0.4, 2.7 (p &lt; 0.03)</td>
</tr>
<tr>
<td>BASS 8 wk</td>
<td>2.7±0.6, 2.6</td>
<td>2.8±0.9, 2.8 (p &lt; 0.02)</td>
</tr>
<tr>
<td>BASS 14 wk</td>
<td>2.9±0.6, 2.1</td>
<td>2.6±0.2, 2.5</td>
</tr>
<tr>
<td>ST PRE</td>
<td>2.9±0.8, 2.0</td>
<td>2.1±1.1, 2.5</td>
</tr>
<tr>
<td>SE Post</td>
<td>1.4±0.2, 2.0 (p &lt; 0.04)</td>
<td>1.6±1.2, 2.0</td>
</tr>
<tr>
<td>SE 8 wk</td>
<td>1.3±0.2, 2.0 (p &lt; 0.002)</td>
<td>1.6±1.2, 2.0</td>
</tr>
<tr>
<td>SE 14 wk</td>
<td>1.8±1.1, 2.0</td>
<td>1.6±1.2, 2.0</td>
</tr>
<tr>
<td>CESD 8 wk</td>
<td>27.2±9.2, 28.0</td>
<td>22.2±13.4, 18.0</td>
</tr>
<tr>
<td>CESD 14 wk</td>
<td>25.6±10.5, 27.5</td>
<td>20.8±13.0, 17.0 (p &lt; 0.02)</td>
</tr>
<tr>
<td>Positive PANAS Pre</td>
<td>24.7±8.6, 26.9</td>
<td>31.6±7.3, 30.5</td>
</tr>
<tr>
<td>Positive PANAS Post</td>
<td>21.2±4.2, 22.0 (p &lt; 0.01)</td>
<td>31.3±12.3, 36.0</td>
</tr>
<tr>
<td>Positive PANAS 8 wk</td>
<td>20.1±5.2, 19.0 (p &lt; 0.0007)</td>
<td>28.8±13.2, 25.0</td>
</tr>
<tr>
<td>Positive PANAS 14 wk</td>
<td>21.8±3.0, 19.5</td>
<td>31.9±18.3, 30.0</td>
</tr>
<tr>
<td>Lupus Symptom Scale Pre</td>
<td>63.5±49.8, 62.5</td>
<td>57.0±25.4, 62.5</td>
</tr>
<tr>
<td>Lupus Symptom Scale Post</td>
<td>59.6±23.2, 26.0</td>
<td>60.9±28.2, 66.6</td>
</tr>
<tr>
<td>Lupus Symptom Scale 8 wk</td>
<td>57.5±17.3, 57.5 (p &lt; 0.03)</td>
<td>58.7±26.7, 66.6</td>
</tr>
<tr>
<td>Lupus Symptom Scale 14wk</td>
<td>59.3±34.4, 62.5 (p &lt; 0.05)</td>
<td>61.2±26.1, 66.6 (p &lt; 0.02)</td>
</tr>
<tr>
<td>NIHQOL Pre</td>
<td>66.7±33.3, 39.9</td>
<td>74.8±18.8, 38.5</td>
</tr>
<tr>
<td>NIHQOL Post</td>
<td>65.0±20.1, 56.5</td>
<td>78.5±18.2, 47.5</td>
</tr>
<tr>
<td>NIHQOL 8 wk</td>
<td>59.7±17.3, 39.3</td>
<td>74.1±25.4, 42.6</td>
</tr>
<tr>
<td>NIHQOL 14 wk</td>
<td>55.5±58.3, 58.3</td>
<td>76.9±19.9, 86.1 (p &lt; 0.004)</td>
</tr>
</tbody>
</table>

Partner-Assisted Therapy (PAT) for Perinatal Depression

Results: The mean age of the intervention and control group were 42.7±10.9 and 39.7±7.8 yrs respectively. Body Mass Index, disease duration and disease activity were not different between the study groups or time periods. BILS and NIHQOL (Table 1) improved over time in the intervention group. Also noted were improvements in depression (CES-D), Lupus Symptom Scale (LSS) and N-HRQOL in the intervention group, while worsening in self esteem (SE), positive PANAS and Lupus Symptom Scale (LSS) were noted over time in the control group (Table 1). On longitudinal analysis, change in BI lead to changes in SE, CES-D and State Anxiety over the study duration.

Conclusions: Body Image is modifiable in SLE. The proposed intervention was successful; improvements in BI persisted at 14 weeks after the intervention and lead to significant improvements in other health outcomes. Further research is ongoing.
18 OPTIMIZATION OF LDL CHOLESTEROL, TRIGLYCERIDES, HDL CHOLESTEROL AND AMELIORATION OF CARDIOVASCULAR EVENTS IN 326 PATIENTS, AGE ≥50 AT ENTRY, WHO HAD ≥10 YEARS FOLLOW-UP: WITH MEDIAN AGE 79 AT LAST FOLLOW-UP


Background: We prospectively examined the effect of optimization of LDL-C, HDL-C and triglycerides (TG) in 175 patients free of CVD events at entry and 151 patients with CVD events at entry, all of whom had ≥10 years follow-up. We were ≥2 age 60 at last follow-up.

Methods and Results: We conducted a prospective cohort study in 326 patients, 310 Caucasians, 14 African Americans, 2 others, and recorded the number of mortal and morbid cardiovascular disease (CVD) events (myocardial infarction, angina, angioplasty, coronary artery bypass graft, claudication, ischemic stroke, transient ischemic attack) at study entry baseline and up to the last follow-up. Among 326 patients included in the study, 54% had no CVD events at entry and 46% had CVD events at entry. Diet, statins, fibrin acids, and long chain marine polyunsaturates were used to optimize lipid levels, as summarized below. Treatment successfully lowered median LDL-C, HDL-C and TG safely and continued throughout age 79, optimization of LDL-C, HDL-C and TG safely reduced new CVD events to 1.85%/year in patients who were CVD free at median age 63 at entry, and to 4.5%/year when CVD events were present at entry.

<table>
<thead>
<tr>
<th></th>
<th>Mean±SD</th>
<th>Median</th>
<th>P paired Wilcoxon (initial vs last follow-up)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>Initial Follow-up</td>
<td>224±49</td>
<td>160±37</td>
</tr>
<tr>
<td>TG</td>
<td>Initial Follow-up</td>
<td>706±158</td>
<td>118±60</td>
</tr>
<tr>
<td>HDL</td>
<td>Initial Follow-up</td>
<td>47±14±15</td>
<td>45±49</td>
</tr>
<tr>
<td>LDL</td>
<td>Initial Follow-up</td>
<td>131±41</td>
<td>85±50</td>
</tr>
<tr>
<td>BMI</td>
<td>Initial Follow-up</td>
<td>27.1±4.3</td>
<td>27.0±4.5</td>
</tr>
<tr>
<td>AGE</td>
<td>Initial Follow-up</td>
<td>63±17</td>
<td>71±7</td>
</tr>
<tr>
<td>Treatment duration (years)</td>
<td>14±3.3</td>
<td>14</td>
<td></td>
</tr>
</tbody>
</table>

19 ANOMALOUS RIGHT CORONARY ARTERY AND LEFT VENTRICULAR OUTFLOW TRACT OBSTRUCTION: A RARE COMBINATION

MZ Bawany, B Saeed, A Mutgi Toledo, OH. University of Toledo Medical Center.

Left ventricular outflow tract obstruction (LVOT) prevalence in general, population ranges between 0.16–0.29 percent and the incidence of anomalous left coronary artery origin and LVOT are reported in the literature, but none has been seen with anomalous origin of RCA. A 29-year-old Caucasian male presented to emergency department with worsening chest pain for one month. Pain was pressure like, retrosternal, 8/10 in intensity and associated with shortness of breath. Patient also had 2–3 episodes of syncope in the last month or two not associated with chest pain. Past medical history was significant for hypertension of unknown etiology since the age of 19 years. The patient did not have any significant family history of sudden cardiac death or coronary artery disease. Smoking history was significant for a pack of cigarettes daily for ten year. His blood pressure upon presentation was 132/70 mm Hg, pulse 77 beats per minute, respirations 16 per minute and temperature 98.3° F. Physical exam was significant for midystolic murmur, which accentuated with Valsalva. Her Hb was 15.8 g/dl, WBC 8.3 Platelets 210,000, Na 136, K 3.9, BUN 24, Cr 1.0, CK-MB 4.7 and Troponin 0.03. Chest x-ray was unremarkable and EKG showed normal sinus rhythm without ST changes. Patient was started on ACS protocol and his symptoms improved. Serial cardiac enzymes and EKG remained with normal limits. Patient underwent echocardiogram, which revealed EF 55%, moderate left ventricular hypertrophy with increased inter-ventricular septal thickness in proximal portion and systolic anterior motion of mitral valve. Patient had cardiac catheterization for the evaluation of severity of obstruction, which revealed resting gradient of 10 mmHg across aortic valve and 65-mmHg post nitroglycerine infusion. Beside this RCA could not be selectively cannulated, which raised the possibility of anomalous origin of RCA. A CAT scan of the heart confirmed the origin of RCA from the left cusp and the vessel was markedly compressed between the pulmonary outflow tract and aortic root explaining his symptoms. Considering the malignant course of RCA and LVOT, cardiothoracic surgery recommended surgery and the patient underwent septal myectomy and CABG, without complications. We present a case report highlighting the extremely uncommon association of two diverse clinical conditions with both the conditions carrying a high mortality rate and successful surgical intervention.

20 PANCREATO-LIVER ABDOMIS IN A PATIENT WITH HISTORY OF CHRONIC PANCREATITIS

MZ Bawany, T Sodeman Toledo, OH. University of Toledo Medical Center.

Liver abscess accounts for 48% of visceral abscess and presents with significant morbidity and mortality. The overall incidence of pyogenic liver abscess is 3.6 per 100,000 populations. The morbidity and mortality rate for liver abscess ranges from 2-12% depending on the severity of underlying co-morbidities. A 36-year-old African American male with history of chronic pancreatitis presented to the emergency department for abdominal pain in the epigastric area along with nausea, vomiting, diarrhea, fever. His symptoms began 3–4 days before presentation. Abdominal pain was dull in nature and 6/10 in intensity, non-radiating. Past medical history was significant for HTN, diabetes mellitus and chronic diarrhea secondary to chronic pancreatitis. On admission patient was alert and oriented, blood pressure was 97/44 mm Hg, heart rate 16 beats per minute, respiration 16 per minute, oxygen saturation 94% on room air and temperature 102°F. Abdominal examination revealed hyperactive bowel sounds and tenderness in epigastriac & RUQ. Liver span was 14 cm. Rest of the examination was unremarkable. Laboratory work revealed: Hemoglobin 9.8 g/dl, WBC 22.1 with segs of 81% and 9% bands; BUN 54, Cr 4.7, total protein 10.4, albumin 1.8, total bilirubin 1.1, direct bilirubin 0.3, AST 98, ALT 38, alkaline phosphatase 250, amylase 81, lipase 10, lactate 2.3 and INR 1.39. Patient was started on fluids and meropenem for broad spectrum coverage. But his condition worsened and he developed acute respiratory distress syndrome due to sepsis necessitating intubation. Considering his abdominal pain patient underwent CT scan of the abdomen which revealed multiple liver abscesses; largest measuring 7.5cm in right lobe of the liver with pancreatic calcifications. As the patient condition did not improve, he underwent liver abscess drainage and a catheter was left in place for continuous drainage. Fluid analysis showed Ph 4.0, LDH 39 units/L, Glucose 81 mg/dl, Protein 1.6 g/dl, lipase 68 units/L. Even though his blood and fluid cultures remained negative during the hospital stay he was continued on antibiotics, considering the possibility of initial antibiotic therapy rendering the blood cultures negative. The success of management was assessed with the hepatic CT 10 days post drainage and was demonstrated by the observation of improvement in the patient’s general condition, as indicated by normal temperature, decreased draining catheter output and the resolution of deranged laboratory values. The catheter was then removed and the patient was discharged. In summary, we present a case of pancreato-liver abscess in a patient with the history of chronic calcified pancreatitis. It was treated with antibiotics and percutaneous drainage, with satisfactory resolution.

21 CORRELATION OF POLYMORPHONUCLEAR LEUKOCYTOSIS ON ENDOCERVICAL GRAM STAIN WITH A FINAL DIAGNOSIS OF CHLAMYDIA AT A SEXUALLY TRANSMITTED INFECTIONS CLINIC IN SANGAMON COUNTY, ILLINOIS

V Sundreeshan, D Hunt, NM Khardori Springfield, IL. Southern Illinois University.

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Background: The unique advantage of seeing patients in Sexually Transmitted Infections (STI) clinics run by public health departments is that they are diagnosed, treated and in most cases cured at the same site. The preliminary microbiological tests used in these clinics (including gram stain, wet mount and KOH preparation of endocervical specimen) are simple to perform and unambiguous to interpret. The diagnoses are then confirmed with more sophisticated tests including culture and Nucleic Acid Amplification Tests (NAAT). The guidelines for treatment of STIs are outlined very specifically by CDC. As per the guidelines, a group of female patients with elevated polymorphonuclear leukocytes on gram stain of endocervical specimen but no other microscopic findings (such as yeast, trichomonas, clue cells or gram negative intracellular diplococci (GNIID)), are not treated in the same visit until confirmatory tests (PCR) for Chlamydia and gonorrhea return. However, some specialists consider an increased number of polymorphonuclear leukocytes on endocervical Gram stain as being useful in the diagnosis of cervicitis, but this criterion has not been standardized. Chlamydia is one of the important causes of cervicitis. On the other hand, males with leukocytes on gram stain but no GNID are treated with azithromycin or doxycycline orally for a presumptive diagnosis of non gonococcal urethritis (NGU). One of the most common bacterial causes of NGU is Chlamydia trachomatis. Additionally, Ureaplasma urealyticum and Mycoplasma genitalium can cause NGU.

Aim: To ascertain correlation of leukocytes in vaginal secretion and a final diagnosis of Chlamydia.

Methods: This is a retrospective study of female patients seen in the Samargon County STI clinic between 2005 and 2009 with elevated leukocytes on endocervical gram stain at initial visit. We determined the percentage of patients with the isolated leukocytes on initial gram stain who were confirmed to have Chlamydia.

Results: CDC reports that in Illinois State, prevalence of Chlamydia in women in 2008 was 66.1 per 100000 population. From Samargon County public health clinic the estimated prevalence of Chlamydia in women in 2009 was 5.64 per 100,000. A total of 2271 women were seen in this period at the clinic and data from 2240 female patients is available. Table 1 below summarises the number and percentage of female patients seen between 2005 and 2009, with and without leukocytes on gram stain in relation to those with a confirmed diagnosis of Chlamydia.

Conclusions: Based on the above information, Chlamydia should be suspected with 2+ or 3+ leukocytes on gram stain. It may be reasonable to offer presumptive treatment with azithromycin in female patients with 2+ or 3+ PMNs on the endocervical gram stain at initial presentation and without alternative diagnosis to explain the same even prior to confirmed diagnosis of chlamydial cervicitis by culture or NAAT. In high prevalence areas this easy to perform, inexpensive screening test on initial visit may treat and prevent transmission in a larger number of patients as compared to a low prevalence area like ours.

### Table 1.

<table>
<thead>
<tr>
<th>Polymorphonuclear Leukocytes (PMNs)</th>
<th>Number with diagnosis of Chlamydia</th>
<th>Total Number of patients</th>
<th>Percentage with confirmed diagnosis of Chlamydia</th>
</tr>
</thead>
<tbody>
<tr>
<td>0/0 (0 PMNs)</td>
<td>45</td>
<td>795</td>
<td>6.03%</td>
</tr>
<tr>
<td>1/1 (10-20 PMNs)</td>
<td>72</td>
<td>641</td>
<td>11.67%</td>
</tr>
<tr>
<td>2/2 (20-30 PMNs)</td>
<td>105</td>
<td>688</td>
<td>15.26%</td>
</tr>
<tr>
<td>3/3 (30 PMNs)</td>
<td>22</td>
<td>139</td>
<td>15.8%</td>
</tr>
</tbody>
</table>

22 REFRACTIVE IDIOPATHIC THROMBOCYTOPENIC PURPURA

N Mishra, A Chandra Brooklyn, NY. Coney Island Hospital.

Case Report: A 26-year-old woman presented to Coney Island Hospital (CIH) 11/21/09 after she developed oral bleeding along with petechial rash on trunk and lower extremities. This was the second episode of such symptoms with the first occurring 4 to 5 weeks prior to her presentation, requiring admission at Lutheran hospital. There she was diagnosed with immune thrombocytopenic purpura (ITP) and treated with steroids. Patient was discharged with a prednisone taper and presented to CIH with a platelet count of 3,000/µL, accompanied by recurrent bouts of epistaxis and gingival hemorrhage. Other hematologic malignancies were ruled out by normal bone marrow biopsy and cytogenetic testing. She was treated with high dose intravenous steroids, but her platelet count remained between 5,000 to 8,000/µL after 2 weeks of therapy. Patient was then given intravenous immunoglobulin’s (IVIG) and later WinRho but her platelet counts did not improve. At that point Rituxan was administered, briefly increasing the platelet count to 15,000/µL, which promptly fell back to 4,000/µL in 2 days. She also received doses of vincristine, which did not produce any effect. Finally, splenectomy was planned. On the day prior to splenectomy her platelet count improved to 70,000 but splenectomy was done anyhow given the long course of no response. On follow up, her oral purpura and petechial rash had resolved, however her platelet counts had fallen down to 10,000/µL. The refractory ITP (now recurring after splenectomy) had responded possibly to either rituxan or vincristine prior to splenectomy. She was restarted on these agents weekly. Platelet count rose to 105,000/µL on one week follow up.

Discussion: Primary immune thrombocytopenia, also referred to as idiopathic thrombocytopenic purpura, is a autoimmune disorder in which antibody-coated or immune complex-coated platelets are destroyed prematurely by the reticuloendothelial system. It is important to rule out conditions like pseudothrombocytopenia caused by platelet clumping and dilutional thrombocytopenia resulting from massive blood loss and transfusional support with packed RBCs. The principal goal in the management of thrombocytopenia is prevention of death from bleeding. All current treatments are designed to decrease platelet destruction, either by immunosuppression or by splenectomy. However there are newer agents being developed that enhance platelet production. Steroids, IVIG, anti-Rh(D) immunoglobulins, and splenectomy remain the mainstays of treatment in ITP. For patients with severe and symptomatic thrombocytopenia, following failure of initial treatment with glucocorticoids, splenectomy is the most effective treatment option, with the most success for achieving durable complete remissions. Patients are not usually considered refractory until splenectomy has failed. Transient increases of the platelet count are frequently reported with administration of vinblastine or vincristine, but durable complete remissions are rare. Rituxan, a chimeric monoclonal antibody targeted against CD20 antigen expressed on B-lymphocytes has B-cell-depleting and immunomodulatory capabilities and has been shown to be greatly efficacious in the treatment of patients with refractory ITP.

Conclusion: Although splenectomy is the most effective treatment for refractory idiopathic thrombocytopenic purpura (ITP), many post-splenectomy patients have recurrent thrombocytopenia refractory to multiple medical therapies. No single agent or regimen has been shown to provide long-term success for such patients, and for most treatments it is difficult to assess whether benefits outweigh risks.

23 EVIDENCE BASE FOR GENETIC TESTING AND SCREENING IN SUDDEN CARDIAC DEATH

SM Modell Ann Arbor, MI. University of Michigan School of Public Health. 

Objectives: Provide a descriptive capsule of the heritable ion channelopathies and cardiomyopathies associated with SCD; (2) Describe and provide evidence for the use of family history and genetic testing for these conditions; (3) Lay-out evidence-based screening strategies and recommendations; and (4) Offer preliminary data from public health trainees - attitudes towards risk assessment for SCD.

Background: Sudden cardiac death (SCD) accounts for 400,000 deaths annually in the U.S. Approximately 22,000 of these deaths occur in young, seemingly healthy people <45 years of age. Up to 10% of SCD cases in general, and 25% of cases involving a family history, are thought to have an hereditary basis. The expanding ability to associate specific genetic mutations with specific risks for sudden death offers hope of improved secondary prevention of life-threatening arrhythmias in susceptible individuals and primary prevention in at-risk family members. Information for the various genetic causes of SCD is now coming together to a degree that evidence-based assessment of genetic testing for the various syndromes has become a real possibility.

Methods: Knowledge synthesis in this area depends on 4 major sources of information: (1) individual case and molecular work-up reports of varied size and study design; (2) mutational databases (e.g., European Society of Cardiology Inherited Arrhythmias Database, CardioGenomics FHC Mutations Database); (3) reviews of syndromes and applicable technologies; and (4) working group consensus statements. Using the author’s 2006 Long QT Syndrome (LQTS) Human Genome Epidemiology Review (covering gene variants, clinical and population testing) as a backbone, this presentation utilizes all 4 sources in looking at the broader set of SCD ventilar
arrhythmia syndromes and the evidence base for genetic testing. Major criteria used: test validity and utility; applicability of genomic region and populations assessed; role of family history; study replicability and generalizability; and ethical considerations.

**Findings:** Clinical test sensitivity varies with condition, e.g., LQTS - 75%; catecholaminergic ventricular tachycardia and hypertrophic cardiomyopathy - 50%; familial dilated cardiomyopathy >30%. The comparative value of clinical assessment and availability of a tested family member are critical to the decision to employ genetic testing. Use of family history to help identify family members at risk for SCD has been validated prospectively. Various assessment strategies, based on sequential checking of major suspected and lesser genes, allow optimization of cost-effectiveness. The SCD-related syndromes form a spectrum of validity for widespread mutation testing in groups at risk, with LQTS on one end (most certain), and arrhythmogenic cardiomyopathy and Brugada syndrome on the other.

**Conclusions:** Genetic testing for SCD is feasible at present, but its widespread use as a frontline means of assessment depends on the screening strategy (e.g., population targeted, phenotypically-directed, familial cascade approaches) used, and on the suspected condition. Risk assessment can start with personal presentation, family history, or decondent information. Mutation testing is now routinely available for some conditions; combinations with phenotypic assessment is usually warranted. Screening in several high frequency populations has begun, though its adoption in particular racial-ethnic groups requires closer statistical and ethical scrutiny.

### 24

**CHANGES IN GLUCOSE REGULATION DURING CALORIC RESTRICTION COMBINED WITH PARTIAL SLEEP LOSS**

JN Booth, Chicago, IL. University of Chicago.

Short sleep is associated with obesity and increased risk of type 2 diabetes. Controlled human experiments also indicate that sleep deprivation is accompanied by reduced glucose tolerance and decreased insulin secretion and action. Since diet-induced weight loss represents an important strategy for metabolic risk reduction, we examined the effects of caloric restriction combined with partial sleep loss on glucose regulation in overweight adults. Ten participants (3F/7M; mean [SD] age 40 [5]y; BMI 27.4 [2.0]kg/m2) each completed two 14-day studies in random order at least 3 months apart. Studies were carried out in the laboratory with 5.5 or 8.5-h time-in-bed (TIB) nightly sleep opportunity and nutritionally balanced caloric intake equal to 90% of the subjects’ initial resting metabolic rate (carbohydrate 50-25%, fat 30-35%, protein 15-20% of energy content). Body weight (scale) and composition (DEXA) were measured before and after each 2-week intervention. Sleep was monitored by polysomnography. Oral and intravenous glucose challenges were performed to measure glucose tolerance, glucose effectiveness, insulin secretion, and insulin sensitivity after each intervention. We also measured 24-h blood concentrations of the glucose-regulatory hormones ghrelin, growth hormone, cortisol, epinephrine, and norepinephrine. Data were analyzed using generalized estimating equations regression models which controlled for order-of-treatment and differences in body composition (SPSS, Version 16.0). Subjects slept 5 h 14 min (± 6 min) and 7 h 25 min (± 32 min), respectively, during the 5.5-h and 8.5-h TIB condition (P<0.01), and the corresponding weight loss during each treatment was similar (3.7 ±1.0 vs. 3.5 ±1.2 %, NS). However, while approximately half of the weight loss during the 8.5-h TIB condition was fat, the loss of adipose tissue was significantly reduced during the 5.5-h TIB condition when only 25% of the weight loss was fat and the remaining 75% were related to markedly increased loss of lean body mass (P<0.01). Independent of final body composition, sleep restriction was accompanied by lower fasting blood glucose concentrations, reduced insulin secretion and sensitivity, and decreased glucose effectiveness, without deterioration in glucose tolerance. The 24-h concentrations of cortisol and norepinephrine were similar during both sleep conditions, whereas acylated ghrelin and growth hormone concentrations increased, and epinephrine concentrations decreased during the 5.5-h TIB intervention. Our results indicate that combining a dietary intervention for weight loss with recurrent sleep restriction results in reduced loss of fat and increased loss of lean body mass. This was accompanied by changes in the human neuroendocrine response to caloric restriction characterized by peripheral insulin resistance and decreased beta-cell function, similar to the pattern of ghrelin-mediated changes in peripheral glucose metabolism. In the setting of preserved oral glucose tolerance and lower fasting glucose concentrations, these findings suggest that sleep loss can trigger a set of adaptations to enhance carbohydrate partitioning towards glucose-dependent tissues at times of restricted energy availability. Supported by NIH grants P01-AG11412, R01-HL089637, CTSA-RR 04999 and P60-DK020595 Keywords: change in glucose regulation, caloric restriction, partial sleep loss.
It has been estimated that a third of the American population, and two thirds of those over 65 have Hypertension; and that 35% of these are not adequately controlled. In 2007 we identified 68,000 Aetna Medicare Advantage members with Hypertension, accounting for over 25,000 hospital admissions and over 17,000 emergency room visits in the preceding year. In December 2007 we randomly selected 10,000 of these members and used an Interactive Voice Response (IVR) call to offer participation in a program for management of blood pressure. Members who agreed to participate were given commonly used pressure cuffs for home use, along with instructions encouraging them to call the dedicated IVR for this program at least monthly with their blood pressure readings. The IVR program provided immediate feedback and education when the blood pressure reading was high, and alerts when high enough to be of short term concern. It also provided education about blood pressure and its management including the value of lipid screening and management. Outbound IVR calls were also made quarterly to solicit blood pressure and provide education and feedback. The program was overseen by a clinical nurse manager who evaluated the blood pressures and alerts, and who facilitated case manager involvement as indicated for specific members. The program was administered February 2008 through April 2009. It drew 1192 participants who were similar to the non-participants in age (mean 72 vs. 73), sex, diagnosis, hypertension severity, medical risk score and location. Results were analyzed for all participants who were continuously enrolled throughout the study and who called in at least two valid readings a minimum of 90 days apart. For these (674) participants, systolic mean blood pressure was reduced 1.40 (p=0.038) and diastolic mean was reduced 2.17 (p=0.0001), compared to their initial blood pressure reading. Those with adequate control (<140/90) or well controlled (<120/80) increased from 67.8% to 73.3% of the participants. 217 of these 674 were initially out of control - and 18% of these transitioned to well or adequate control (p<0.01). The median time in the study for this population was 346 days. LDL screening during the year increased from 86.8% to 91.3% of participants (p<0.01). This automated program clearly had a salutary impact on blood pressure in an elderly population with hypertension. This was observed, even though the proportion of the population with adequate control at the start of the study was greater than previous published information suggested - perhaps resulting in less than anticipated opportunity for impact. In addition, the increase in LDL screening should also be noted. Based on economic modeling for a population over 64 years in age and 8% participation, this impact on blood pressure would result in 23 fewer strokes, 22 fewer coronary artery disease events and 16 fewer deaths per 100,000 per year if the effect were maintained. Demonstration of a significant and measurable effect on a major risk factor with an automated program is a significant potential benefit to Public Health. A modification of this program will be implemented in 2010 on a larger scale for Aetna Medicare Advantage members. Modifications based on this experience should improve impact. In addition, programs such as this, combining home equipment with interactive voice feedback and skilled clinical oversight, might be applied to other risk factors.

27 LACTOFERRIN MODULATION OF MYCOBACTERIAL CORD FACTOR Trehalose 6,6'-dimycolate induced granulomatous response

KJ Welsh, SHwang, ML Kruzel, JK Actor Houston, TX.UTHSC Houston. Tuberculosis (TB) is the leading bacterial cause of death worldwide. The immune system responds to TB infection by the formation of granulomas, which initially provide protection against dissemination of the organism. However, immune-mediated destruction of lung tissue is a cause of significant morbidity and contributes to transmission of the disease. Our laboratory is actively investigating lactoferrin, an iron-binding glycoprotein with immunomodulatory properties, due to its ability to decrease tissue destruction and promote a TH1 immune response essential for controlling TB infection. The cord factor trehalose 6,6'-dimycoclated (TDM) model of granuloma formation mimics many aspects of TB infection, including granuloma formation and the production of proinflammatory cytokines. Wild-type C57BL/6 mice were intravenously injected with TDM in a water and oil emulsion. A subset of mice was given 1 mg of bovine lactoferrin 24 hours post-TDM challenge. Lung tissue was subsequently analyzed for histological response, and for production of chemotactic and proinflammatory mediators. Wild-type C57BL/6 mice demonstrated the formation of granulomas that correlated with increased production of IL-12, IL-6, TNF-α, IL-12p40, IFN-γ, and IL-10 protein and RNA. Mice treated with lactoferrin 24 hours post-challenge had fewer and smaller granulomas compared to mice given TDM alone. Proinflammatory and TH1 cytokines that are essential to the control of mycobacterial infections, such as TNF-α and IFN-γ, were not altered in the mice given lactoferrin. However, the anti-inflammatory cytokines IL-10 and TGF-β were increased, providing a potential mechanism for decreased tissue damage seen in the lactoferrin treated mice. Lactoferrin modulation of the immune response may be a useful adjunct in the treatment of TB.

28 TRANSIENT RECEPTOR POTENTIAL VANILLOID 2 (TRPV2) STIMULATION IS CARDIOPROTECTIVE

A Anjak, L Haar, T Forde, M Jiang, X Ren, N Weintraub, K Jones, J Rubinstein Cincinnati, OH. University of Cincinnati. Background: Ischemic preconditioning has been shown to attenuate myocardial damage. Recent studies have shown that peripheral stimulation of the skin with specific agonist can mimic this effect, possibly through Transient Receptor Potential (TRP) proteins. The TRP proteins are cationic channels that have been discovered mostly in the renal and nervous systems. Abdominal skin nerve stimulation of the Vanilloid (TRPV) subtype 1 receptor with selective agonists has been recently shown to have a cardioprotective effect in mouse models of ischemia/reperfusion (IR). Furthermore, TRPV1 knockout (KO) models have been shown to have worse outcome after ischemic injury and pressure overload. Based on chemical and functional similarities we hypothesized that TRPV2 is expressed in the heart and peripheral nerves and that activation of TRPV2 would lead to changes in cardiac function and response to IR injury. Methods: Steady state levels of TRPV2 mRNA were measured in tissue samples using previously reported primer sequences. For in vivo functional studies, we applied probenacid, an established TRPV2 agonist, to evaluate changes in myocardial function. Subsequently, it was applied at two different doses (1 molar of 0.0025 molar and 0.025 molar in gel) at the same abdominal location as previously reported for TRPV1 agonists for 30 min prior to coronary occlusion. Occlusion was maintained for 45 min, followed by 24h reperfusion. Subsequently, there was a full echocardiographic assessment and histological measurements of infarct size. Results: We demonstrated a low level of TRPV2 mRNA in naive samples with 1.9 fold higher expression in the atria compared to the ventricle and a 1.8 fold induction of the mRNA 3h after coronary occlusion in the ventricle. There were dramatic changes noted in the in vivo studies. Peripheral application of probenacid (0.0025 molar) resulted in an increase (pre- and post-treatment) in the ejection fraction of 26.4 ± 9% in comparison to 2.8 ± 9% for the sham group (P=0.002). Post-IR, there was a significant reduction in infarct size normalized to risk region (55.8 ± 7.9, 20.2 ± 16.4% and 51.2 ± 14.6, for low dose, high dose and sham, respectively; P<0.05). Preliminarily, in vivo echocardiographic analyses, showed decreased radial displacement in the anterior and lateral walls (0.16 ± 0.02 and 0.12 ± 0.07 mm vs. 0.32 ± 0.1 and 0.22 ± 0.05 mm) and decreased circumferential strain in the same walls (6.5% and 9.4% vs. 19.9% and 20.2%) in the groups treated with low dose vs. high dose probenacid. Conclusions: TRPV 2 is expressed in the myocardium. We found that peripheral TRPV2 stimulation with probenacid stimulates cardiac contracility, and initiates a cardioprotective effect against myocardial infarction similar to activation of the same field with TRPV1 agonists. It is possible that TRPV2 stimulation of peripheral nerves might stimulate ischemic preconditioning with subsequent cardioprotection. This is a novel finding with far reaching implications as TRPV agonists may be a powerful adjunct therapy for MI patients in the near future. The precise mechanism of action and potential uses are under study.

29 GERMLINE POLYMORPHISM DISCOVERED VIA A CELL-BASED GENOME-WIDE APPROACH PREDICTS PLATINUM RESPONSE IN OVARIAN AND HEAD AND NECK CANCERS


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Identifying patients prior to treatment that are less likely to benefit from or most likely to experience adverse events from chemotherapeutic agents is essential. Early identification of subtypes is the most relevant system, phenotypic and genotypic discovery in the field of oncology is plagued by difficulties in executing large clinical trials and confounding factors such as co-morbidities, dosage, and concomitant medications. Therefore, we developed a genome-wide cell-based approach evaluating single nucleotide polymorphisms (SNPs) and gene expression to predict chemotheraphy-induced response and toxicity and validated our findings in clinically relevant patient cohorts. Our model utilizes the International HapMap lymphoblastoid cell lines (LCLs). Genome-wide association studies were performed to identify SNPs significantly associated with carboplatin sensitivity through their effects on mRNA expression. The significant findings were evaluated in an independent LCL replication set and in patient samples obtained from the Australian Ovarian Cancer Study (AOCSS) and two Phase II head and neck clinical trials (UC12019 and UC13881) conducted at the University of Chicago. Four hundred and nine ovarian cancer patients receiving carboplatin and paclitaxel were analyzed in AOCSS; while 60 and 32 head and neck cancer patients were evaluated in UC12019 and UC13881 trials. Carboplatin was used as induction and concomitant chemo-radiotherapy therapy in UC12019 and UC13881 trials, respectively. Using LCLs, our genome-wide model identified 31 SNPs that are significantly associated with the carboplatin sensitivity phenotype through the expression of 26 genes. Four of them were replicated in a separate set of LCLs. In AOCSS, SNP (rs1649942) was significantly associated with progression-free survival (Plog-rank=0.009) and overall survival (Plog-rank=0.03). In the head and neck cancer trials, 2 other SNPs (rs180094 and rs169056) were identified and replicated in both trials; including the association between SNP (rs4946514) and overall response to carboplatin, and the association between SNP (rs7134205) and post treatment plateau changes. Given the obstacles to performing large, replicable pharmacogenomic studies in patients, the cell-based model is proven to be an effective alternative in novel pharmac-snpo discovery. We demonstrate germ-line SNPs identified through the cell-based genome-wide approach are clinically important predictors of chemotherapy response and toxicity.

30 EPHRINB2 REGULATES TUMOR MICROENVIRONMENTAL CONSTITUENTS

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The tumor microenvironment consists of heterogeneous populations of hematopoietic, vascular, and stromal cells and plays important roles in tumor growth. Microenvironmental vascular cells, which reside at the interface between tumors and the circulatory system, regulate inflammatory constituents. Molecules known to play key roles in vascular development have also been implicated in the control of leukocyte migration across endothelial barriers as well as activation. EphrinB2, which is an important regulator of vascular patterning and endothelial cell/pericyte interactions in developing embryos, is expressed in tumor endothelial cells and facilitates in vitro monocyte transmigration. We therefore hypothesized that ephrinB2 signaling in tumor endothelial cells promotes the recruitment of pro-growth inflammatory cells to the tumor microenvironment in vivo. Using an established model to assess ephrinB2 expression, the ephrinB2tau-lacZ/Wt mouse, we previously characterized the vasculature of invasive solid tumors including KR158 glioma and B16 melanoma (Westly et al, CSCCR 2009). Tumor invasion led to ephrinB2 upregulation in adjacent muscle, suggesting a role for ephrinB2 within tumors as well as adjacent tissues. To define the role of ephrinB2 signaling in tumor growth and invasion, we used a known pharmacological inhibitor of ephrinB2 signaling, soluble EphB4 (sEphB4). Inhibition of ephrinB2 led to growth delays of KR158 (PBS 187.9 mm2 vs. sEphB4 517 mm2, p = 0.03) and B16 (2420 mm2 vs. 1658 mm2, p = 0.002) tumors despite modest effects on vascular density and no effect on pericyte coverage. We analyzed bone marrow-derived cells in tumors using GFP positive bone marrow donors and C57BL6 wild type hosts to generate GFP / WT chimerics. Analysis of implanted KR158 tumors demonstrated bone marrow-derived GFP+ / LYVE1+ cells that were recruited specifically to the tumor/muscle interface. Furthermore, sEphB4 treatment led to decreased recruitment of LYVE1+ cells (40 versus 22 cells per field, p = 0.03), suggesting that ephrinB2 signaling promotes the recruitment of pro-growth angiogenic myeloid populations. These studies demonstrate the therapeutic potential of targeting tumor microenvironments by inhibiting angiogenic signaling pathways. Understanding the complex interplay between vascular associated molecules and hematopoietic cells will be critical for developing novel tumor therapies designed to manipulate tumor microenvironments.

31 ATORVASTATIN ATTENUATES LRPS/5SOX9 MEDIATES DEGENERATIVE JOINT DISEASE AND CARDIOVASCULAR CALCIFICATION IN THE HYPERCHOLESTEROLEMIC APOE NULL MICE


Background: Degenerative joint disease is a common clinical problem in the aging population which is responsible for significant morbidity and suffering in patients who have this disease. The cellular mechanisms of osteoarthritis in joints are under intense investigation. We have previously shown that Lrp5 mediates abnormal bone formation in the cardiovascular calcification via osteoblast differentiation pathway. We hypothesize that lipids may play a role in the development of this degenerative joint disease in parallel to the bone formation in the aortic valves.

Methods: In this study we tested the effect on lipids with and with atorvastatin in the ApoE mouse model to determine the gene and protein expression of the Lrp5/Sox9 pathway in the joints and in the valves. ApoE-/- mice (n=60). Group I (n=20) normal diet, Group II (n=20) 0.25% chol diet (w/w), and Group III (n=20) 0.25% (w/w) chol diet+atorv for the development of abnormal matrix synthesis in the joints over 24 weeks. The aortic valve (AVA) was examined for calcification, Lrp5 and Sox9. Bone formation was assessed by micro Computed Tomography (micro-CT). Matrix synthesis in the joint was assessed by Masson Trichrome Staining. Aortic Valve Disease was assessed by Visual Sonics Mouse echocardiography.

Results: The cholesterol diet induced complex bone formations in the calcified AV and thickened knee joints with an increase in Sox9, Lrp5 receptor expression and Cyclin as compared to the Control(-3-fold,p<.05). Atorvastatin attenuated all markers in the valves and in the joints. Echocardiography demonstrated a mild increase in the peak jet velocity across the aortic valve but not statistically significant. Masson Trichrome visual assessment demonstrated a marked increase in the matrix synthesis as compared to the controls and atorvastatin treated joints.

Conclusion: These results demonstrate that Lrp5 mediated bone formation in the hypercholesterolemic aortic valves and abnormal matrix synthesis in the knee joints is associated with aortic valve disease and is attenuated with atorvastatin. Providing further evidence in an atherosclerotic mouse model of joint disease and valvular heart disease, that statins play a role in the treatment of this disease.

32 GRANULATION TISSUE DERIVED STEM CELLS PREVENT RENAL DAMAGE AND ACCELERATE RECOVERY FROM ACUTE RENAL FAILURE


In earlier studies we showed that when a foreign body such as a polycyclic tube is placed in the subcutaneous tissue of rats, it rapidly induces formation of a new tissue that encapsulates the foreign body. The new tissue (called the subcutaneous granulation tissue) is a regenerating tissue that is well organized and supplied by new blood vessels (Singh et al., 2007). Further, we successfully isolated, cultured and characterized mesenchymal stem cells from this tissue, which a) produce high levels of growth factors, b) are capable of differentiating into multiple lineages (osteogenic, chondrogenic, adipogenic), and c) when systemically injected migrate only to injured organs in the body. These stem cells are called granulation tissue derived stem cells (GTSC) (Patel et al., 2009). Here we test the efficacy of GTSC to ameliorate acute renal failure (ARF) in rats. ARF (ischemia/reperfusion injury of the kidney) was induced in a group of Fischer (F344) in-bred rats. This was performed by unilateral (right) nephrectomy, occlusion of the left renal pedicle for 45 minutes using non-traumatic clamps followed by de-occlusion and reperfusion of the kidney. ARF is characterized by a rapid increase in plasma creatinine and histological changes in the kidney showing tubular necrosis, congestion and casts. After inducing ARF injury, the rats were divided into two groups. Four hours after injury, group 1 (treated; n=5) rats received one intravenous injection of GTSC (2-4 X 106 cells in 0.7 ml...
volume) and group 2 (control; n=5) rats received 0.7 ml of vehicle. Group 3
(n=5) were sham operated. Sham operated rats had normal renal function
(plasma creatinine = 0.7 mg/dL). Fusion of the activated omentum to an injured organ facilitates
repair and regeneration of injured tissue (Singh et al., 2009). Here we tested
whether activated omentum could ameliorate chronic kidney disease in rats.
Chronic kidney disease was induced in rats by renal mass reduction (5/6
nephrectomy by removal of right kidney and excision of the two poles of the
left kidney). Rats were divided into two groups. Group 1 rats (n = 5; treated)
received intraperitoneal injection of poloxadexan gel particles to activate the
omentum and promote its fusion to the kidney, while in Group 2 rats (n = 5;
control) omentum was prevented from fusing to the kidney by performing
omentectomy. Group 3 (n = 5) were sham-operated rats. In group 1 (treated)
rats there was fusion of the activated omentum with the cut pole of the kidney.
Group 2 (control) rats showed no such fusion. Both treated and control
groups showed a rise in plasma creatinine after 2 weeks of surgery. At week 6
the treated rats had much lower plasma creatinine (1.6 ± 0.05 mg/dL) than the
control rats (2.2 ± 0.08 mg/dL) (p < 0.05). At week 12, plasma creatinine
remained high (2.3 ± 0.07 mg/dL) in the control rats but it decreased to 1.4 ±
0.07 mg/dL in the treated rats (p < 0.05). Consistent with the plasma
creatinine levels creatinine clearance at week 12 was higher in treated rats
(p < 0.05). The average weight gain in treated rats was higher than in control
rats over the 12 weeks of observation. Both groups had moderate proteinuria
(30–60 mg/day; p = n.s.). Histology of the kidney by periodic acid Schiff
staining at week 12 showed significantly less glomerulosclerosis, tubular
dilation and interstitial fibrosis in treated rats as compared to control rats by
dilatation and interstitial fibrosis in treated rats as compared to control rats by
kidney injury score. Sham operated rats showed no changes in the renal
parameters during the 12 weeks of observation. These results suggest that fusing
activation of omentum to the kidney prevents progression of chronic kidney
disease and may even improve recovery from kidney damage.

33 FUSION OF ACTIVATED OMENTUM WITH KIDNEY PREVENTS PROGRESSION OF CHRONIC KIDNEY DISEASE

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In our previous work we showed that an omentum activated by an inert
foreign body (poloxadexan gel particles) greatly expands its milky-spot tissue
and becomes rich in stem cells and growth factors (Litbarg et al., 2007; Singh
et al., 2008). Fusion of the activated omentum to an injured organ facilitates
repair and regeneration of injured tissue (Singh et al., 2009). Here we tested
whether activated omentum could ameliorate chronic kidney disease in rats.
Chronic kidney disease was induced in rats by renal mass reduction (5/6
nephrectomy by removal of right kidney and excision of the two poles of the
left kidney). Rats were divided into two groups. Group 1 rats (n = 5; treated)
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kidney injury score. Sham operated rats showed no changes in the renal
parameters during the 12 weeks of observation. These results suggest that fusion
activation of omentum to the kidney prevents progression of chronic kidney
disease and may even improve recovery from kidney damage.

34 A RANDOMIZED CONTROLLED TRIAL OF A PRIMARY CARE INTERNET BASED DEPRESSION PREVENTION INTERVENTION FOR ADOLESCENTS (CATCH-IT): 12-MONTH OUTCOMES

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Objective: Depressive disorders affect 25% of adolescents by age 24 and
cause considerable morbidity and mortality (suicide). However, no low cost,
acceptable and widely available prevention/intervention program currently
exists. To address this need, we developed a primary care Internet-based
depression prevention intervention for adolescents analysis 14–21 (CATCH-IT).
A critical question is what form of physician engagement would be necessary
to ensure adherence to the Internet-based behavior change program.
Motivational interviewing (MI, patient centered, physician seeks to help
the adolescent develop their own rationale for participation) and brief advice
(BA, physician centered, physician recommends the intervention based medical
recommendation) are two contrasting approaches to increasing adherence. We hypothesized that the longer, more time intensive MI would be
superior to BA in engaging into the program and preventing depressive episodes.

Methods: We evaluated two versions of this intervention: primary care
physician (PCP) motivational interview + Internet program (MI) versus PCP
brief advice + internet program (BA) in thirteen primary care sites of four US
health systems. The intervention includes an initial and follow-up interview
in primary care, 14 Internet-based modules based targeting risk behaviors and
an accompanying parent combined program. Patients were screened for risk
of major depression (depresion exhibited below the level of major depression)
and evaluated by phone to confirm inclusion criteria (depressed mood) and
exclusion (current major depression or other mental disorder) and assigned
randomly to the BA (N=40) or MI groups (N=43) and then evaluated by
blinded assessors.

Results: We enrolled 83 adolescents (mean age = 17.2 years; 40 % ethnic
minority and no significant between group differences). Physicians demonstrated satisfactory fidelity to the MI and BA interview models and
MI was of longer duration (6 minutes versus 1.7, p-value =0.003) CES-D
(centers for Epidemiologic Studies Depression Score, depressed mood)
scores decreased in both groups from baseline to 12 weeks with statistically
significant reductions and sustained out to 52 weeks follow-up (MI baseline
24.03 SD=12.2, 6-weeks 17.5 SD=11.7, 12 weeks 14.9 SD=8.8, 52 weeks 14.0
SD=9.6, all p-values < 0.005; BA baseline 25.1 SD=12.5, 6 weeks 15.5
SD=11.0, 12 weeks 14.88 SD= 10.5, 52 weeks 14.1 SD=10.6, p-values
comparisons < 0.02). Both groups demonstrated significant improvements in
social support from peers with moderate to large effect sizes (MI: 0.76, 95%
CI: 0.18, 1.32; BA: 1.39, 95% CI: 0.75, 1.99; all: 1.09, 95% CI: 0.64, 1.52).
MI had significantly higher levels of engagement than BA for measures
including total time on site (143.7 minutes versus 100.2 minutes, p=0.03),
number of sessions (8.16 versus 6.00, p=0.04), longer duration activity
(46.2 days versus 29.34 days, p=0.04), and with more characters typed into
exercises (3532 versus 2004, p=0.01) and more favorable ratings of the
intervention with the physician (“Trust”, 4.18 versus 3.74, p=0.05). The MI
group demonstrated fewer depressive episodes compared to the BA group by
12 months (4.76% 95% CI 0.11 versus 30% 95% CI 15, 44). The MI group
was significantly less likely than the BA group to experience a depressive
episode (4.65% versus 22.5%, p = 0.023) or to report hopelessness (MI group
of 2% versus 15% for the BA group, p=0.044) by 12 weeks.

Conclusion: Adolescents demonstrated sustained reductions in depressed
mood and suicidal ideation out to 52 weeks. Motivational interviewing, when
coupled with the Internet intervention, increases adherence to the Internet
program and may confer additional protection against depressive episodes,
hopelessness, and self-harm ideation.

35 DELAYED CYTOPROTECTION MEDIATES AMIFOSTINE PROTECTIVE EFFECTS ON MOUSE MODEL OF VENTILATOR-INDUCED LUNG INJURY

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Rational: Over distension of lung tissue induces excessive oxidative species
(ROS), which has been linked to the pathogenesis of ventilator-induced lung
injury (VILI). Amifostine, a reducing reagent with phosphorothioate, has
been demonstrated the protective effect against lipopolysaccharide-induced
acute lung injury by virtue of its ability to reduce oxidative stress.

Objective: To investigate the protective effect of amifostine on VILI and to
test whether the delayed cytoprotection is the mechanism underlying the
protective effect of amifostine.

Method: In vivo, male C57BL/6j mice received triple i.p. injection of
amifostine (25 mg/kg) followed by mechanical ventilation with high tidal
volume (30 ml/kg) for 4 hrs. Lung injury was assessed by bronchoalveolar
lavage (BAL) performance and histological examination. Pulmonary vascular
permeability was assessed by Evans Blue assay. Superoxide dismutase 2
(SOD2), catalase enzyme activity and MDA content in lung tissue were
measured to 0.17 to 0.17 tissue oxidative stress. In vitro, human pulmonary artery
endothelial cells (HPAEc) were pretreated with amifostine (0.5 mM and
1 mM) daily for 3 days, then subjected to 18% cyclic stretch for 2 hrs
followed by 0.01 U/ml thrombin stimulation 5 min prior to the end of cyclic
stretch. Western blot was performed to analyze oxidative sensitive signaling
pathways.

Results: Amifostine significantly reduced BAL protein concentration and
neutrophil accumulation in alveolar space induced by mechanical ventilation

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(0.23±0.019mg/ml vs 0.13±0.025mg/ml; 3.46±0.33/ml vs 1.26±0.07/ml, respectively). Moreover, amifostine markedly reduced Evans blue accumulation, suggesting the beneficial barrier protective effect of amifostine. In contrast, single dose of amifostine failed to show protective effect against VILI. HTV induced significant oxidative stress in lung tissue indicated by enhanced MDA content and impaired catalase activity, which were attenuated by amifostine treatment. In addition, amifostine significantly upregulated SOD activity. Signaling analysis showed cyclic stretch in combination with thrombin stimulation induced significant ROS production in endothelial cells (EC) and activation of oxidative stress sensitive inflammatory regulators, such as p38, JNK MAP kinase, which was completely abrogated by pretreatment of the cells with amifostine.

**Conclusions:** We conclude that 3 days low dose amifostine treatment up-regulated intracellular anti-oxidant defense system, which counteracts oxidative stress induced by HTV. Therefore, the protective effect of amifostine is attributed to the delayed effect rather than the ability of amifostine as reactive oxygen species scavenger.

36
THE NOVEL S-PHOSPHONATE ANALOG OF FTY720 REGULATES PULMONARY VASCULAR PERMEABILITY

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**Rationale:** A significant and sustained increase in vascular permeability is a hallmark of acute inflammatory diseases such as acute lung injury (ALI). Unfortunately, effective therapies for preserving or reconstituting the vascular barrier are lacking. We have demonstrated that FTY720, an analog of the potent barrier-enhancing phospholipid, sphingosine 1-phosphate (S1P), has potent vascular barrier-enhancing effects. However, FTY720 causes bradycardia and immunosuppression by inducing lymphopenia that may be detrimental in patients with ALL.

**Methods/Results:** Because of these limitations, we have developed several novel FTY720 analogs for vascular barrier enhancement. The FTY720 S-phosphonate analog exhibits potent effects on cultured human pulmonary artery endothelial cell (EC) barrier function in vitro (as measured by transendothelial electrical resistance(TER)) with a wider protective concentration range (1-50 μM) and greater potency than either S1P or FTY720 and significantly reduces lung permeability (>60% reduction in BAL protein, relative to saline controls) in murine ALI. Reduction of S1P1 receptor expression with siRNA significantly attenuates S-phosphonate TER elevations. Importantly, incubation with S-phosphonate maintains EC S1P1 expression in contrast to reductions of at least 50% after treatment for 4 hrs with S1P, FTY720, or other analogs. S-phosphonate induces rapid cortical actin formation in EC, while actin depolymerization with cytochalasin blocks S1P, FTY720, or other analogs. S-phosphonate induces rapid cortical actin formation in EC, while actin depolymerization with cytochalasin blocks S-PHOS and calcium tolerance during REP, suggesting that protection provided by PC is mediated by preventing mitochondrial permeability transition pore opening, rather than direct improvement of OXPHOS. Thus, ISC damaged mitochondria can still be modulated during REP in order to attenuate mitochondrial-driven cardiac injury.

38
DEVELOPMENT OF FUNCTIONAL NATURAL KILLER CELLS FROM HUMAN PLURIPOTENT STEM CELLS

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Hematopoietic cell transplantation (HCT) is routinely being utilized as an effective mean to treat and potentially cure many patients with hematologic malignancies such as leukemia, lymphoma, or myeloma. However, despite advances in cell-based therapies, many patients still succumb to these cancers. Anti-tumor immunotherapy can provide an important alternative treatment, and promising results using T cell or natural killer (NK) cell-based therapies highlight the exciting therapeutic potential of harnessing the cellular immune system to fight hematologic malignancies. Our research has focused on use of human embryonic stem cells (hESCs) as an important and novel starting point to study human hematopoiesis and lymphopoiesis. We have previously demonstrated that hESC-derived CD34+ cells (or sub-populations of CD34+ cells) can effectively produce NK cells in vitro with potent anti-tumor activity against several tumor targets, similar to umbilical cord blood (UCB) derived NK cells. Here, we demonstrate the production of functional NK cells from human induced pluripotent stem (iPS) cells. Briefly, iPS cells are somatic cells that are “reprogrammed” using defined genes to become the functional equivalent of ES cells. Human iPS cells can differentiate into multiple cell lineages, including hematopoietic cells. Using similar methods that reliably generate hematopoietic progenitors from hESCs we isolated populations of CD34+CD45+ cells that can effectively produce NK cells in vitro with potent anti-tumor activity against several tumor targets, similar to umbilical cord blood (UCB) derived NK cells. Here, we demonstrate the production of functional NK cells from human induced pluripotent stem (iPS) cells. Briefly, iPS cells are somatic cells that are “reprogrammed” using defined genes to become the functional equivalent of ES cells. Human iPS cells can differentiate into multiple cell lineages, including hematopoietic cells. Using similar methods that reliably generate hematopoietic progenitors from hESCs we isolated populations of CD34+CD45+ cells that can effectively produce NK cells in vitro with potent anti-tumor activity against several tumor targets, similar to umbilical cord blood (UCB) derived NK cells. Here, we demonstrate the production of functional NK cells from human induced pluripotent stem (iPS) cells. Briefly, iPS cells are somatic cells that are “reprogrammed” using defined genes to become the functional equivalent of ES cells. Human iPS cells can differentiate into multiple cell lineages, including hematopoietic cells. Using similar methods that reliably generate hematopoietic progenitors from hESCs we isolated populations of CD34+CD45+ cells that can effectively produce NK cells in vitro with potent anti-tumor activity against several tumor targets, similar to umbilical cord blood (UCB) derived NK cells. Here, we demonstrate the production of functional NK cells from human induced pluripotent stem (iPS) cells. Briefly, iPS cells are somatic cells that are “reprogrammed” using defined genes to become the functional equivalent of ES cells. Human iPS cells can differentiate into multiple cell lineages, including hematopoietic cells. Using similar methods that reliably generate hematopoietic progenitors from hESCs we isolated populations of CD34+CD45+ cells that can effectively produce NK cells in vitro with potent anti-tumor activity against several tumor targets, similar to umbilical cord blood (UCB) derived NK cells. Here, we demonstrate the production of functional NK cells from human induced pluripotent stem (iPS) cells. Briefly, iPS cells are somatic cells that are “reprogrammed” using defined genes to become the functional equivalent of ES cells. Human iPS cells can differentiate into multiple cell lineages, including hematopoietic cells. Using similar methods that reliably generate hematopoietic progenitors from hESCs we isolated populations of CD34+CD45+ cells that can effectively produce NK cells in vitro with potent anti-tumor activity against several tumor targets, similar to umbilical cord blood (UCB) derived NK cells. Here, we demonstrate the production of functional NK cells from human induced pluripotent stem (iPS) cells. Briefly, iPS cells are somatic cells that are “reprogrammed” using defined genes to become the functional equivalent of ES cells. Human iPS cells can differentiate into multiple cell lineages, including hematopoietic cells.
Systolic blood pressure (SBP) increases with age in both men and women. While SBP is lower, on average, among women compared to men prior to age 60, this pattern reverses after age 60. The serum concentration of 17β-estradiol declines with menopause and it has been suggested that lower levels of this hormone contribute to higher rates of hypertension among women compared to men after age 60. We recently identified an inverse relationship between systolic blood pressure (SBP) and serum 16α-hydroxyoestrone, a metabolite of 17β-estradiol, in postmenopausal women. 16α-hydroxyoestrone is an antioxidant and induces endothelial cell production of prostacyclin, a potent vasodilator. Formation of 16α-hydroxyoestrone is catalyzed primarily by CYP3A7, a cytochrome P450 enzyme. The goal of this study was to evaluate the relationships between known dietary and behavioral modifiers of CYP3A7 activity and serum 16α-hydroxyoestrone concentration in a population-based sample of 34 postmenopausal women aged 50-65 living in Cook County, Illinois. We used mass spectrometry to measure the serum concentration of 16α-hydroxyoestrone. Survey questionnaires were administered to assess dietary and behavioral patterns, as well as demographic factors. We hypothesized that exercise and dietary fiber from fruits and vegetables (known inducers of CYP3A7) would be positively associated with serum 16α-hydroxyoestrone while fiber from grains would not be associated with serum 16α-hydroxyoestrone. We also hypothesized a negative association between African-American race and serum 16α-hydroxyoestrone, given the higher rate of hypertension in this population. Serum 16α-hydroxyoestrone values exhibited a positively skewed distribution so they were subjected to natural log (ln) transformation prior to multivariate regression analysis. After adjusting for age, body mass index, and race/ethnicity, we found positive relationships between ln serum 16α-hydroxyoestrone and both exercise (B = 0.971, p = 0.038) and dietary fiber from fruits and vegetables (B = 0.059, p = 0.047). Dietary fiber from grains was not associated with ln serum 16α-hydroxyoestrone but African-American race (B = 0.047). Dietary fiber from grains was not associated with ln serum 16α-hydroxyoestrone and both exercise and dietary fats and vegetables. In addition, our results suggest a mechanism by which African-Americans experience higher blood pressure compared to other racial/ethnic groups. Further research is needed regarding additional behavioral and genetic factors that may influence CYP3A7 activity, as well as the relative importance of 16α-hydroxyoestrone to SBP among both women and men.

40 RESPIRATORY SYNCTIAL VIRUS LIMITS α SUBUNIT OF TRANSLATION INITIATION FACTOR (eIF2α) PHOSPHORYLATION TO MAINTAIN TRANSLATION AND VIRAL REPLICATION
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Background: Respiratory syncytial virus (RSV) causes bronchiolitis in infants, exacerbations in patients with obstructive lung disease, and pneumonia in immunocompromised hosts. We have shown that RSV increases the amount of protein kinase R (PKR) protein, a cellular kinase triggered by a double-stranded RNA (ds-RNA) intermediate during replication of the virus. In most instances, ph-PKR targets the α subunit of translation initiation factor eIF2α (eIF2α) protein via phosphorylation, leading to an inhibition of translation of cellular and viral protein. We hypothesize that RSV maintains translation of its own viral proteins by limiting phosphorylation of eIF2α and maintaining overall cellular protein translation.

Methods: A549 airway epithelial cells were exposed to control media or RSV (MOI 2) for 24 hours. PKR, ph-PKR, and ph-eIF2α protein were determined with Western blot. Immunoprecipitation of eIF2α with PKR, PPK, and PP2A were determined. Immunoprecipitation of PKR with specific RSV proteins was also measured, and subsequently, immunoprecipitation of PKR with RSV N protein expressed in a vaccinia virus construct was evaluated. The effect of RSV on cellular translation of nascent protein was measured. The effect of PKR siRNA on viral replication was also determined.

Results: Although ph-PKR increases in RSV infection, significant eIF2α phosphorylation is not observed, and inhibition of protein translation does not occur. RSV infection attenuates eIF2α phosphorylation by favoring phosphatase rather than kinase activity. RSV sequesters PKR away from eIF2α by binding of the kinase to the RSV N protein. This occurs in conjunction with an increase in the association of the phosphatase, PP2A, with eIF2α following PKR activation. The result is limited phosphorylation of eIF2α and continued translation of cellular and viral proteins and viral replication. Further, although treating the cells with PKR siRNA markedly decreased PKR protein, there was no difference in viral replication with PKR siRNA as compared to cells with a control siRNA.

Conclusion: Binding of the RSV N protein to PKR reduces the association of the kinase with eIF2α and increases the association of the PP2A phosphatase with eIF2α limiting phosphorylation and maintaining translation. These observations suggest that the binding of RSV N protein to PKR may be an important target for therapy in RSV infection. VA Merit Review grant; NIH: HL089392-02, HL073967-01, HL077431-01, HL075559-04, RO1 HL079901-01A1, AI 063520, and RR00059 from the General Clinical Research Centers Program and UL1RR024979 from the NCRR, NIH.

41 STIMULATION OF NCC ACTIVITY BY ALODOSTERONE
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Aldosterone increases NCC function and protein expression in whole animal studies at 72 hours. Earlier effects are present in other transport proteins, but more acute effects of aldosterone on NCC activity have not been studied. NCC functional studies in mammalian cells have utilized low Cl- pre-incubations to stimulate activity, but such treatments activate SPAK/OSR1, kinases which may be involved in aldosterone signaling. To assess changes in NCC activity without stimulating SPAK/OSR1, mDCT cells were subcloned via cloning rings to select for cells with enhanced baseline NCC activity, therefore eliminating the need for a low Cl- pre-incubation. NCC activity was measured by radiotracer uptake. Cells were grown to 90% confluence, incubated in a 22Na+ containing medium, then washed and lysed. Radioactivity was counted and uptakes normalized to total protein. Thiazide-sensitive uptake was given by the difference of the uptakes with and without thiazide. A subclone designated mDCT15 demonstrated greatly increased thiazide-sensitive 22Na+ uptake compared to mDCT cells after a 30 minute low Cl- pre-incubation (1853.8 ± 234.6 nmol/mg/20min vs. 78.8±026.3, nmol/mg/20min, n=6, p<0.01). Uptakes without a low Cl- pre-incubation remained robust (1237.2±29.0 nmol/mg/20min). To confirm that this 22Na+ uptake represented NCC activity, uptakes were performed in uptake solutions with and without chloride to assess Cl- dependence. Essentially zero thiazide-sensitive 22Na+ uptake was observed in the absence of Cl-, indicating that the 22Na+ uptake was both thiazide-sensitive and chloride dependent, the hallmarks of NCC activity. The acute effects of aldosterone were assessed by incubating serum-starved mDCT15 cells in a serum-free media containing 100nm aldosterone for 6 and 24 hours prior to radiotracer uptake. Aldosterone stimulated NCC activity by 26% at 6 hrs and by 52% at 24 hrs (1941.0 ±287.8 nmol/mg/20min at 6 hours, 2341.8±25.6 nmol/mg/ 20min at 24 hours compared to 1539.6±19.7 nmol/mg/20min for vehicle, n=4, p<0.01 vs. vehicle for both). This represents the first report of an early effect of aldosterone on NCC function. Further work is necessary to characterize the mechanism by which this effect occurs.
streptozotocin to induce islet inflammation. Further, decreased SERCA2 expression was found to correlate with elevated basal (Ca2+)-levels, impaired Ca2+ response to glucose, and insulin secretory defects. To investigate the mechanisms underlying these observations, an in vitro model intended to mimic the molecular milieu of T2DM was created by treating rat islets (INS-1) cells with high glucose (25 mM) and the pro-inflammatory cytokine IL-1β. Using this model, we observed similar decreases in SERCA2 expression. Further, in the presence of 25 mM glucose and IL-1β, we observed a decrease in total SERCA2 protein levels, as well as increased degradation and inactivation of SERCA2 protein. Previous studies have suggested that pharmacologic PPAR-γ activation in T2DM improves β cell function, independent of PPAR-γ effects to lower blood glucose and free fatty acids. Treatment with the PPAR-γ agonist, pioglitazone, restored SERCA2 expression in diabetic mouse and human islets and improved Ca2+ homeostasis in db/db mice. Moreover, it was also able to restore SERCA2 protein levels and prevent SERCA2 degradation in the in vitro INS-1 model. To determine if the transcriptional effects were direct, we scanned the SERCA2 promoter for PPAR-responsive elements (PPREs) and identified 6 putative elements. Luciferase assays were performed in INS-1 cells using different fragments of the SERCA2 promoter. Results from these assays suggest that a region 259 bp upstream of the transcriptional start site, which contained 2 potential PPREs, is sufficient to confer PPAR-γ transactivation of the SERCA2 gene. These results were confirmed by chromatin immunoprecipitation assays, which demonstrated that PPAR-γ directly binds the SERCA2 promoter. Taken together, these data suggest that transcriptional and post-translational dysregulation of SERCA2 in the islet may contribute to the β cell dysfunction observed in T2DM. Our results also suggest that PPAR-γ has a direct effect on the islet to regulate transcription of the SERCA2 gene, which may explain, in part, the beneficial islet-specific effects of PPAR-γ agonists. Further studies will be performed to characterize the pathways through which SERCA expression is altered in T2DM and to clarify the mechanisms by which PPAR-γ prevents SERCA2 protein inactivation and degradation.

43 LOW SERUM 25(OH) VITAMIN D LEVELS (<32 ng/ml) ARE ASSOCIATED WITH REVERSIBLE MYOSITIS-MYALGIA IN STATIN-TREATED PATIENTS

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Our specific aims were to determine whether low serum 25(OH) vitamin D (D2+D3) (<32ng/ml) was associated with myalgia in statin-treated patients and whether the myalgia could be reversed by vitamin D supplementation while continuing statins. After excluding subjects taking corticosteroids or vitamin D therapy, 221 myalgic and 721 asymptomatic patients (26.4±7.0 to 43.7±11.7 ng/ml, p<0.0001), were more likely to be black (11% vs 6%, p=0.013) and female (63% vs 42%, p=0.0001). By analysis of variance, adjusted for race, gender and age, least square mean (±SE) serum vitamin D was lower in the 221 patients with myalgia than in the 721 asymptomatic patients, 23.5±1.1 vs 27.5±0.9 ng/ml, p=0.001. Serum 25 (OH) D was low (<32 ng/ml) in 165/221 (75%) patients with myalgia vs 43/721 (61%) asymptomatic patients (χ2=13.9, p=0.0002). Of the 155 vitamin D deficient, myalgic patients, while continuing statins, 88 were given vitamin D (50,000 units/week for 4.3±2.5 months), with a resultant increase in serum vitamin D from 20.4±7.0 to 24.3±7.1 ng/ml p<0.0001). In these 88 patients, 84 (95%) had no myalgia at their last visit, and 67 (76%) had normalized vitamin D. We speculate that symptomatic myalgia in statin-treated patients with concurrent vitamin D deficiency may reflect a reversible interaction between vitamin D deficiency and statins on skeletal muscle.

44 MECHANISMS OF ALDOSTERONE ACTION ON NCC

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Aldosterone increases NCC function and protein expression in whole animal studies at 72 hours but more acute effects of aldosterone on NCC activity have not been studied. We have recently demonstrated that in a mammalian cell model with robust native NCC activity (mDCT15), aldosterone stimulated NCC activity by 26% at 6 hrs and by 72% at 24 hrs. However, the mechanism by which this acute stimulation was mediated is unknown. To examine this question, mDCT15 cells were incubated with either 100nM aldosterone or vehicle (DMSO) for 6 or 24 hours. Next day, open surface biotinylated and surface expression was assessed by Western blotting. Total NCC protein expression remained unchanged compared to control in both the 6 and 24 hour treatment groups. Furthermore, NCC surface expression did not change in the 6 or 24 hour aldosterone treated groups compared to control. This suggests that the effects of aldosterone upon NCC are not primarily mediated by increased surface expression, but instead due to an increase in individual transporter activity. Further work is needed to characterize this mechanism.

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45 GENETIC DELETION OF NOS3 INCREASES LETHAL CARDIAC DYSFUNCTION FOLLOWING MOUSE CARDIAC ARREST

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Study Aims: A significant proportion of cardiac arrest victims exhibit severe myocardial dysfunction and cardiovascular collapse during the first minutes to hours following successful return of spontaneous circulation (ROSC). Recent work suggests that nitric oxide (NO) signaling may impact resuscitation success and post-ROSC cardiovascular outcomes. We studied the role of endothelial NO synthase (eNOS or NOS3) deficiency on hemodynamic outcomes and NO production following cardiac arrest and resuscitation in genetically-modified mice.

Methods: Adult female wild-type (WT) and NOS3-deficient (NOS3−/−) mice were anesthetized, intubated, and instrumented with left-ventricular pressure-volume catheters. Cardiac arrest was induced with intravenous potassium chloride (KCl). After 8 min of untreated arrest, cardiopulmonary resuscitation (CPR) was performed. Cardiac function, whole-blood nitrosylhemoglobin (HbNO) concentrations, whole heart NOS3 expression and phosphorylation (p-NOS3) were assessed during the first 120 min following ROSC.

Results: Following cardiac arrest and CPR, WT animals displayed higher ROSC rates than NOS3−/− mice (82.4% [14/17] vs 47.6% [10/21], p<0.005). Successfully resuscitated mice displayed left-ventricular dysfunction and cardiovascular collapse within 120 min of ROSC that was greater in NOS3−/− versus WT mice. Cardiac arrest and resuscitation induced both NOS3-independent and -dependent increases in HbNO. In addition, significant modulation of heart tissue NOS3 expression and phosphorylation was observed.

Conclusions: Our results demonstrate that genetic deletion NOS3 decreases cardiopulmonary resuscitation success and worsens post-ROSC left-ventricular function in a mouse model of asystolic cardiac arrest. Post-ROSC cardiac dysfunction may be mediated by cardiomyocyte NOS3 signaling or enzyme-dependent generation of circulating NO metabolites.

46 TRANSCRIPTIONAL REGULATION OF HYPEROXIA-INDUCED NOX4 EXPRESSION IN LUNG ENDOTHELIAL BY NRF2-ANTIOXIDANT RESPONSE ELEMENT

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Rationale: Nox4 is highly expressed in lung endothelium and we have demonstrated earlier that hyperoxia-mediated Nox4 expression and ROS production is regulated by Nrf2 via MAPKs in mouse lung and human pulmonary artery endothelial cells (HPAECs). Here we have investigated transcriptional control of Nox4 expression by hyperoxia through antioxidant response element (ARE) in Nox4 gene through deletions of ARE on the promoter sequence.

Methods/Results: Exposure of HPAECs to hyperoxia (1–24 h) activated Nrf2 as determined by Nrf2 translocation to the nucleus and increased phosphorylation. Down-regulation of Nrf2 with siRNA attenuated basal expression; however, enhanced superoxide/Ros generation under both normoxia and hyperoxia (1–3 h). In vivo studies with Nrf2−/− mice showed that Nox4, but not Nox2, is down-regulated in response to hyperoxia. In silico analysis revealed presence of partial ARE consensus sequences in Nox4 promoter region. Alignment of ARE consensus sequences with Nox4 promoter sequence revealed at least three potential ARE sites at −438 to −458;
47 CIGARETTE SMOKE INDUCES CELLULAR SENESCENCE VIA P53-MEDIATED WRN PROTEIN DOWNREGULATION

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Rationale: Werner’s syndrome is a genetic disorder that causes premature aging due to loss-of-function mutations in the WRn gene encoding a member of the RecQ helicase family. Both WRn protein defects and cigarette smoke accelerate cellular senescence. We recently found that cigarette smoke induces cellular senescence via WRn protein downregulation in cultured lung fibroblasts. Based on these findings, we hypothesized that cigarette smoke-induced WRn protein downregulation by activating the senescence-inhibiting proteins, p53 and p16.

Methods: We generated p53- and p16-deficient lung fibroblasts by a retroviral vector encoding shRNA to knockdown each protein. p53- and p16-deficient lung fibroblasts were cultured in the presence or absence of cigarette smoke extract (CSE). Cellular senescence and WRn protein expression were determined.

Results: p53 deficient cells were significantly more resistant to CSE-induced WRn protein downregulation and cellular senescence compared with normal lung fibroblasts. In contrast, p16 knockdown prevented neither WRn protein downregulation nor cellular senescence by CSE.

Conclusions: Cigarette smoke induces WRn protein downregulation and cellular senescence via the p53 dependent pathway.

48 THE ASSOCIATION OF CD 14 LEVELS WITH THE DEVELOPMENT OF ABDOMINAL AORTIC ANEURYSMS

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Background: Five percent of individuals over the age of 65 have an abdominal aortic aneurysm (AAA), a major cause of morbidity and mortality. The etiology of AAA is unknown. Numerous studies in humans and animal models have associated inflammation with AAA formation. The goal of this investigation was to determine the association between soluble CD14 and AAA formation in both a murine model and a case-control study in humans.

Methods: Phase 1 of the project examined the effect of CD14 gene deletion on AAA formation in two widely accepted murine models: angiotensin II (AngII) and elastase perfusion. Male apolipoprotein E-deficient (apoE-/-) mice were infused with Ang II at 1000 ng/kg per minute via osmotic mini-pumps. In the second model we performed elastase perfusion of the infrarenal aorta in C57Bl/6 wild type or CD14-/- mice. At 14 days for both models, abdominal aortic wall and maximal diameter were determined, and aortic tissues were harvested for immunohistochemistry, zymography, and histology. Aneurysms were defined as a diameter increase of 30% from baseline aorta size. In phase 2 we performed a case-control analysis of the relationship between soluble CD14 levels and AAA formation. Patients were identified by chart review of an ongoing cancer screening program. Cases were defined as those with a documented AAA in a diagnostic test report (CT scan or ultrasound), an operative report from AAA repair, or a pathology report. Controls were comprised of a similar age and sex-matched cohort who had CT scans confirming that a AAA was not present. Banked serum samples from the closest screening exam prior to surgery were tested using an ELISA for CD14. A Fisher’s Exact test was used to compare proportions of patients with and without AAA formation.

Results: In phase 1, CD14 gene deletion attenuated AAA formation in both models. Infusion of AngII produced AAA in 92%, and thoracic aortic aneurysms (TAA) in 42%, of apoE-/- mice, whereas no animals infused with saline developed AAA. Secondly, deletion of CD14 led to a significant reduction in aortic diameter consistent with reduced AAA formation (Figure 1). Likewise, infusion of elastase induced an increase in aortic diameter in C57Bl/6 mice consistent with AAA formation. In contrast, none of the CD14-/- mice developed AAA following elastase infusion. (Figure 2) Elastin degradation, MMP activity, and macrophage infiltration were also decreased in CD14-/- mice in both models. In phase 2 we analyzed blood samples from 28 controls and 21 cases. Subjects with AAA had significantly higher mean CD14 levels than those subjects without AAA (1634 vs 1958 ng/ml, p=0.023)(Figure 3).

Conclusions: CD14 levels were associated with AAA formation in both animal and human models. A larger prospective study is needed to confirm these findings as well as delineate the mechanism of AAA formation with the aim of developing therapy to combat this deadly disease process.

49 POLYMYALGIA RHEUMATICA MASQUERADING AS STATIN INDUCED MYALGIA

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Background: Myositis and myalgia are commonly reported clinical side effects in patients taking statins, often reversibly associated with vitamin D deficiency. Our specific aim was to focus diagnostic attention to polymyalgia rheumatica as an important differential diagnosis in women with intractable generalized myalgia attributed to statins, often with concomitant low 25 (OH) vitamin D levels. In 3 cases reported to us for myalgia on statins, we found that symptomatic on newly established statin even after vitamin D replacement, the subsequent diagnosis of polymyalgia rheumatica was made. Case #1 A 65 year old white female receiving Vytorin 10/80 mg for high LDL cholesterol complained of fatigue and generalized muscle aches and was found to have serum low vitamin D 30.8 (normal range 22–100 ng/ml) and normal CPK 24 (normal range 24–175 U/L). She remained symptomatic on newly established statin after serum vitamin D was normalized. Further laboratory evaluation revealed a high erythrocyte sedimentation rate (ESR) 127 (normal range 0–20 mm/hr) and high C-reactive protein (CRP) 125.3 (normal range 0–4.9 mg/ml)normocytic anemia was also present, homoglobin 10.7 (normal range 11.5–15 gm/dl). Polymyalgia rheumatica was diagnosed as the etiology for the persistent generalized muscle pain. Case #2 A 47 year old white female with high LDL cholesterol could not tolerate Lipitor or Pravachol at any dose level due to myalgia. She took Vicodin for muscle pain which persisted even after statins were discontinued. She was found to have low vitamin D 14.8ng/ml and normal CPK 53 U/L. She remained symptomatic on newly established statins after vitamin D replacement. Further laboratory evaluation revealed elevated ESR 22 mm/hr) and CRP 5.1 mg/L. Polymyalgia rheumatica was diagnosed as the etiology for the persistent generalized muscle pain. Case #3 A 65 year old white female receiving 1.2 g/day of Lpid for hypotriacyleridemia has developed generalized body aches and muscle stiffness. She had normal serum vitamin D 35ng/ml and CPK 35 U/L with elevated ESR 127 mm/hr) and CRP 181.3 mg/ml. Based on the above clinical assessment and lab criteria, we concluded that generalized pain was related to polymyalgia rheumatica. Myalgia-myopathy was entirely relieved on corticosteroid treatment, while continuing on Lopid.

Discussion: Generalized myalgia, often associated with low serum vitamin D, is one of the most common symptoms reported by patients treated with statins for high LDL cholesterol. Persistent myalgia-myopathy with normal CPK levels after correction of vitamin D deficiency and re-institution of statins or Lopid should raise diagnostic suspicion of polymyalgia rheumatica. Polymyalgia rheumatica is characterized by generalized myalgia, stiffness of the shoulder and pelvic girdles, high sedimentation rate and C reactive protein levels, and (usually) rapid clinical response to corticosteroids. Polymyalgia rheumatica is not a known side effect of statins, but the myalgia-myopathy can be misdiagnosed as statin-induced. Further studies are needed to determine whether the prevalence of polymyalgia rheumatica is higher in statin-treated patients.

Conclusion: In patients evaluated because of putative statin-induced myalgia and inability to tolerate statins, persistent after correction of vitamin D deficiency, the diagnosis of polymyalgia rheumatica needs to be considered.

50 ASBESTOS INDUCES AN ENDOPLASMIC RETICULUM STRESS RESPONSE IN ALVEOLAR EPITHELIAL CELLS

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Rationale: Asbestos causes asbestosis and malignancies by mechanisms that are not fully elucidated. The extent of alveolar epithelial cell (AEC) injury and repair are critical determinants of the fibrogenic potential of toxic agents such as asbestos. We previously showed that iron-derivated reactive oxygen species (ROS) from the mitochondria mediate asbestos-induced AEC DNA damage and apoptosis by a mitochondria-regulated (intrinsically) death pathway. The endoplasmic reticulum (ER) and mitochondria are interconnected physically and functionally. The ER stress response affords a protective cellular mechanism following exposure to various noxious stimuli, including ROS, but can trigger intrinsic apoptosis. Recent studies show that ER stress occurs in AEC of patients with idiopathic pulmonary fibrosis (IPF) by mechanisms that remain uncertain. We hypothesized that ER stress has a role in regulating asbestos-induced intrinsic AEC apoptosis.

Methods: We measured amosite asbestos-induced ER Ca2+ release by a Fura-2 assay. We used real time RT-PCR and Western Blotting to measure mRNA and protein expression, respectively, of the proteins implicated in the ER stress response (e.g., CHOP, XBP1, IRE-1, and GRP78) in human A549 and rat AT2 cells.

Results: Asbestos rapidly mobilizes A549 cell ER Ca2+ release within 5 min then decreases but remains elevated over 20 min. Asbestos increases A549 cell mRNA expression of ER stress proteins at 30 min (Table) and CHOP and XBP1 mRNA remain elevated at 24 h. These changes occurred with doses of asbestos that trigger intrinsic apoptosis (25 μg/cm2) as well as a lower dose that does not (5 μg/cm2). Preliminary studies show that asbestos also augments XBP1, IRE and CHOP protein expression in A549 (n=2) and rat AT2 (n = 1) cells. Similar findings are evident following H2O2 exposure, which also induces intrinsic AEC apoptosis.

Conclusions: Asbestos induces an ER stress response in AEC. ER Ca2+ release and an ER stress response may be important for inducing mitochondria-regulated AEC death. We speculate that ER and mitochondria crosstalk is important in AEC survival / death signaling that is crucial in the pathogenesis of pulmonary fibrosis.

Funding: VA Merit Award (DK).

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* Fold control normalized to 18s; mean ± SEM (n=6); t p < 0.05 vs. control

51 OX40 INDUCES CCL20 EXPRESSION IN THE CONTEXT OF ANTIGEN STIMULATION: AN EXPANDING ROLE OF CO-STIMULATORY MOLECULES IN CHEMOTAXIS

Z Zhang, W Zhong, D Hinrichs, JT Rosenbaum Portland, OR. Oregon Health & Science University. T lymphocytes are a vital component of the adaptive immune system. OX40 is an inducible co-stimulatory molecule expressed by activated T cells. It plays an important role in the activation and proliferation of T lymphocytes. Recent studies have shown that some co-stimulatory molecules not only prime T cell activation but also direct their trafficking. Chemotaxis is an essential step for attracting activated lymphocytes to achieve an effective immune response. CCL20 is an important chemokinesis produced by activated T cells. In this study, using DO11.10 mice whose transgenic T cell receptor (TCR) specifically recognizes ovalbumin (OVA), we demonstrate that OVA induces OX40 expression primarily in CD4+ T lymphocytes. Further stimulation of OX40 by OX40 activating antibody up-regulates CCL20 production in a dose-dependent manner. Both NF-kB dependent and independent signaling pathways are implicated in the induction of CCL20 by OX40. Finally, we primed the DO11.10 splenocytes with or without OX40 activating antibody in the presence of OVA. Intranasal administration of the cell lysates derived from the cells with OX40 stimulation results in a more severe leukocyte infiltration in the lung of DO11.10 mice. This marked airway inflammation is substantially attenuated by CCL20 blocking antibody. Taken together, this study has shown that activation of OX40 induces CCL20 expression in the presence of antigen stimulation. Thus, our results broaden the role of OX40 in chemotaxis and provide an insight into a novel effect of co-stimulatory molecules in orchestrating both T cell up-regulation and migration.

52 AROMATIC AMINES EXERT CONTRASTING EFFECTS ON THE ANTI-COAGULANT EFFECT OF ACETALDEHYDE UPON APTT

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AROMATIC AMINES EXERT CONTRASTING EFFECTS ON THE ANTI-COAGULANT EFFECT OF ACETALDEHYDE UPON APTT. A.S.Brecher, La'Tees Hall, and Sarah J. Murrey. Bowling Green State University, Bowling Green, OH 43403. The effects of pharmacological levels of D-amphetamine (AMP), DOPA, procarine (PRO), procainamide (PNH2), isoproterenol (IP), and atenolol (ATEN) upon APTT and upon the anti-coagulant influence of acetaldehyde (AcH) were investigated. AMP and IP exerted pro-coagulant effects upon APTT, whereas PRO and ATEN exerted anti-coagulant effects. DOPA and PNH2 had no effect on clotting as measured by APTT. When the test compounds were mixed with AcH prior to addition to plasma, contrasting effects were observed. AMP-AcH pre-mixtures reduced APTTs relative to AcH alone, suggesting a detoxication of the AcH effect. ATEN-AcH pre-mixtures similarly exhibited lower APTTs relative to AcH alone. Pre-mixtures of PRO with AcH had an additive anti-coagulant effect on APTT (p=0.0013). We also explored the possibility that each APTT pre-mixture may be acting independently in plasma. PNH2-AcH pre-mixtures did not have statistically significant effects upon APTT relative to AcH alone. However, there was a statistically significant difference between the pre-mixtures and mixtures obtained upon adding PNH2 first to plasma for 10 min @ RT and, consequently AcH for an additional 10 min @ RT. The differences in effects on AcH by ATEN and IP are interesting in view of their respective agonist and antagonist effects on beta-adreno-receptors. (The authors gratefully acknowledge the gift of coagulation reagents by Mr. Robert Harr, Chair of the Medical Technology Program, BGSU.)

53 PROMOTER VARIANT OF PIK3C3 LINKED TO SCHIZOPHRENIA SUSCEPTIBILITY IS ASSOCIATED WITH SEROLOGIC PROFILE AND SERUM IFN-α IN LUPUS PATIENTS

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Objective: SLE associated autoantibodies and high serum interferon-alpha (IFN-α) are two important heritable phenotypes in Systemic Lupus Erythematosus (SLE), and they are thought to play a role in disease pathogenesis. A case-case genome wide association study (GWAS) was carried out to determine the genetic factors associated with these two inter-related phenotypes. Single nucleotide polymorphisms (SNPs) in the 5’ region near the PIK3C3 gene were implicated in this study, and we followed up a functional promoter variant SNP which has previously been linked to schizophrenia susceptibility.

Methods: The rs3813065 SNP (~442 C/T) in the promoter region of the PIK3C3 gene was genotyped in an independent multi-ancestral cohort comprising 511 cases and 739 matched controls (cases include 255 African-American, 162 European-American, and 94 Hispanic-American subjects). Genotyping was performed using Taqman genotyping probes, and IFN-α was measured using a sensitive reporter cell assay.

Results: We detected an association between the C allele of rs3813065 and increasing number of autoantibody specificities in SLE patients from each ancestral background analyzed separately, with the strongest association in African-American patients (p=0.0103). We also explored possible associations between individual autoantibody specificities and the C allele of rs3813065 in each ancestral background using logistic regression modeling. The strongest associated autoantibody profile was found with anti-Ro combined with anti-Sm antibodies in African-American SLE patients (p=0.0001). There were no significant differences in allele frequencies between cases and controls in our cohort, although the C allele trended toward enrichment in the cases (OR=1.09, p=0.48).

Conclusions: Genetic variation in the promoter region of PIK3C3 is associated with serologic phenotype in lupus patients. It is interesting that this variant has also been linked to schizophrenia, as there is some overlap between these conditions clinically, and one study has previously demonstrated...
familial aggregation of anti-Sm antibodies in families multiplex for schizophrenia. Our data further support the idea of shared pathogenic factors between these two conditions.

54  LRRRC20 IS A NOVEL GENE ASSOCIATED WITH SERUM INTERFERON ALPHA IN LUPUS PATIENTS
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Objective: Interferon alpha (IFN-α) is a heritable risk factor for systemic lupus erythematosus (SLE), although the genetic regulation of this risk factor is complex and not well understood currently. We performed a small scale multi-ancestral background case-case genome-wide study of SLE patients stratified by extremes of phenotype in autoantibodies and serum interferon alpha (IFN-α) to detect novel genes associated with IFN-α in SLE. A SNP in the leucine-rich repeat containing 20 (LRRC20) gene demonstrated an association signal, which is followed up in this study.

Methods: We used a multi-step screening algorithm to select candidate genes from the top hits in our GWAS for validation. We chose to follow up a SNP in the 3’ UTR region of the LRRC20 gene (rs10762360) in 511 independent cases and 739 matched controls from our multi-ancestral local cohort (cases include 255 African-American, 162 European-American, and 94 Hispanic-American subjects). Genotyping was performed using Taqman genotyping probes, and IFN-α was measured using a sensitive reporter cell assay.

Results: The minor allele of rs10762360 was associated with higher serum IFN-α in our cohort in a dose-response fashion (p=0.0002). This pattern was shared across all of the different ancestral backgrounds included in our study. Additionally, we used logistic regression modeling to detect associations between autoantibodies and this allele in each ancestral background separately. We discovered a strong association between the minor allele of rs10762360 and anti-La antibodies in African-American SLE patients (OR=2.28, p=0.0007), which was not observed in the other ancestral backgrounds. While autoantibodies have been associated with higher serum IFN-α in SLE, this antibody association did not account for the differences we observed in serum IFN-α by genotype. There were no significant differences in allele frequencies between cases and controls in our cohort.

Conclusions: We used a case-case subphenotype strategy to discover a novel gene associated with increased serum IFN-α and autoantibody profile in a multi-ancestral SLE cohort. These data suggest that LRRC20 plays a role in IFN-α signaling in SLE, and will thus mediate disease phenotype and severity.

55  REGULATION OF HEME-OXYGENASE-1 PROTEIN EXPRESSION VIA EXPRESSION OF MICRORNAS 377 AND 217
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Heme oxygenase-1 (HO-1) enzyme plays critical role in metabolizing the excess heme generated during hemolysis in pathological conditions, such as sickle cell disease. We and others have previously demonstrated that during chronic intravascular hemolysis the expression of HO-1 protein is not sufficient to reduce the oxidative burden of free heme in the vasculature, leading to oxidative stress and vascular inflammation. This led us to hypothesize that a post-translation mechanism of control exists for HO-1 expression. MicroRNAs (miRNAs) are ~21–22 nucleotides in length and affect post-translational expression of genes by interacting with complementary target sites within the 3’ non-coding sequence, which stabilizes the translated region (3’UTR) of the messenger RNA. We performed in silico analysis using TargetScan for the human hmx gene and identified candidate microRNA binding sites within the 60bp human hmx 3’UTR region. Two candidate microRNAs, hsa-miR-377 and hsa-miR-217, were cloned into separate hairpin expression vectors and co-transfected with a luciferase vector containing the human hmx 3’UTR region. The combination of hsa-miR-377 and hsa-miR-217 produced a significant (p<0.01) 2.4-fold reduction in hmx1 luciferase reporter expression compared to controls, with hsa-miR-377 and ~217 constructs alone showing 1.6 and 1.3-fold reductions respectively. HEK293 cells were then treated with 10 μM hemin chloride for 1 h, followed by a 24 h incubation to induce HO-1 protein expression. Cells which were transfected with the combination of miRNA-377 and miRNA-217 exhibited a significant 1.6-fold reduction in HO-1 protein expression compared to untransfected hemin-stimulated controls. Transfection with either miRNA-377 or miRNA-217 alone did not produce a decrease in HO-1 protein expression. Over expression of the microRNA constructs did not affect hmx1 mRNA expression. We conducted time-course evaluation of miRNA-377 and miRNA-217 expression in cells exposed to 10 μM hemin chloride for 1 h, then incubated for 8 and 24 hours. Exposure to hemin induced a significant (p<0.02) decrease in miRNA-217 expression and a trend toward decreased miRNA-377 expression (p=0.07) after 8 hours. Combined this data suggests that miRNA-377 and miRNA-217 help regulate HO-1 protein expression in the presence of hemin. Further evaluation of the expression of these miRNAs during pathological hemolysis is warranted.

56  GENETIC VARIATIONS INFLUENCE EFFECTS OF ALCOHOL: POSSIBLE MECHANISM FOR VULNERABILITY TO ALCOHOL DEPENDENCE
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Alcohol dependence (AD) is a highly prevalent disorder that is associated with serious morbidity and mortality. AD has a substantial genetic component. Because the GABAα neurotransmitter receptor is an important mediator for several behavioral effects of alcohol, genes encoding GABA-related proteins are functional candidates to influence risk of AD. Family-based and case-control genetic studies indicate that genetic variations throughout the GABAα-1 receptor subunit gene and the adjacent GABAα-2 receptor subunit gene are associated with AD. Given that reduced sensitivity to alcohol is a risk factor for the development of AD, we determined if sensitivity to the acute subjective effects of alcohol is related to genetic variation in GABAα-receptor subunit genes.

Methods: 27 healthy social drinkers [70% males, all Caucasian, 24.1 mean age (y) (SD=2.8) participated in an alcohol sensitivity challenge session. Subjects received a placebo drink at time 0, followed by three equal alcohol doses at 45 min intervals, achieving a mean peak alcohol concentration of 105 mg/dL (SD=22). The major outcomes were subjective measurements on validated scales: the Bialpse Alcohol Effects Scale (BAEs), Subjective High Assessment Scale (SHAS) and Visual Analog Drug Effect Questionnaire (VAS) which were completed at baseline, following each alcohol drink and at 45-minute intervals for the session duration. Results.Individual single nucleotide polymorphisms (SNP) analysis. Genotype effects were analyzed using longitudinal analysis by generalized estimating equations (GEE) methods, while controlling for gender, time and blood alcohol levels (BAL). Consistent with prior reports, subjects homozygous or heterozygous for the minor allele for SNPs in the GABAα-2 region reported on average significantly lower alcohol “high” effects (SHAS) (rs 11530214 p=0.01; rs 16899354 “p=0.01) and lower “lack” effects (VAS) of alcohol (rs 503734 p=0.01) compared to those homozygous for the common allele. Subjects homozygous for the AD associated minor allele for SNPs in the GABAα-1 region reported on average significantly lower alcohol “high” effects (SHAS) (rs 1497577; p=0.03) compared to those heterozygous and homozygous for the common allele. Reconstruction of haplotypes in the study cohort. The program Haplovie (v4) was used to delineate haplotype blocks. PHASE2 method was used to reconstruct haplotypes for SNPs within the same haplotype block. Three main haplotype blocks were observed. One included 3 SNPs in GABAα-1 region. The second haplotype block extended from GABAα-1 receptor subunit gene intron 7 and extended 1kb 5’ of this gene. The third haplotype block extended from the intergenic region 5’ GABAα-1–2 GABAα-α to intron 3 of GABAα-2 gene. Haplotype analysis. Haplotype effects were analyzed using longitudinal analysis by GEE methods, controlling for gender, time and BAL. Subjects who were carriers of the GABAα-1 common allele TATTCTGAC haplotype (block 2, H1) had significantly higher SHAS total scores (p=0.03) compared to non-carriers. In addition, subjects who were carriers of the GABAα-2 minor allele TTTGACCTTCTAGCAGC haplotype (block 3, H4) had significantly lower alcohol “stimulant” effects (BAEs) (p=0.01) compared to subjects who were non-carriers.

Conclusion: Our results suggest that the proposed variations modulate subjective responses to alcohol and thus may increase susceptibility to AD. Greater understanding of the role of genetic variation mediating alcohol effects may contribute to the development of pharmacogenetic approaches in the screening and management of alcohol use disorders. [Supported by NIH]
57 GUT STERILIZATION REDUCES SEVERITY OF INFLAMMATION, DISEASE ACTIVITY, LEUKOCYTE INFILTRATION AND ANGIogenesis IN THE MOUSE DSS COLITIS MODEL


Introduction: Microbial species are thought to contribute to immunological homeostasis within the healthy gut, and there is abundant evidence that they may be involved in the pathogenesis of inflammatory bowel disease (IBD). Commensal enteric bacteria have a wide range of effect on the gastrointestinal tract including modulating of inflammation through a direct effect on angiogenesis and on proliferation and differentiation of epithelium. Various antibiotics have been shown to decrease gut inflammation and are commonly used in the management of IBD in certain situations. We tested the hypothesis that colitis, angiogenesis and leukocyte infiltration is less severe in DSS induced colitis in a germ free sterile gut mouse model.

Methods: Mice were divided into 4 treatment groups; control group, antibiotic (ABX) group, DSS group and DSS with antibiotic (DSS+ABX) group. During the first 3 days of the study, no group received DSS. Groups scheduled to receive antibiotics received a combination of vancomycin, neomycin, and metronidazole. During the 2nd phase (1 day), the antibiotics were continued and 3% DSS added to ‘colitis’ groups. At the end of the study, the mice were sacrificed and tissue sections harvested. Disease Activity Index (DAI) was determined by average score of % weight loss (0-10%), 1:1-5%, 2:5-10%, 3:10-15%, 4:>15%), stool form (0:normal form, 2:loose stools, 4:liquid stools), and clinical occult blood. Histopathological (HP) scoring was done on colon sections using established criteria. Inflammation severity (0:none, 1:slight, 2:moderate, 3:severe), extent of injury (0:none, 1:mucosal, 2:mucosal and submucosal, 3:transmural), crypt damage (0:none, 1:basal 1/3rd, 2:basal 2/3rd, 3:only surface epithelium intact, 4:loss of entire crypt and epithelium) was calculated and value was then multiplied by an extent index (1.0-25%, 2.26-50%, 3.51-75%, 4.76-100%). Maximum possible HP score was 40.

Results: Histopathological scores in controls (0.00) and in ABX (1.00 +/- 0.8165) were significantly (p<0.001) lower than DSS (12.65+/-0.00). The DSS group had significantly (p<0.001) higher HP score compared to ABX+DSS (4.1 +/- 1.225). The severity of inflammation, extent of injury, and crypt damage were all significantly improved in ABX +DSS compared to DSS group. The Disease Activity Index score (day 1) was significantly worse in DSS group compared to ABX+DSS group. Stool blood and form scores were also significantly improved in ABX+DSS group. Importantly, myeloperoxidase was significantly reduced in ABX+DSS (0.045+/- 0.027) compared to DSS group (0.1942 +/- 0.1528), indicating that neutrophil infiltration was blocked in the gut. There was no significant difference in MPO activity between control (0.022+/- 0.006) and ABX+DSS group. Also, in this model angiogenesis was significantly attenuated by gut sterilization (DSS: 84.37% +/- 7.20, ABX+DSS: 41.66% +/- 5.34, p=0.001).

Conclusion: Our study shows that the clinical and histopathological severity of colitis was significantly worse in the DSS colitis group compared to ABX+DSS group supporting the hypothesis that development of IBD is likely to be less severe with appropriate antibiotic treatment. In particular, gut sterilization effectively reduces leukocyte dependent (PMN) injury and angiogenesis results to improve outcomes and both may be an important target for therapy in IBD.

58 GENETIC VARIATION AT THE IRF7/PHRF1 LOcus IS ASSOCIATED WITH AUTOANTIBODY PROFILE AND SERUM INTERFERON ALPHA ACTIVITY IN LUPUS PATIENTS

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Objective: Interferon alpha (IFN-α) is a heritable risk factor for systemic lupus erythematosus (SLE). Genetic variation near interferon regulatory factor 7 (IRF7) is implicated in SLE susceptibility. SLE-associated autoantibodies can stimulate IFN-α production through the Toll-like receptor/IRF7 pathway. We hypothesized that variants of IRF7 may cause risk of SLE by increasing IFN-α production, and that autoantibodies may be important to this phenomenon.

Methods: 492 SLE patients were studied (236 African-American, 162 European-American, and 94 Hispanic-American subjects). Serum IFN-α was measured using a reporter cell assay, and SNPs in the IRF7/PHRF1 locus were genotyped.

Results: In European-American and Hispanic-American subjects, rs702966C was associated with anti-dsDNA antibodies (OR=1.83, p=0.0069 in joint analysis). The rs702966 CC genotype was only associated with higher serum IFN-α in European-American and Hispanic-American patients with anti-dsDNA antibodies (joint analysis p=4.1×10^-5 in anti-dsDNA positive vs. 0.99 in anti-dsDNA negative patients). In African-American subjects, anti-Sm antibodies were associated with the rs9463128 SNP near IRF7 (OR=1.95, p=0.0017). rs4963128 CT and TT genotypes were associated with higher serum IFN-α only in African-American patients with anti-Sm antibodies (p=0.0012). In African-American patients lacking anti-Sm antibodies, the anti-dsDNA/rs702966C interaction upon serum IFN-α was observed, similar to the other ancestral backgrounds (overall joint analysis p=1.0×10^-6). In European-American and Hispanic-American patients, the IRF5 SLE-risk haplotype showed an additive effect with rs702966C upon IFN-α in anti-dsDNA positive patients.

Conclusions: IRF7/PHRF1 variants correlate with SLE-associated autoantibodies to result in higher serum IFN-α, providing a biologic relevance for this locus at the protein level in human SLE in vivo.

59 EPigenetic regulation of soluble ST2 by LPA in human bronchial epithelial cells

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Lysophosphatidic acid (LPA), a naturally occurring bioactive lysophospholipid, plays a critical role in airway inflammation and resolution by inducing expression and release of pro-inflammatory and anti-inflammatory mediators in airway epithelial cells. Our previous studies showed that post-treatment with LPA attenuated inflammation in a LPS-induced murine model of acute lung injury. This study provides new evidence that LPA plays an anti-inflammatory effect by increasing expression and release of interleukin-1 (IL-1) receptor like protein, soluble (s) ST2, a decoy receptor for IL-33, in primary human bronchial epithelial cells (HBEpCs). LPA (0.1–5 μM) treatment of HBEpCs increased sST2 mRNA expression and protein release in a dose- and time-dependent fashion. Immunocytochemical analysis showed that LPA (1 μM, 3h) induced sST2 expression and localization in secretory bodies of HBEpCs. Inhibition of LPA receptor1/3 by Ki16425 (10 μM, 1h) or Gai with pertussis toxin (Gai/βδγ/γ) attenuated LPA-induced sST2 release. Pretreatment with NF-κB pathway inhibitor (Bay11-7082) or JNK inhibitor (JNKIII) partially prevented sST2 release by LPA. These results demonstrate that LPA induces sST2 expression and release through Gai-coupled LPA receptors-mediated activation of NF-κB and JNK pathways in HBEpCs. Pretreatment with LPA (1 μM, 3h)-treated conditional medium attenuated LPS (5 μg/ml)-induced decrease in transepithelial resistance (TER). Furthermore, sST2 was detected in bronchial lavage (BAL) fluids from LPA intratracheally challenged mice suggesting release of sST2 during lung injury. LPA induces sST2 mRNA expression and protein release through LPA receptors-mediated NF-κB and JNK/AP-1 signaling pathways. Chip data shows LPA can increase the acetylation level in ST2 promoter regions, both in distal and proximal promoters. Also, TSA (Histone deacetylase inhibitor) can enhance the induction of sST2 by LPA in HBEpCs. These results suggest a novel mechanism of regulation of sST2 by LPA that may be of physiological relevance to airway inflammation and remodeling.

60 MACROPHAGES MEDIATE CHRONIC CARDIAC REJECTION THROUGH MONOCYTE-INDUCED BY IFNγ (MIG)

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Introduction: Chronic rejection (CR) in cardiac transplantation leads to allograft dysfunction and is the leading cause of transplant related heart failure. Attempts to prevent acute rejection (AR), so to prevent CR, are directed at T cell processes which obviously are insufficient. We investigated...
the role of the macrophages and in particular monocyte-induced by IFN-γ (MIG) and IFN-inducible protein 10 (IP-10), known T cell chemottractants, in chronic allograft rejection. **Methods:** BALB/c (H-2d) hearts were transplanted into C57Bl6 (H-2b) recipients (n=6). Group AR had no treatment given. In group CR, 1mg i.p. of GK1.5 (anti CD4) was given Day −1, 0, 7. The hearts were analysed using qRTTPCR and immunohistochemistry. The spleens underwent FACs analysis. **Results:** In group AR, all hearts rejected at day 7. In group CR, the functioning hearts were harvested at day 30 and trichrome analysis of the hearts confirmed the presence of CR. The allografts underwent qRTTPCR analysis and whilst there was a significant increase in allograft MIG and IP-10 in group AR, relative to isografts (p<0.05), only MIG was significantly upregulated in group CR relative to both group AR and isografts. (p<0.05) This corresponded with a significant increase in the ICH staining for macrophages in the group CR allografts compared to both the group AR allografts and the isografts. FACs analysis of the splenocytes in group CR detected macrophages to be twice as abundant compared to the group AR (40% vs 22%, respectively, p=0.05). **Conclusions:** Chronic cardiac allograft rejection, which is a significant clinical problem, may be a macrophage dependent process, potentially mediated through the chemokine MIG, both at the allograft and systemic level.

61 THE ‘EMPTY HEART SYNDROME’: ANALYSIS OF PR-INTERVAL SHORTENING AND THE DEVELOPMENT OF NEUROCARDIOGENIC SYNCOPES DURING TILT TABLE TEST

L. Saju, K Mayuga, J Bena, H Wang, F Fouda-Tarazi Cleveland, OH, Davis, CA and New Symrna Beach, FL. Cleveland Clinic. **Background:** Shortening of the PR-interval, thought to reflect decreased time for left ventricular filling, may be a predisposing factor for the development of neurocardiogenic syncope as seen on Head-Up-Tilt Test (HUT). **Patient Population:** 92 patients (pts.) (35 male, 57 female; 8-80 years old (yo)) with a history of orthostatic intolerance underwent HUT from 04/2008 to 04/2009 in a single center. Only pts. who exhibited a negative test [normal response (NR), n=42, 23 male, 19 female, 20-80yo] or a symptomatic sudden drop in BP and HR [vasovagal response (VVR), n=50, 12 male, 38 female, 20-80yo] were included in the study. Pts. with pacemakers or junctional rhythm at baseline were excluded. Those with VVR were further subdivided according to BP and HR changes before the development of VVR: Progressive Orthostatic Hypotension (POH-VVR, n=19), Postural Orthostatic Tachycardia syndrome (POTS-VVR, n=3), both POTS and POH (POTS+POH-VVR, n=16) and VVR Without preceding BP or HR changes (W-VVR). POTS was defined as >30bp increase in HR during upright tilt which was gradual fall in HR up to 100bpm. HUT was also associated with different clinical manifestations and the underlying molecular pathogenesis of SLE differ significantly by ancestry. Additionally, clinical features in SLE may demonstrate significant associations with each other, and these associations between manifestations may also differ by ancestry. We used logistic regression modeling to establish the network of associations between different clinical manifestations in large SLE cohorts from several different ancestries, and examined the correlation between ACR criteria and serum IFN-α levels to detect cytokine-phenotype associations in each background. **Methods** We analyzed data reporting presence or absence of ACR criteria as well as IFN-α levels from 724 SLE patients from the LEFF registry at OMRF. IFN-α levels were binned as a high vs. low categorical variable using a cut-off value of 2 standard deviations above the mean of healthy controls. The cohort was first stratified by ancestral background into 224 African-American patients, 105 Hispanic-Americans and 395 European-Americans. Iterative logistic regression was performed in each background using each of the ACR criteria and the IFN-α variable as an outcome variable serially, with the other variables used as predictor variables. Variables from this initial analysis with a p-value below 0.20 were then used in a repeat logistic regression, and results with p<0.05 in this analysis were considered significant. Positive and inverse correlations between different clinical variables as determined by β-coefficients were included in a final network diagram, and the strength of these linkages was evaluated based on odds-ratio calculations. Results Of over 100 million possible associations between ACR criteria in our background stratified population we found 36 unique associations, forming network maps of relatively sparse density in each background. Of those, only 9 associations were shared by more than one different ancestral background. Using a Chi-square analysis, we found that this differed significantly from a model in which associations between clinical manifestations would be shared between at least 2 of the three ancestral backgrounds (p<10−7). The network maps of interactions between clinical features were strikingly different in different ancestries. IFN-α showed no common associations between different ancestral backgrounds. In European-Americans, high IFN-α is linked to malar rash and hematologic manifestations of SLE. In African-Americans, it is linked to immunological manifestations of SLE. In Hispanic-Americans, it is linked to the absence of arthritis, oral ulcers and photosensitivity. Conclusions We found strikingly different associations between ACR criteria in different ancestral back-grounds, and IFN-α was also associated with different clinical manifestations in each background. These data suggest that both the patterns of clinical manifestations and the underlying molecular pathogenesis of SLE differ significantly by ancestry. **Conclusion:** Pts. who exhibited VVR on HUT were more likely to be younger and female, as well as more likely to have a shorter baseline PR, shorter final PR, and a greater PR decrease compared to NR pts. This suggests a role of decreased left ventricular filling, or “empty heart syndrome”, in the pathogenesis of neurocardiogenic syncope. Further study is needed to define this mechanism.

62 ANCESTRAL DIFFERENCES IN ASSOCIATIONS BETWEEN DISEASE MANIFESTATIONS AND SERUM IFN-α IN SLE PATIENTS

C. Weecker, B Franek, J Kelly, G Bruner, J James, J Harley, T Niewold Chicago, IL and Oklahoma City, OK. University of Chicago Medical Center. Background IFN alpha (IFN-α) is a primary pathogenic factor in systemic lupus erythematosus (SLE), and high IFN-α levels may be associated with particular clinical manifestations. SLE disease manifestations are highly variable between patients, and the prevalence of individual clinical features differs significantly by ancestry. Additionally, clinical features in SLE may demonstrate significant associations with each other, and these associations between manifestations may also differ by ancestry. We used logistic regression modeling to establish the network of associations between different clinical manifestations in large SLE cohorts from several different ancestries, and examined the correlation between ACR criteria and serum IFN-α to detect cytokine-phenotype associations in each background. Methods We analyzed data reporting presence or absence of ACR criteria as well as IFN-α levels from 724 SLE patients from the LEFF registry at OMRF. IFN-α levels were binned as a high vs. low categorical variable using a cut-off value of 2 standard deviations above the mean of healthy controls. The cohort was first stratified by ancestral background into 224 African-American patients, 105 Hispanic-Americans and 395 European-Americans. Iterative logistic regression was performed in each background using each of the ACR criteria and the IFN-α variable as an outcome variable serially, with the other variables used as predictor variables. Variables from this initial analysis with a p-value below 0.20 were then used in a repeat logistic regression, and results with p<0.05 in this analysis were considered significant. Positive and inverse correlations between different clinical variables as determined by β-coefficients were included in a final network diagram, and the strength of these linkages was evaluated based on odds-ratio calculations. Results Of over 100 million possible associations between ACR criteria in our background stratified population we found 36 unique associations, forming network maps of relatively sparse density in each background. Of those, only 9 associations were shared by more than one different ancestral background. Using a Chi-square analysis, we found that this differed significantly from a model in which associations between clinical manifestations would be shared between at least 2 of the three ancestral backgrounds (p<10−7). The network maps of interactions between clinical features were strikingly different in different ancestries. IFN-α showed no common associations between different ancestral backgrounds. In European-Americans, high IFN-α is linked to malar rash and hematologic manifestations of SLE. In African-Americans, it is linked to immunological manifestations of SLE. In Hispanic-Americans, it is linked to the absence of arthritis, oral ulcers and photosensitivity. Conclusions We found strikingly different associations between ACR criteria in different ancestral back-grounds, and IFN-α was also associated with different clinical manifestations in each background. These data suggest that both the patterns of clinical manifestations and the underlying molecular pathogenesis of SLE differ significantly by ancestry. **Conclusion:** Pts. who exhibited VVR on HUT were more likely to be younger and female, as well as more likely to have a shorter baseline PR, shorter final PR, and a greater PR decrease compared to NR pts. This suggests a role of decreased left ventricular filling, or “empty heart syndrome”, in the pathogenesis of neurocardiogenic syncope. Further study is needed to define this mechanism.
Severe hepatitis occurs in approximately 1 in 30,000 patients anesthetized with halothane. Recently, we characterized a murine model of severe halothane hepatitis that recapitulates many of the pathological features, histopathological features, and risk factors that occur in humans, including female sex, genetics, and middle age. Whereas the mechanism of halothane-induced hepatitis is unknown, clinical and animal studies implicate the innate immune system in the pathogenesis. We hypothesize that natural killer (NK) or natural killer T (NKT) cells contribute to the development of severe halothane hepatitis in mice. Female wild-type BALB/cJ (WT) mice, interferon-gamma knock out (IFN-γ KO) mice, and mice deficient in NKT cells (CD1d KO) were administered halothane (15 mmol/kg, ip) and liver and plasma samples were collected for evaluation of liver injury by histopathologic analysis and determination of plasma alanine aminotransferase (ALT) activity. Halothane treatment resulted in severe liver injury in WT mice, characterized by plasma ALT activity of ~6000 U/L at 12 hrs and histopathological features of severe centrilobular necrosis. INF-γ concentration in plasma was markedly elevated in halothane-treated mice (500 pg/ml) compared to vehicle-treated mice (<100 pg/ml) at 24 hrs. IFN-γ KO mice were resistant to severe halothane-induced liver injury (ALT ~ 500 U/L) compared to halothane-treated WT mice (ALT ~ 6500 U/L). NK depletion by anti-AsG1 treatment attenuated halothane induced liver injury mice (ALT ~ 2787 U/L), whereas rabbit IgG co-treatment caused severe halothane-induced liver injury (ALT ~ 7500 U/L). Halothane-treated CD1d KO mice developed an exacerbated halothane-induced liver injury (ALT 12,000 U/L) compared to halothane-treated WT mice (ALT ~ 6,000 U/L). These results indicate that IFN-γ is critical to the development of severe halothane hepatitis in mice. Also, the data show that NKT cells, NK cells, and CD1d are involved in the pathogenesis, presumably through IFN-γ release. These results should inform our thinking about the mode(s) of action of human halothane hepatitis and may prove to be useful in future studies of mechanisms underlying idiopathic adverse drug reactions from halothane and other drugs.

64 ENHANCED TYPE I INTERFERON ACTIVITY IN NEUROMYELITIS OPTICA OPTICA AND LUPUS COMPARED TO MULTIPLE SCLEORIS

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Objective: To compare IFN response in NMO, SLE, MS, healthy controls. Methods: Serum type I IFN-alpha/beta activity was measured by RT-PCR induction of 3 genes (sensitivity, 0.1 U IFN-a/b/ml). In vitro IFN-beta-induced activation of phospho-tyrosine STAT1 transcription factor (P-Y-STAT1), phospho-serine STAT1 (P-S-STAT1), and IFN-induced MxA were quantitated with Western blots in mononuclear cells from NMO, SLE, MS, and healthy controls.

Results: Serum IFN-a/b activity is significantly higher in untreated and partially immunosuppressed NMO patients compared to fully immunosuppressed NMO patients. Serum IFN-a/b activity was highest in SLE patients compared to NMO and controls, and lowest in MS. In vitro, activated P-Y-STAT1 and P-S-STAT1 levels were high in NMO patients even at baseline compared to therapy-naive MS and controls. Kinetics of in vitro IFN-beta stimulation from 30 minutes to 24 hours showed high levels of P-S-STAT1 and P-Y-STAT1 in NMO and SLE, but not in MS and normal controls. IFN-beta-induced marker proteins (MxA) were markedly elevated in NMO and SLE compared to MS and controls.

Discussion: Serum IFN-a/b and responses to IFN are abnormally up-regulated in NMO patients versus MS and controls. Similar to SLE patients, NMO patients also have enhanced IFN-induced responses. Our data parallel observations that NMO disease worsens after IFN-beta treatment. We argue that type I IFN therapy should be avoided in NMO, that there are fundamental abnormalities of IFN regulation in these diseases, and that IFN responses can be used to discriminate between these diseases.

65 EXPRESSION OF MATRIX METALLOPROTEINASES ARE SIGNIFICANTLY ENHANCED IN THE PAPILLARY THYROID CARCINOMA CELL LINE, BCPAP AND ENHANCE INVASION BY EXTRACELLULAR MATRIX DEGRADATION

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Papillary thyroid carcinoma (PTC) is the most common thyroid and endocrine malignancy, accounting for ~80% of all thyroid cancer. PTC arises from a gain-of-function mutation in the RET, RAS or BRAF genes which can lead to linear signaling cascade for ERK activation; aggressive disease is associated with the BRAFV600E mutation (accounting for ~60% of PTC). While the average 5-year survival is generally good (96.0%), recurrent or persistent disease prevails in up to 40% of thyroidectomy cases with a poor prognosis when aggressive cancer is evident. Little is known about the pathogenesis of aggressive disease and research in this area may yield translational findings that could improve patient management by expanding treatment options and developing early detection biomarkers. Degradation of extracellular matrix (ECM) proteins by proteases is a hallmark of aggressive and invasive carcinoma. Matrix metalloproteinases (MMPs) represent a family of secreted zymogens with diverse functions such as ECM degradation, facilitating cell migration, cell signaling, mitigating apoptosis, promoting tumor growth, and activating other zymogens. MMPs have been implicated in the pathogenesis of a number of aggressive carcinomas, and here we seek to investigate their involvement in PTC. Expression and function of MMPs and their regulatory counterpart, tissue inhibitors of metalloproteinases (TIMPs), were investigated in the BRAFV600E positive PTC cell line, BCPAP, and normal thyroid cell line, NTHY-ori, by protein array, real-time RT-PCR, and invasion/migration assay. MMP up regulation was demonstrated in BCPAP by 16.90 fold (SE=0.79) for MMP-1, 1.80 fold (SE=0.41) for MMP-3, 1.40 fold (SE=0.08) for MMP-9, 7.12 fold (SE=2.14) for MMP-10, and 12.60 fold (SE=6.90) for MMP-13; also, NTHY-ori demonstrated a 2.38 fold (SE=1.04) fold increase in TIMP-4. Spot densitometry from protein array data revealed similar increases in MMP protein levels in BCPAP conditioned media compared to NTHY-ori. Fold increases in the optical concentration for MMPs in BCPAP were 29.48 (SE=1.92) for MMP-1, 16.80 (SE=1.07) for MMP-3, 1.66 (SE=0.068) for MMP-9, 3.66 (SE=0.55) for MMP-10, and 1.59 (SE=0.11) for MMP-13; additionally, TIMP-4 was increased 1.27 fold (SE=0.056) in NTHY-ori. Inhibition of MMPs with 1-10 phenanthroline significantly reduced BCPAP invasion across an ECM barrier, decreasing the invasion/migration index from 20.49 (SE=1.78) to 7.48 (SE=1.4). Decreased migration was also observed in NTHY-ori. MMP inhibition showed fold down-regulation in PTC cell line and that they contribute in invasion across an ECM barrier. Further investigation into the role these agents play in the pathogenesis of aggressive PTC may yield insight into future treatment and screening alternatives.

66 FUNCTIONAL VARIANTS OF SPHINGOSINE-1-PHOSPHATE RECEPTOR 1 GENE ASSOCIATE WITH ASTHMA SUSCEPTIBILITY

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Asthma is a complex genetic disease that arises from a gain-of-function mutation in the RET, RAS or BRAF genes which can lead to linear signaling cascade for ERK activation; aggressive disease is associated with the BRAFV600E mutation (accounting for ~60% of PTC). While the average 5-year survival is generally good (96.0%), recurrent or persistent disease prevails in up to 40% of thyroidectomy cases with a poor prognosis when aggressive cancer is evident. Little is known about the pathogenesis of aggressive disease and research in this area may yield translational findings that could improve patient management by expanding treatment options and developing early detection biomarkers. Degradation of extracellular matrix (ECM) proteins by proteases is a hallmark of aggressive and invasive carcinoma. Matrix metalloproteinases (MMPs) represent a family of secreted zymogens with diverse functions such as ECM degradation, facilitating cell migration, cell signaling, mitigating apoptosis, promoting tumor growth, and activating other zymogens. MMPs have been implicated in the pathogenesis of a number of aggressive carcinomas, and here we seek to investigate their involvement in PTC. Expression and function of MMPs and their regulatory counterpart, tissue inhibitors of metalloproteinases (TIMPs), were investigated in the BCPAP conditioned media compared to NTHY-ori. Fold increases in the optical concentration for MMPs in BCPAP were 29.48 (SE=1.92) for MMP-1, 16.80 (SE=1.07) for MMP-3, 1.66 (SE=0.068) for MMP-9, 3.66 (SE=0.55) for MMP-10, and 1.59 (SE=0.11) for MMP-13; additionally, TIMP-4 was increased 1.27 fold (SE=0.056) in NTHY-ori. Inhibition of MMPs with 1-10 phenanthroline significantly reduced BCPAP invasion across an ECM barrier, decreasing the invasion/migration index from 20.49 (SE=1.78) to 7.48 (SE=1.4). Decreased migration was also observed in NTHY-ori. MMP inhibition showed fold down-regulation in PTC cell line and that they contribute in invasion across an ECM barrier. Further investigation into the role these agents play in the pathogenesis of aggressive PTC may yield insight into future treatment and screening alternatives.
tagging SNPs were selected for subsequent genotyping (iPLEX Gold platform and TaqMan allelic discrimination assays). Association studies performed in three case-control studies of unrelated individuals from Chicago and New York (502 cases and 559 controls) found association of SNP rs2038366 (−1557/G/T) with asthma in European Americans (p = 0.03). For African Americans, the intronic SNP rs7531994 (c.−164+170A/G) was associated with both asthma and severe asthma (p = 0.006 and p = 0.040, respectively), and the promoter SNP rs93917557 (−532C/G) conferred decreased severe asthma susceptibility (p = 0.028). The associated promoter SNPs (−1557/T and −532C/G) were cloned into a luciferase reporter vector and assessed for functionality in transplanted human lung endothelium. Promoter SNP −1557/G/T significantly increased luciferase promoter activity, whereas SNP −532C/G increased its activity. These data strongly indicate that S1PR1 variants influence susceptibility and severity of asthma. (X. Sun and S.F. Ma equally contributed to this work).

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TWO FUNCTIONAL VARIANTS OF SPHINGOSINE-1-PHOSPHATE RECEPTOR 3 GENE DECREASE humAN SUSCEPTIBILITY TO SEVERE SEPSIS-ASSOCIATED ACUTE LUNG INJURY

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Rationale: The genetic mechanisms underlying acute lung injury (ALI) is poorly understood. We have previously shown that sphingosine-1-phosphate (S1P) and its receptor S1PR3, are intimately involved in lung inflammatory responses particularly in vascular barrier regulation.

Objective: We explored the functional contribution of S1PR3 gene polymorphisms to sepsis-induced ALI susceptibility.

Methods: Common gene variants were identified by direct DNA sequencing of exons, exon-intronic boundaries, and 2 kb of upstream and downstream of the S1PR3 in a multiethnic panel of 27 samples and 9 cosmopolitan tagging SNPs were selected for subsequent genotyping. Association studies were performed in case-control samples of unrelated individuals from Chicago 218 cases and 378 controls. Genotyping was performed using iPLEX Gold platform and TaqMan allelic discrimination assays. The associated SNPs with predicted in silico functionality were cloned into a luciferase reporter vector and assessed for functionality in transfected endothelial cells.

Results: A total of 80 S1PR3 variants were identified (51 novel SNPs). In European Americans, the promoter SNPs rs7022797 (−1358 T/G) and rs11137480 (−1244 G/C) significantly decreased the risk of both severe sepsis and sepsis-induced acute lung injury (p < 0.05). Compared to promoter with −1244G and −1358T, promoter with −1244C and SNP −1358G significantly decreased luciferase promoter activity in endothelial cells (40% and 50% reduction, respectively (p = 0.05)).

Conclusion: This data indicates that S1PR3 variants influence risks of sepsis and sepsis-associated ALI (S.-F. Ma and X. Sun equally contributed to this work).

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ASSOCIATION BETWEEN INTRACARDIAC T WAVE ALTERNANS AND SPONTANEOUS VENTRICULAR TACHYCARDIA AND FIBRILLATION AFTER CORONARY ARTERY OCCLUSION IN A CANINE MODEL

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Introduction: T wave alternans (TWA) has been investigated as a risk factor for lethal ventricular tachycardia (VT). Here we studied intracardiac TWA as a predictor of spontaneous VT or fibrillation (VF) in a canine model of coronary artery occlusion (CAO).

Methods: Nineteen anesthetized, open chest dogs were studied. Electrograms from intracardiac bipolar electrodes (IBE) were assessed for TWA and spontaneous VT or VF before and up to 20 min after left CAO. TWA was measured on IBE by visual estimate defined as ST or T wave voltage change on every other complex without a change in IBE QRS. In each heart we examined 62 electrograms measured in the risk zone and surrounding normal sites, filtered from 3–1300 Hz. Ischemia was measured as % of all IBE recorded which had QRS voltage drop >45%. Mapping localized the 3-D origin of spontaneous VT or VF. Data was grouped from dogs with VF (n=4), VT (n=8) or controls (no VT or VF, n=7) and analyzed before CAO, at the 20th min after CAO and, in the cases of VT and VF, immediately preceding an episode.

Results: Before CAO, number of IBE with TWA as a percentage of the total (% TWA) was comparable in controls (24 ± 6 (SEM)), VT (21 ± 6) and VF (23 ± 8). Percent TWA increased in all groups by 20 min (40 ± 4 versus 35 ± 4 versus 45 ± 3) and roughly corresponded to the size of ischemic zone. Immediately preceding VT/VF, % TWA increased to a maximum of 38 ± 6 in VT versus VF (56 ± 7, p < 0.05) and magnitude of TWA increased in 65% and 80% of cases respectively. We analyzed endocardial IBE alone showing that % TWA increased only in the VF group (from 31 ± 10 to 60 ± 3, p < 0.05). 3-D mapping identified focal mechanisms in most VT/VF; 50% of these sites were endocardial. At these sites, TWA (58 ± 6%) was more prevalent than ischemia (42 ± 7%, p < 0.05).

Conclusions: In summary, intracardiac TWA correlated with spontaneous VT and VF in a clinically applicable canine ischemic model. Since alteration in intracellular calcium transport has been implicated in TWA, interventions directed at calcium homeostasis may protect against VT and VF. Intracardiac TWA may be a better indicator of VT and VF occurrence than ischemia.

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THE F11 RECEPTOR/JUNCTIONAL ADHESION MOLECULE-A (F11R/JAM-A) ON ACTIVATED SMOOTH MUSCLE CELLS IN ATHEROSCLEROSIS: IMPLICATIONS FOR PLAQUE REMODELING

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Background: The F11 Receptor/Junctional Adhesion Molecule-A (F11R/JAM-A) is a cell adhesion molecule (CAM) and is a member of the immunoglobulin superfamily that is present on the surface of human platelets and in endothelial cell (EC) tight junctions. Previously we have shown F11R/JAM-A to be upregulated in EC by inflammatory cytokines and that it adheres platelets to inflamed endothelium; suggesting that F11R/JAM-A plays a crucial role in inflammatory thrombosis and atherosclerosis. In this study, F11R/JAM-A expression was examined in atherosclerotic plaques from human coronary arteries and identified a novel source of F11R/JAM-A expression on dedifferentiated vascular smooth muscle cells of the atherosclerotic plaque. Further experiments examined F11R/JAM-A expression in cytokine stimulated (TNFa, INFg and IL-1b) human aortic smooth muscle cells (SMC) and potential functions of F11R/JAM-A in SMC migration and proliferation.

Methods: Human coronary arteries were immunofluorescence stained using markers for EC (anti-vWF), macrophages (anti-CD68), platelets (anti-CD61) and SMC (anti-actin). Human aortic SMC were grown in culture, treated with cytokines and F11R/JAM-A expression was measured by realtime PCR, immunofluorescence staining and western blot. The induced F11R/JAM-A expression on SMC was silenced using siRNA and assayed for migration across a cell free scratch wound and for proliferation by MTT and trypan blue exclusion assays.

Results: The results presented here are the first report of F11R/JAM-A expression on SMC. The expression of F11R/JAM-A was found to be exclusive to the activated SMC of the vessel intima and absent from SMC of the vessel media. Additionally, SMC F11R/JAM-A levels were higher in stable plaques compared to unstable plaques. Expression of F11R/JAM-A was found to be induced in cultured SMC following exposure to inflammatory cytokines. These results indicate F11R/JAM-A is involved in atherosclerotic plaque remodeling.

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TIE1 ATTENUATION RESULTS IN A LOCATION-SPECIFIC AND SHEAR STRESS-DEFINED REDUCTION IN ATHEROSCLEROSIS

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Blood flow inflicts on the vascular endothelial surface a frictional force per unit area known as hemodynamic shear stress and the transcriptional response of endothelial cells exposed to non-laminar flow with low shear stress similar to atherosclerosis prone areas is distinct from that elicited by atheroprotective laminar flow with high shear stress. However, the critical mediators of endothelial cell mechanotransduction involved in atherosclerosis have not been clearly delineated. Tie1 is an orphan receptor tyrosine kinase.
renal phosphate wasting due to FGF23 excess can also be induced by iron severity. We previously demonstrated that the variable phosphate phenotype may demonstrate a waxing and waning of renal phosphate wasting, while respectively. In both conditions, the phosphate wasting is mediated by excess X-linked hypophosphatemia (XLH) are rare phosphate wasting disorders.

Methods:

Background:

Autosomal dominant hypophosphatemic rickets (ADHR) and EA Imel, A Gray, MJ Econs

4. Not in XLH

Serum iron concentrations are related to FGF23 regulation in ADHR. FGFR3 is a transcription factor that can regulate the expression of FoxM1 is a transcription factor that can regulate the expression of glucose homeostasis in mice. We hypothesized that Tie1 plays an essential role in endothelial response to atherogenic shear. We first documented a 38% decrease in atherosclerosis in Tie1 heterozygous/apoE null mice. In our data, we confirmed that atherogenic shear stress increases Tie1 expression, which may play a novel pro-inflammatory role in atherosclerosis. Atheroprotective laminar shear stress decreases Tie1 expression and attenuation of Tie1 in vivo results in a dose dependent reduction in atherosclerosis.

71 SERUM IRON CONCENTRATIONS ARE RELATED TO CIRCULATING FGF23 CONCENTRATIONS IN ADHR, BUT NOT IN XLH

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Background:

Autosomal dominant hypophosphatemic rickets (ADHR) and X-linked hypophosphatemia (XLH) are rare phosphate wasting disorders caused by mutations in fibroblast growth factor 23 (FGF23) and PHED, respectively. In both conditions, the phosphate wasting is mediated by excess circulating FGF23. ADHR can present with childhood rickets, or with delayed onset of penetrance in adulthood. In addition patients with ADHR may demonstrate a waxing and waning of renal phosphate wasting, while XLH presents in childhood, without clear individual variation in clinical severity. We previously demonstrated that the variable phosphate phenotype of ADHR was related to variations in circulating FGF23 concentrations. Renal phosphate wasting due to FGF23 excess can also be induced by iron infusions in anemic patients. We hypothesized that FGF23 concentrations may be related to serum iron status in ADHR, but not in XLH.

Methods:

We measured serum phosphorus, iron, TIBC and ferritin concentrations in 37 subjects with ADHR mutations from 4 kindreds (3 kindreds R176Q; 1 kindred R179W), and in 24 untreated XLH subjects. We measured intact FGF23 concentrations from EDTA-plasma using the Kainos Intact FGF23 ELISA. FGF23 concentrations were log transformed for analysis.

Results: 22 females and 15 males (age range 14–85 years; mean 44.7 years) with ADHR mutations, and 18 females and 6 males with XLH (age 1–53 years; mean age 19.2 years) were tested. Though the range of phosphorus values for subjects with ADHR mutations were similar to XLH subjects, ADHR subjects had a higher mean serum phosphorus (p=0.034), and lower serum FGF23 (52 ± 67 versus 134 ± 60 pg/ml; p=0.014) than XLH subjects. There was a linear relationship between serum phosphorus and log FGF23 (R2=0.151, p = 0.018) in ADHR subjects, and a borderline significant relationship in XLH (R2=0.168, p = 0.052). Total iron binding capacity was not different between groups, but mean serum iron was slightly higher in ADHR subjects (90.4 ± 34.5 versus 73.3 ± 24.9 mcg/dl; p=0.041). There was an inverse linear relationship between serum iron and log FGF23 in subjects with ADHR (R2 = 0.317; p<0.001). There was no relationship between serum iron and log FGF23 in XLH subjects (R2 = 0.014, p = 0.58).

Conclusion: We demonstrate a relationship of iron to FGF23 in ADHR, a disorder associated with clinical waxing and waning of the biochemical phosphate wasting phenotype, but not in another phosphate wasting disorder (XLH) which does not manifest waxing and waning. This may provide mechanistic clues into the cause of waxing and waning of FGF23 concentrations in ADHR. In ADHR, iron deficiency may influence FGF23 overproduction, or iron itself may inhibit FGF23 production or increase its degradation. Although we cannot rule out a confounding factor affecting both iron status and FGF23 production, these data provide an interesting clue into FGF23 regulation in ADHR. Additional longitudinal studies are needed to confirm the relationship of iron to FGF23 metabolism.

72 CASE REPORT SPLINTER HEMORRHAGES: A SIGN OF THORACIC OUTLET SYNDROME

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Splinter hemorrhages are a classic finding in patients with endocarditis and other disorders such as SLE, rheumatoid arthritis, antiphospholipid syndrome, peptic ulcer disease, malignancies, oral contraceptive use, pregnancy, psoriasis, and trauma. Association between splinter hemorrhages and Thoracic Outlet Syndrome is not well described. We present a patient with previously asymptomatic thoracic outlet obstruction syndrome whose presenting symptoms included Reynaud’s like symptoms and distinct splinter hemorrhages. A 28-year-old female presented with right arm pain and numbness associated with transient bluish discoloration of her fourth and fifth fingers. Her examination was normal except for splinter hemorrhages of fingernails bilaterally. Arterial and venous Doppler ultrasound of the right upper extremities revealed no abnormalities. No associated atrial fibrillation or arterial bruits were noted. The patient underwent multiple investigations, which included a CTA of Chest, which revealed a normal arch, ascending and descending aorta. A C-Spine MRI, which revealed a CS-C6 broad based disk, with possible small right herniation. The patient underwent an extensive connective tissue disease evaluation including a negative anti-DNA, ANA, rheumatoid factor, sedimentation rate and hypercoag work-up which revealed a mildly elevated CRP at 0.9. MRI of the brain showed no signs of stroke. Her past medical history was significant for smoking, psoriasis, asthma, and GERD. The patient was treated with aspirin, clopidogrel, and naproxen. A week later, the patient presented to the emergency room complaining of acute onset of pain, paleness, and numbness in the right arm. An Arteriogram of the right upper extremity was performed and revealed occlusion of the right radial artery at its origin. There was also a thrombus seen at the origin of the ulnar artery and the distal brachial artery. It also revealed bilateral Thoracic Outlet Syndrome. An emergency embolectomy restored the arterial patency but patient had persistent splinter hemorrhages a week later. The patient was stable. For follow up after her discharge from the hospital at the GIM clinic. On presentation, the patient was in no distress. Blood pressure was 138/80 mmHg, pulse 136 beats/min, temperature 99.7F. Physical examination was significant for splinter hemorrhages on both upper extremities in the index finger. The rest of the examination was within normal limits. We present a case of thromboembolic disease due to thoracic outlet syndrome with signs of classic splinter hemorrhages. We suggest that in patients with splinter hemorrhages clinicians should consider thoracic outlet syndrome in the differential diagnosis in addition to the usual well known diseases.

References:

73 FOXM1 IS UPREGULATED BY NON-DIABETIC OBESITY AND STIMULATES PANCREATIC β-CELL PROLIFERATION

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Background: The expansion of β-cell mass is one mechanism by which obese animals compensate for insulin resistance and forestall the onset of diabetes. FoxM1 is a transcription factor that can regulate the expression of multiple cell cycle genes and is necessary for the maintenance of adult β-cell mass, β-cell proliferation, and glucose homeostasis in mice. We hypothesized
that FoxM1 is upregulated by non-diabetic obesity in mice and humans, and can initiate a transcriptional program leading to β-cell proliferation.

Methods and Findings: We performed gene expression analysis on islets from the non-diabetic C57BL/6 ob/ob mouse, the diabetic BTBR ob/ob mouse, and an F2 ob/ob population derived from these two strains. We identified obesity-driven coordinated upregulation of islet FoxM1 and many of its target genes in the non-diabetic strain, correlating with β-cell mass expansion and proliferation. This upregulation was absent in the diabetic strain. In the F2 ob/ob population, increased expression of FoxM1 and its target genes segregated with higher insulin and lower glucose levels. We next studied the effects of overexpression of FoxM1 on isolated mouse and human islets. We found that FoxM1 stimulated mouse and human β-cell proliferation by activating many phases of the cell cycle. We asked if FoxM1 expression is also responsive to obesity in human islets by collecting RNA from 19 human islet donors with a BMI range of 24–51. We found that the expression of FoxM1 and many of its target genes is positively correlated with BMI in human islets.

Conclusions: We find that FoxM1 is sufficient to stimulate human β-cell replication. Our data also suggest that β-cell proliferation occurs in adult obese humans in an attempt to expand β-cell mass to compensate for insulin resistance, and that the FoxM1 transcriptional program plays a key role in this process.

74 Cdk1/Cyclin B1 Mediated Bcl-xL/Bcl-2 Phosphorylation Pro-Apotic Signal Following Mitotic Arrest and Microtubule Inhibitor Treatment

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The molecular signals that control cell death after prolonged spindle checkpoint activation remains elusive. This information would be useful to determine how microtubule inhibitors (MTIs), critical and often used anti-cancer agents, induce cell death following prolonged mitotic arrest. One signal is the extensive phosphorylation of anti-apoptotic proteins Bcl-xL and Bcl-2. These proteins can drive tumorigenesis or chemotherapeutic resistance in specific tumor types. Evidence suggests that phosphorylation of Bcl-xL and Bcl-2 disables their anti-apoptotic activity. However, the responsible kinase has remained elusive, which may represent one signaling partner toward the execution of cell death. Previously, we presented an assay for Bcl-xL kinase and data now indicate Cdk1/cyclin B catalyzes mitotic arrest-induced Bcl-xL/Bcl-2 phosphorylation. Data presented here further validates, in cells, that Cdk1/cyclin B transiently and incompletely phosphorylates these proteins during normal mitosis. Furthermore, and unexpectedly, when mitosis is prolonged in the absence of microtubule inhibition, Bcl-xL and Bcl-2 become highly phosphorylated. Transient overexpression of non-degradable cyclin B1 was used to prolong mitosis without microtubule perturbation while inducing apoptosis. Co-overexpression with a phospho-defective Bcl-xL mutant but not a phospho-mimic Bcl-xL mutant blocked cell death induced by overexpression of non-degradable cyclin B1. These results confirm Bcl-xL as a key target of pro-apoptotic Cdk1/cyclin B signaling. This signal during normal mitosis may provide a level of post-mitotic cell death regulation in normally dividing cells through a minor and transient level of Bcl-xL/Bcl-2 phosphorylation. In contrast, following prolonged mitosis, as induced by MTIs, complete Bcl-xL/Bcl-2 phosphorylation may be a Cdk1/cyclin B1 pro-apoptotic switch. Thus, phosphorylation of anti-apoptotic Bcl-2 proteins acts as a sensor for Cdk1/cyclin B signal duration and as a functional link coupling mitotic arrest to apoptosis. To determine the nature of this signal in primary human tumors will provide information of their sensitivity to MTIs. To take this beyond into in vitro cell culture observations, we present data analyzing Bcl-xL and Bcl-2 phosphorylation in human peripheral blood lymphocytes isolated from patients with breast cancer before and after taxane therapy. The goal was to analyze Bcl-xL/Bcl-2 phosphorylation following taxane treatment directly from human samples, which has not been reported to-date. Two other potential and separate outcomes were also predicted. First, it analyzes if Bcl-xL/Bcl-2 phosphorylation and Cdk1/cyclin B1 activity in peripheral blood lymphocytes can be used as a surrogate marker for breast cancer cell and normal cell sensitivity to taxane MTIs. Thus, a molecular marker of cell death will be analyzed to predict treatment response (tumor cell death) and/or side effects (normal cell death). Secondly, this study could also serve as a negative control for future studies that analyze Bcl-xL/Bcl-2 phosphorylation and Cdk1/cyclin B activity directly in breast tumor cells if Bcl-xL/Bcl-2 phosphorylation is not observed. To date, the data on several human samples indicates no Bcl-xL phosphorylation but potential degradation of Bcl-2 after taxane therapy. Further studies are underway to determine the cell population before and after taxane therapy in peripheral blood lymphocytes, which may necessitate isolating only mitotic cells to determine Bcl-xL and Bcl-2 phosphorylation.

75 TGF-β Activation of AMPK Occurs Through Activation of CAMKK and PKC ζ

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AMP activated protein kinase (AMPK) is involved in energy regulation in the cell. In the low energy state, it protects cells against apoptosis and stimulates energy producing processes while inhibiting energy consuming processes. Additionally, inhibition of AMPK by ethanol has been found to play an important role in the development of alcoholic fatty liver. AMPK activity is regulated by a number of kinases. LKB1, which is activated by PKC ζ, is thought to be the major AMPK kinase. CaMKK (calcium/calmodulin activated kinase kinase) has also been found to play an important role in AMPK activation independently of LKB1. Other known AMPK kinases include Tak1 (TGF-β activated kinase 1) and ATM (ataxia telangiectasia mutated kinase). TGF-β is a pleiotropic cytokine involved in numerous cellular processes, including induction of apoptosis and regulation of tissue remodeling and repair. In alcoholic liver disease, TGF-β levels are increased leading to the development of hepatic fibrosis, and ultimately, cirrhosis. TGF-β has been shown to activate AMPK in HepG2 and HeLa cells, while AMPK has been demonstrated to impair activity of Smad3, a transcription factor activated by TGF-β, in human mesangial cells. This suggests that AMPK activation may provide negative feedback for TGF-β signaling. Ethanol may impair this feedback through its inhibition of AMPK, potentially implicating AMPK not only in alcohol-induced hepatic steatosis, but also in alcohol-induced hepatic fibrosis. The pathway by which TGF-β activates AMPK has not been definitively defined. Thus, we sought to determine the mechanism of TGF-β induced activation of AMPK to better understand the means by which ethanol could affect this pathway.

Methods: H4IIEC3 cells and HeLa cells were used for these experiments. After growth to confluence and culturing in serum-free medium overnight, cells were treated as described below. All kinase inhibitors were added thirty minutes before stimulation with TGF-β. Two hours after stimulation, cells were harvested and subjected to western blotting.

Results: Treatment with TGF-β increased AMPK phosphorylation in H4IIEC3 cells. Pretreatment with STO-609, an inhibitor of CaMKK, or with GO 6983, an inhibitor of PKC ζ, prevented this effect. KU-55933, an inhibitor of ATM, had no effect on TGF-β induced AMPK phosphorylation. Interestingly, LKB1 phosphorylation was also prevented by inhibition of PKC ζ or of CaMKK. To determine whether LKB1 activation was necessary for TGF-β stimulation of AMPK, similar experiments were performed in the LKB1 deficient HeLa cells. TGF-β was still able to induce phosphorylation of AMPK in these cells. Inhibition of CaMKK prevented TGF-β induced AMPK phosphorylation in HeLa cells, while neither ATM inhibition nor PKC ζ inhibition had an effect. Recent reports suggest that AMPK may be able to (reciprocally) phosphorylate LKB1. To determine whether this occurs in the TGF-β signaling pathway, H4IIEC3 cells were treated with the AMPK inhibitor compound C prior to TGF-β stimulation. This resulted in decreased levels of phospho-LKB1.

Conclusions: TGF-β activation of AMPK appears to involve both CaMKK and PKC ζ, but not ATM. LKB1 is phosphorylated in response to TGF-β, but this may be a consequence rather than a cause of AMPK activation. Future experiments to confirm the role of PKC ζ and CaMKK using siRNA and to determine the possible role of Tak1 using a dominant negative kinase are currently in progress. Supported by P60AA 07611 (DWC), R01 AA15070 (DWC), K08 AA016570 (SL), and F32 AA017800 (MS).
AMP-activated protein kinase (AMPK) is a major regulator of lipid and energy metabolism in the cell. Activated AMPK stimulates fatty acid oxidation, while inhibiting fat synthesis. Peroxisome proliferator-activated receptor alpha (PPARα) is a member of the nuclear hormone receptor family that dimerizes with the retinoid X receptor (RXR). When activated, PPARα translocates to the nucleus and regulates gene expression. AMPK activity is further increased by AMPK activators such as AICAR or metformin. Therefore, elucidating the molecular mechanisms regulating this key transporter and its role in lipid and energy metabolism is of paramount physiologic and potentially therapeutic importance.

**Methods:** H4IIEC3 cells, a rat hepatoma cell line, were transfected with a PPARα expression plasmid. PPRE (PPAR response element) luciferase reporter plasmids and a retinoic acid receptor (RAR) luciferase reporter plasmid. 

**Results:** Activation of AMPK by either AICAR or metformin in cells transfected with PPARα and PPRE decreased reporter activity compared to controls. Stimulation of PPARα activity by WY-14,643 was also impaired by AICAR and metformin. 

**Conclusions:** Contrary to our expectations, activation of AMPK led to impaired activity of PPARα. This was unexpected considering the mechanism of activation, as both metformin and AICAR decreased PPARα activity. PPARα activity was also decreased by metformin and AICAR. Treatment with compound C prevented the effects on PPARα and PPARγ, suggesting that the decrease in activities was mediated by AMPK. The effects of AICAR and metformin do not appear to be mediated through RXR, as they did not inhibit RAR reporter activity, another nuclear receptor which complexes with RXR, and there was no further effect of compound C in cells treated with the AMPK activators. Compound C treatment nearly doubled PPRE reporter activity induced by either WY-14,643 or rosiglitazone. RAR reporter activity stimulated by AM580 (RAR agonist) was nearly doubled PPRE reporter activity induced by either WY-14,643 or rosiglitazone. RAR reporter activity stimulated by AM580 (RAR agonist) was also significantly increased by compound C, though to a much lesser extent.

**78 CHOLERICINIC SIGNALING AND REGULATION OF INTESTINAL OXALATE TRANSPORT**

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The vast majority of kidney stones are composed of calcium oxalate, and minor changes in urinary oxalate affect the stone risk. Knockout mouse studies have indicated that intestinal oxalate secretion mediated by anion exchanger SLC26A6 plays a major constitutive role in limiting net absorption of ingested oxalate, thereby preventing hyperoxaluria and calcium oxalate urolithiasis. This indicates that defects in the function or regulation of this transporter are potential molecular mechanisms predisposing to calcium oxalate urolithiasis in humans. Therefore, elucidating the molecular mechanisms regulating this key transporter is of paramount physiologic and potentially therapeutic importance. We previously reported that PCK-8 activation negatively regulates SLC26A6 expression in mouse duodenal tissue. To identify physiologic agonists acting upstream of PCK-8, we used the human intestinal cell line T84, which endogenously expresses SLC26A6. We measured DIDS-sensitive [14C]oxalate uptake in the presence of an outward Cl gradient as an assay of Cl-oxalate exchange activity, and we observed transport characteristics described for SLC26A6 when expressed in Xenopus oocytes. To characterize T84 cells as a valid model to study SLC26A6 regulation, it is critical to confirm that the observed oxalate transport is indeed mediated by SLC26A6. To this end, we knocked down SLC26A6 expression in T84 cells using shRNA, and we observed >70% reduction in SLC26A6 mRNA and >55% reduction in Cl-oxalate exchange activity. These findings suggest that the Cl-oxalate exchange activity observed in T84 cells is largely mediated by SLC26A6. The choleretic agonist carbachol is known to modulate intestinal ion transport through signaling pathways including PKC activation. We therefore examined whether carbachol affects Cl-oxalate exchange activity in T84 cells. We found that carbachol significantly inhibited oxalate transport by T84 cells, an effect blocked by the relatively selective PCK-8 inhibitor rötterlin. Under the same conditions, carbachol also led to significant translocation of PKC-8 from the cytosol to the membrane of T84 cells, thus providing further evidence that PKC-8 is the involved PKC isoform. We used pharmacological inhibitors to demonstrate that carbachol inhibition of oxalate transport resulted from activation of the M3 muscarinic receptor and phospholipase C. We also found that carbachol caused significant stimulation of cSrc phosphorylation, and that inhibition of oxalate transport by both carbachol and PMA was significantly attenuated by the Src family kinase inhibitor PP2. Taken together, these results indicate that carbachol inhibits oxalate transport by T84 cells through signaling pathways including the M3 muscarinic receptor, phospholipase C, PCK-8 and c-Src kinase. These findings suggest that intestinal oxalate secretion is subject to choleneric regulation, and future studies will be directed at testing this hypothesis in native tissue.
therapy. In addition to generating valuable patient-oriented, translational data, this project serves as a launching point for Dr. Van Poznak to master the evolving field of pharmacogenetics and biomarkers.

Relevance: Patients, dentists and medical oncologists will benefit from an evolving field of pharmacogenetics and biomarkers.

Objectives: We have demonstrated that cholesterol crystals (CC) are present abundantly in thrombus occluding coronary arteries. However, the effect of CC on thrombolysis is unknown. Therefore, we evaluated the effect of CC on thrombolysis with tissue plasminogen activator (TPA).

Methods: Fresh human whole blood was collected in sodium citrate. Thrombi (n=84) were made in a circular mold (15 mm x 4 mm) by adding CaCl2 and thrombin. Half the thrombi (n=42) had CC added (25 mg/ml) while the other half were used as control. After 2 hr, thrombi were incubated either in NaCl solution or PBS with TPA at 37°C. Two doses of TPA (0.25, 0.5 μg/ml) were used. Each sample was obtained from the same thrombus in the same TPA solution. The effect of CC on thrombolysis was unknown. Therefore, we evaluated the effect of CC on thrombolysis with tissue plasminogen activator (TPA).

Results: Compared to the control, thrombolysis occurred faster in the presence of NaCl at both concentrations of 0.25 and 0.5 μg/ml at 120 min respectively (0.50 ± 0.01 vs. 0.59 ± 0.03; p < 0.003; p < 0.004) (Table: Summary for 0.5 μg TPA). Viscosity of thrombus with CC was significantly greater than control (103 vs. 101 Pa-sec; p = 0.003). SEM demonstrated CC embedded within the thrombus disrupting the tight thrombus architecture. This could have occurred by increasing the spaces into which the TPA can reach the fibrin.

Conclusions: Although there was increase in viscosity of thrombus with CC, the presence of CC changed the thrombus architecture leading to enhanced thrombolysis. This could have occurred by increasing the spaces into which the TPA can reach the fibrin.

81 TNF-α INHIBITION REDUCES THE DEVELOPMENT OF SODIUM-SENSITIVE RENAL DISEASE IN RATS RECOVERED FROM RENAL ISCHEMIA/REPERFUSION (I/R) INJURY

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Background: TNF-α is an inflammatory cytokine involved in vascular inflammation and is elevated in kidney disease. This study utilized a model of salt sensitive kidney disease that develops in Sprague-Dawley (SD) rats that have recovered from renal I/R injury. Following acute kidney injury, rats are allowed to recover for 5 weeks; then subsequently exposed to a high NaCl diet. These animals develop hypertension, albuminuria and glomerular and tubular damage in comparison to shams exposed to high salt diet (1). Etanercept, a TNF-α inhibitor used to treat autoimmune conditions, acts as a soluble receptor that binds TNF-α in the bloodstream. Given TNF-α’s role in vascular inflammation and renal disease, we have evidence that TNF-α inhibition results in significantly lower albuminuria as well as a marked decrease in interstitial fibrosis and tubular cystic changes when compared to controls.

Methods: Male SD rats weighing 250-300 gm were fed a low salt (0.4% NaCl) diet. To induce I/R injury, the rats were anesthetized and blood supply to the kidneys was interrupted for 35 min by applying microvascular clamps on the pedicles of both kidneys. Clamps were released and reperfusion was visually confirmed. Other rats were subjected to sham surgery. Plasma creatinine was significantly elevated 5-fold 24 hours after I/R injury, but returned to levels not different from control. Rats recovered for 35 days. Prior to changing to a high salt (4.0% NaCl) diet, urine was collected to assess sodium, albumin and creatinine excretion. Upon initiation of the 4.0% NaCl diet, 5 I/R and 5 sham rats were administered etanercept (0.5 mg/kg sq) twice a week and 4 I/R rats and 4 sham rats were given vehicle twice a week. At the end of three weeks, repeat urine studies were performed and both kidneys were obtained for histology. Specimens were sent for trichrome staining and were reviewed with a renal pathologist.

Results: The results are presented below; all rats demonstrated an increased sodium excretion rate when fed the high salt diet. When NaCl was increased rats that recovered from I/R injury and were treated with etanercept had significantly less albuminuria than rats treated with vehicle alone (p<0.05). Histologic comparisons between I/R etanercept, sham vehicle and I/R vehicle showed evidence of renal protection. I/R vehicle rats had significantly more interstitial fibrosis, tubular injury with dilatation, and microcystic changes, whereas I/R etanercept rats had similar histology to sham rats with minimal fibrosis and maintenance of tubular integrity.

Conclusions: 1. TNF-α inhibition with etanercept reduces the amount of albuminuria in I/R rats compared to vehicle. 2. I/R rats treated with etanercept exhibit histologic characteristics similar to sham rats, while I/R vehicle rats have more fibrosis and tubular injury. 3. TNF-α inhibition with etanercept diminishes the salt-sensitive renal damage that occurs in rats recovered from renal I/R acute kidney injury.

References:
82 SPHINGOSINE KINASE-2 MEDIATED PRODUCTION OF SPHINGOSINE-1-PHOSPHATE: A NEW FUNCTION IN MITOCHONDRIAL RESPIRATION

Sphingosine-1-phosphate (SIP) is a potent lipid mediator that regulates diverse physiological and pathological processes acting through five specific cell surface receptors. The majority of research to date has focused on the activation of these receptors, but there is now evidence to suggest that SIP also exerts intracellular functions independent of its cell surface receptors. We sought to determine if knock-out of sphingosine kinase 2 (SphK2-KO), one of the two enzymes that form SIP, will impact mitochondrial function. Mouse heart mitochondria were isolated by differential centrifugation from both Sphk2-KO and WT mice. Oxidative phosphorylation (ADP-stimulated state 3 respiration and ADP-limited state 4 respiration, nA0/min/mg; respiratory control ratio (RCR) state 3/state 4) was measured. By immunoblotting, SphK2 was present in WT mitochondria (but not KO). Sphk2-KO mitochondria had a lower content of SIP. Sphk2-KO mitochondria displayed a significant decrease in oxidative phosphorylation compared to WT mitochondria. Western blotting of complex I (NADH-ubiquinone oxidoreductase, complex II (succinate-cytochrome c oxidoreductase), complex IV (TMPD-ascorbate) substrates with preserved coupling of respiration (RCR). A new, aberrant band of complex IV was detected by Blue-Native PAGE in Sphk2 KO mitochondria, suggesting a disorganization of complex IV. The absence of Sphk2 leads to a dysfunction in mitochondrial oxidative phosphorylation with the primary defect at complex IV. This finding supports novel actions of SIP in mitochondria.

Oxidative Phosphorylation

<table>
<thead>
<tr>
<th>Glutamate/Malate</th>
<th>Succinate</th>
<th>TMPD-ascorbate</th>
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<tr>
<td><strong>WT</strong></td>
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<tr>
<td>state 3</td>
<td>state 4</td>
<td>RCR</td>
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<tr>
<td>355±18</td>
<td>100±7</td>
<td>3.6±0.1</td>
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<tr>
<td>0.93</td>
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<td>0.92</td>
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<tr>
<td>897±32</td>
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<tr>
<td><strong>SphK2-KO</strong></td>
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<tr>
<td>192±4</td>
<td>33.2±4</td>
<td>2.76±1.8</td>
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<tr>
<td>84.9</td>
<td>2.5±0.2</td>
<td>71±5.5*</td>
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Mean ± SEM, n=5 per group, *p<0.05 vs. WT

83 INHIBITORY EFFECT OF ETHANOL ON AMPK PHOSPHORYLATION IS MEDIATED IN PART THROUGH ELEVATED CERAMIDE LEVELS
S Liangpunsakul, M Sozio, E Shin, Z Zhao, Y Xu, R Ross, Y Zeng, D Crabb, Indianapolis, IN. Indiana University School of Medicine.

Ethanol treatment of cultured hepatoma cells and of mouse liver tissue induced the activity of AMPK. Our recent work showed that the inhibitory effect of ethanol on AMPK phosphorylation is exerted through the inhibition of the phosphorylation of upstream kinases, and the activation of protein phosphatase 2A (PP2A). Inhibition of AMPK phosphorylation by palmitate was attributed to ceramide-dependent PP2A activation. We hypothesized that the inhibitory effect of ethanol on AMPK phosphorylation was mediated partly through the generation of ceramide. Methods: The effect of ethanol and inhibitors of ceramide synthesis on AMPK phosphorylation, ceramide levels, and PP2A activity were assessed in rat hepatoma cells (H4IIEC3). The effect of ethanol on hepatic ceramide levels was also studied in C57BL/6J mice fed the Lieber-DeCarli diet. Results: In H4IIEC3 cells, ceramide reduced AMPK phosphorylation when they were treated for between 4 and 12 hrs. The basal level of AMPK phosphorylation in hepatoma cells was increased with the treatment of ceramide synthase inhibitor, fumonisin B1. Ethanol treatment significantly increased cellular ceramide content and PP2A activity by ~18–23%, when the cells were treated with ethanol for between 4 and 12 hrs. These changes in intracellular ceramide concentrations and PP2A activity correlated with the time course over which ethanol inhibited AMPK phosphorylation. The inhibitory effect of ethanol on AMPK phosphorylation was attenuated by the presence of fumonisin B1, and imipramine, an acid SMase inhibitor. There was a significant increase in the levels of ceramide and acid SMase mRNA in the livers of ethanol-fed mice when compared to controls. Conclusions: The effect of ethanol on AMPK appears to be mediated in part through increased cellular levels of ceramide and activation of PP2A.

84 REGULATION OF LUNG ENDOTHELIAL PERMEABILITY BY OXIDIZED PHOSPHOLIPIDS
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Rationale: Oxidized phospholipids appear in the lung circulation as a result of oxidative stress that accompanies acute lung injury and inflammation. Oxidation of phospholipids generates a group of bioactive oxidized phospholipids (OxPLs) exhibiting a wide spectrum of physiological effects including regulation of endothelial barrier. However, mechanisms of differential action of high and low OxPL concentrations on endothelial permeability are unknown.

Methods: The electrical cell substrate impedance-sensing (ECIS) system was used to measure agonist-induced changes in transendothelial electrical resistance (TER) in endothelial cell (HPAEC) cultures. Phosphorylation status of proteins was evaluated using 2-D SDS PAGE phosphoprotein gel staining and anti-phosphotyrosine immunostaining. Phosphorylation of VE-cadherin, FAK, ERK, SRC was assessed with phospho site-specific antibodies. Localization and physical interactions between p120- and beta-catenin involved in formation of cell adhesion complexes were evaluated by co-immunoprecipitation and immunofluorescence.

Results: OxPL at low concentrations (5–20 mcg/ml) caused prolonged increase in TER reflecting barrier-protection effect. In contrast, high OxPL concentrations (50–100 mcg/ml) caused EC barrier-disruptive response, which was accompanied by overall increase in cell protein tyrosine phosphorylation with prominent tyrosine phosphorylation of VE-cadherin and p120-catenin. Low and high OxPL elicited distinct patterns of total protein phosphorylation revealed on 2-D protein gels. High OxPL doses also induced specific phosphorylation of SRC at Tyr 418 suggesting its role in early phosphorylation of VE-Cadherin and p120-catenin. High OxPL doses caused dissociation of p120 from VE-cadherin but had little effect on beta-catenin - VE-cadherin complex.

Conclusions: Our results suggest that differential effects of high and low OxPL doses on VE-cadherin - p120-catenin complex assembly may determine EC permeability response. Profound tyrosine phosphorylation of VE-cadherin and p120-catenin by oxPL may play a key role in dissociation of this complex leading to increased EC permeability induced by high OxPL doses.
promising set of genetic variants are located in or close to solute carrier family 35, member F1 (SLC35F1) on Chromosome 6 at q22 where the best SNP produced an association of sufficient magnitude (Odds Ratio=2.9) with a probability sufficiently small (p<2×10−9) to be significant after the genome-wide correction for the multiple testing (p<5×10−8). Perhaps the best known relative of SLC35F1 is NRAMP1 (SLC11A1), variants of which, for example, influence the severity of Mycobacteria tuberculosis. Other genes that suggest a role in the anti-Protective Antigen responses after AVA vaccination include ST6GALNAC3, a predicted sialyltransferase. SNPs in this gene were detected in the screening experiment (OR=7.3, p=3.6×10−3) and then independently confirmed in independent samples (p=103). A cluster of 8 markers in the human regulator of G-protein signaling 6 (RGS6) were found to be associated by genome screening (OR=0.71, p=1.7×10−10) — two markers from which were significant in the smaller cohort genotyped individually (p<10−4). RGS6 modulates G protein function by activating the intrinsic GTPase activity of the alpha (guanine nucleotide-binding) subunit.

Conclusions: Overall, our results suggest that the genetic approach will identify variants that influence the success of the vaccination to Anthrax and, perhaps, these genes will also be important in responses to other vaccinations.

86 SYSTEMIC AUTOREACTIVE T AND B CELLS INDUCE DEVELOPMENT OF BRONCHUS-ASSOCIATED LYMPHOID TISSUE (BALT) IN THE LUNG

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Rheumatoid arthritis-related intestinal lung disease (RA-ILD) is a common extra-articular manifestation in patients with Rheumatoid arthritis and is associated with significant morbidity and mortality. Studies in humans have found that the incidence of bronchus associated lymphoid tissue (BALT) correlates with the severity of lung disease in these patients. However, the mechanisms underlying the development of BALT in the lung are not known. We have investigated whether systemic autoimmunity in a mouse model of autoimmune arthritis can promote the development of BALT. We have used a novel mouse model derived from the K.BxN autoimmune arthritis model. In our model, mice with the KRN T cell receptor specific for glucose 6 phosphate isomerase (GPI) were crossed to Gpi-specific heavy and light chain knock-in mice producing mice with a majority of T and B cells specific for GPI. We hypothesized that these mice would develop lymphoid aggregates outside the joint tissue due to the high numbers of B and T cells specific for GPI. Mice were sacrificed after the development of severe arthritis as measured by mean ankle thickness. We have found that 67% of the mice have lymphocytic infiltration in the lungs localized to either the perivascular or peribronchial regions. Interestingly, 50% of the mice with lymphocytic infiltrations had lymphoid-like lesions resembling lymphoid tissue with predominantly T and B cells beneath the airway muscosa of the lungs. The large lymphoid-like infiltrates resemble BALT with distinct areas of T and B cell follicles. The T cells infiltrating the lungs in mice with lung pathology have an activated phenotype with increased expression of ICOS, CD44 and CD25 suggesting ongoing T cell activation and antigen presentation in the lungs. Our data suggest systemic autoimmunity promotes BALT development in the lung through the cooperation of autoreactive T and B cells.

87 20-HETE MITIGATES EXPERIMENTAL RENAL ISCHEMIA-REPERFUSION INJURY

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20-Hydroxyeicosatetraenoic acid (20-HETE) regulates renal blood flow, tubular sodium transport, and cell survival through diverse intracellular signal transduction pathways. The present study aimed to evaluate the role of 20-HETE in experimental renal ischemia-reperfusion (IR) injury. First, we compared the susceptibility of SS:LEW 4A+ congenic rats and control Dahl S (S) rats to experimental renal IR injury. The 4A+ congenic strain was previously generated by introgression of a region on rat chromosome 5 - that includes the cytochrome P450 4A (CYP4A) genes that after the renal production of 20-HETE - the Lewis rat to the Dahl S genetic background. The renal expression of CYP4A protein and the production of 20-HETE is greater in the 4A+ congenic strain compared to the control S strain. Baseline plasma creatinine was normal in both the S and 4A+ strains. Following 30 min bilateral renal ischemia and 24 hrs reperfusion, serum creatinine in the S rats rose to 3.8±0.4 mg/dl. In contrast, serum creatinine rose to 1.4±0.5 mg/dl in the 4A+ rats indicating that overexpression of CYP4A protein and increased renal 20-HETE production confers protection from renal IR injury. Additional experiments were then performed to determine whether 20-HETE mediates protection from renal IR injury by interaction with pro-survival intracellular signaling pathways. Proximal tubular epithelial LLC-PK1 cells were exposed to ATP depletion for 4 hrs and then allowed to recover in serum-free media for an additional 2 hrs. Incubation with 20-HETE (20 µM) throughout the injury and recovery periods significantly decreased cytotoxicity as measured by lactate dehydrogenase (LDH) release and enhanced cell viability compared to vehicle-treated cells. Incubation of cells with the stable 20-HETE analogue, 5,14-20-HEDE (2 µM), during the 2 hr recovery phase resulted in a significant increase in cell viability (34.1±3.8%) with decreased cleavage of caspase-3 (88.9±2.5%) compared to vehicle-treated cells. Incubation of either ERK1/2 activation with a MEK inhibitor (U0126) or Akt activation using a PI3 kinase inhibitor (Wortmannin) abolished the protective effect of 5,14-20-HEDE. In summary, we provide evidence that overexpression of renal CYP4A protein and renal 20-HETE production mitigates renal IR injury in rats. The protective effect of 20-HETE in renal IR injury may be due activation of pro-survival intracellular signaling pathways in renal tubular epithelial cells.

88 TRANSCRIPTIONAL REGULATION AND POST-TRANSLATIONAL MODIFICATION OF SPHINGOSINE-1-PHOSPHATE LYASE IN LPS-INDUCED ACUTE LUNG INJURY

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Background: Sphingosine-1-phosphate (S1P), a naturally occurring bioactive sphingolipid metabolite, plays a crucial role in many biological processes such as cell proliferation, migration, angiogenesis and endothelial barrier integrity. It has been demonstrated that administration of S1P attenuates lipopolysaccharide (LPS)-induced acute lung injury (ALI) in animal models. S1P lyase (S1PL) irreversibly degrades S1P to hexadecenal and ethanolamine-phosphate in the terminal step of S1P catabolism. This study provides new insights on the expression, regulation and post-translational modifications of S1PL in a murine model of LPS-induced ALI and in human lung microvascular endothelial cells (HLMVECs).

Methods/Results: S1PL is widely expressed in endothelial as well as epithelial cells in the mouse lung tissue and is primarily localized in the membrane organelles of HLMVECs transiently transfected with pcDNA-S1PL. Mice with one mutated Sgpl allele exhibited reduced S1P levels in both lung tissue and bronchoalveolar lavage (BAL) as determined by LC-MS/MS. Intraperitoneal injection (15mg/kg, 48 h) or intratracheal instillation (5mg/kg, 24h) of LPS up-regulated mRNA and protein levels of S1PL in the mouse lung by 2–3 fold. LPS (100 ng/ml)-mediated up-regulation of S1PL was confirmed in HLMVECs by Western blotting and luciferase reporter assay using a Sgpl promoter construct. LPS-induced lung injury increased S1PL levels in bronchoalveolar lavage fluids. LPS-β-EPK1/2-activated S1PE a MEK inhibitor abolished the protective effect of 5,14-20-HEDE. S1PL controls S1P catabolism and is primarily localized in the membrane organelles of HLMVECs. Down-regulation of S1PL with siRNA or knockdown of one allele of Sgpl gene offered protection against LPS-mediated lung inflammation and pulmonary leak.

Conclusion: LPS not only up-regulated the expression of S1PL in the mouse lung and HLMVECs, but also promoted post-translational modifications such as phosphorylation and nitration of the tyrosine residues of S1PL. The physiological role of phosphorylation and nitration of tyrosine residue(s) of S1PL by LPS in lung injury is unclear and under investigation. Supported by NIH grant HL 079396 to VN.

89 THE ROLE OF THE ANTIAPOPTOTIC PROTEIN C-FLIP IN MULTIPLE MYELOMA

CD Creen, G Zhang, LB VanRyckeghem, H Hansenberg, R Abonour, SS Farag, A Suvannasankha Indianapolis, IN. Indiana University School of Medicine.

Background: An antiapoptotic protein, c-FLIP, is over expressed in multiple myeloma (MM) cells from patients. However, the importance of c-FLIP as a target in MM therapy has not been clearly explored.
Objective: We describe the extent of c-FLIP expression in myeloma bone marrows and the effects of the genetic knockdown of c-FLIP on MM cell growth both in vitro and in vivo.

Methods: c-FLIP expression was evaluated by immunohistochemistry in bone marrow biopsy samples of multiple myeloma patients both at diagnosis and relapse. Genetic c-FLIP knockdown was achieved by expressing a lentiviral vector containing a doxycycline-inducible shRNA against c-FLIP into MM cells.

Results: c-FLIP was overexpressed universally in MM cells, both at diagnosis and relapse, compared to normal plasma cells from bone marrow samples of healthy volunteers. Doxycycline treatment in vitro induced c-FLIP shRNA transcription and reduced c-FLIP levels more than 80% in H929 cells. Correspondingly, we observed a greater than 95% reduction in the growth of H929 cells, compared to a cell line containing an empty vector control. The reduced cell growth was due to apoptosis induction, as we observed cleavage of caspase-8 and caspase-3. The in vivo effects of c-FLIP knockdown were evaluated in subcutaneous and intravenous xenograft models. In NOD/Scid mice subcutaneously transplanted with the H929 cell line containing an inducible c-FLIP shRNA (H929/c-FLIP "sh2"), we observed a drastic shrinkage of tumors after doxycycline treatment. In another model wherein H929 cells containing the inducible c-FLIP shRNA were transplanted intravenously and allowed to home and engraft, treatment with doxycycline decreased the level of human serum kappa light chain, which was used to measure tumor load. In addition, we observed longer survivals in the c-FLIP knockdown groups in both models.

Summary: Our results show that c-FLIP is overexpressed in MM. Also, c-FLIP knockdown induces the apoptosis of MM cells, both in vitro and in vivo, and therefore targeting c-FLIP should be further explored in MM therapy. Further studies will address the regulatory mechanisms of c-FLIP expression and further methods of targeting c-FLIP in animal models.

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PITUITARY-ADRENAL FUNCTION AFTER TOTAL BODY IRRADIATION: EFFECT OF HEAD SHIELDING

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Total body irradiation causes multiorgan injury in humans and rats. Head shielding could significantly modify this response if it affected pituitary function. WAG/Rij rats underwent 10 Gy total body irradiation (TBI) with or without a custom lead shield. Pituitary and adrenal function were assessed at 8 days and at 10 weeks after TBI, in vitro testing of pituitary and adrenal gland function and by measurement of plasma hormone levels. TBI did not change the adrenal cell synthesis of corticosterone or aldosterone, at either time point compared to un-irradiated rats. At both time points in head-protected rats, basal and CRH-stimulated ACTH secretion was higher in irradiated compared to un-irradiated rats (p<0.05). In contrast, at both time points in no-head-protected rats, basal and CRH-stimulated ACTH release was lower in irradiated compared to un-irradiated rats (p<0.002). Data are shown in the table for the 10 week timepoint. At the 10-week time point in head-protected rats, plasma ACTH was significantly higher in the irradiated compared to the un-irradiated rats (p=0.023). In contrast, at 10 weeks in the no-head-protected rats, plasma ACTH was significantly lower in the irradiated compared to the un-irradiated rats (p=0.002). We conclude that 10 Gy partial body irradiation that does not include the head has a stimulatory effect on pituitary corticotroph function, whereas 10 Gy TBI that includes the head suppresses pituitary corticotroph function. Studies are in progress to determine if this effect is mediated by hypothalamic factors.

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CYTOSKELETAL SIGNALING MEDIATES TGF-β1-INDUCED PULMONARY MYOFIBROBLAST DIFFERENTIATION

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Myofibroblast differentiation induced by Transforming Growth Factor beta-1 (TGF-β1), characterized by the expression of SM-α-actin (α-SMA) and stress fiber formation, plays an important role in the pathogenesis of pulmonary fibrosis. We have previously shown that expression and activation of serum response factor (SRF) is required for TGF-β1-induced myofibroblast differentiation of human lung fibroblasts (Sandbo et al., Am J Respir Cell Mol Biol. 2009 Sep;41(3):332-8). However, the signaling mechanisms mediating SRF activation by TGF-β1 remain unclear. In this study, we show that TGF-β1 induces a Rho kinase - dependent formation of actin stress fibers in human lung fibroblasts with a time course that parallels SRF activation and α-SMA expression, suggesting that these events are co-dependent. The pharmacological inhibitor of SRF, CCG-1423, blocks α-SMA expression but does not affect stress fiber formation in response to TGF-β1. In contrast, inhibition of actin polymerization and stress fiber formation by latrunculin-B, blocked TGF-β1-induced SRF activation and α-SMA expression, without affecting canonical Smad3/3 nuclear translocation or Smad-dependent gene transcription in response to TGF-β1. In summary, we describe a novel, Smad-independent signaling cascade utilized by TGF-β1 to stimulate myofibroblast differentiation, which involves Rho kinase-dependent actin polymerization, leading to SRF activation.

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TRANSGENIC EXPRESSION OF S100A12 IN VASCULAR SMOOTH MUSCLE ACCELERATES Atherosclerosis AND PROMOTES CARDIAC DYSFUNCTION

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Background: S100A12 is a chemoattractive and proinflammatory protein that is endogenously expressed in myeloid cells. However, human vascular smooth muscle cells (VSMC) express S100A12 under pathological conditions that have been associated with coronary artery plaque rupture and thoracic aortic aneurysms (TAA). We have previously shown that BLS1 mice that express human S100A12 under control of the SM22α promoter develop VSMC dysfunction leading to medial degeneration of the aorta and formation of TAA. We therefore tested the hypothesis that atherosclerosis prone mice (ApoE null mice) that also express human S100A12 would exhibit marked augmentation of atherosclerosis and possibly features of plaque instability.

Methods: Transgenic (TG) mice expressing human S100A12 in VSMC were backcrossed 4 generations into ApoE null mice. S100A12-ApoE null and wild type (WT)-ApoE null littermates were studied. Mice (n=6 for each group) were kept in our barrier facility and fed a normal rodent chow for 11 months. Aortic root dilation was examined by in-vivo ultrasound. Histology of the innominate artery, aortic arch and distal thoracic aorta was performed and atherosclerosis was quantified for (1) elastic fiber breakdown (VVG stain), (2) fibrosis and thickness of fibrous cap (MT stain), (3) necrotic core size (H&E stain) and (4) calcification (Alizarin red stain).

Results: S100A12-ApoE null mice had significant more aortic root dilation than WT-ApoE (2.1±0.2 vs 1.6±0.2 mm, p<0.01) and more signal scattering suggestive of vascular calcification. S100A12-ApoE null mice had accelerated plaque growth by two fold, associated with features of plaque vulnerability including a thin fibrous cap, increased necrotic core size (8% in WT-ApoE and 34% in S100A12-ApoE null, p<0.05), increased elastic fiber break down (grade 2 in WT-ApoE and grade 4 in S100A12-ApoE, p<0.05). Furthermore, we observed largely increased intravascular calcification in the S100A12-ApoE null mice which was nearly absent in the WT ApoE null mice.

Conclusion: Forced expression of human S100A12 in VSMC is sufficient to accelerate atherosclerosis, promote plaque calcification and medial degeneration in ApoE null mice. S100A12-ApoE null mice could represent a novel in vivo model to test compounds aimed to achieve plaque stability and to prevent plaque rupture.

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THE ROLE OF E2F1 IN THE DEVELOPMENT OF HYPERTROPHIC CARDIOMYOPATHY

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The overexpression of the transcription factor, E2F1, induces hypertrophy and apoptosis with cell cycle re-entry in cardiomyocytes in vitro and in vivo, suggesting that targeting E2F1 may have therapeutic potential. Accordingly, we tested the hypothesis that blocking the E2F1-mediated signal transduction...

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pathway prevents cardiac hypertrophy by treating E2F1 knockout mice (E2F1−/−) with either isoproterenol (ISO, 60 mg/kg/day SQ for 7 days) or Angiotensin II (ANG, 3 mg/kg/day SQ for 14 days). Echocardiography (Siemens Sequoia 15 MHz) was used to derive LV mass index (LVMi) and the myocardial performance index (MPI), a measure of combined systolic and diastolic LV function. In normal mice (E2F1+/+) both ISO and ANG treatments induced cardiac hypertrophy, and in the case of ISO, impaired ventricular function. Unexpectedly, E2F1+/− mice also demonstrated a similar pattern of cardiac hypertrophy and function after both treatments (Table). In addition, the molecular markers of hypertrophy ANP and BNP and necropsy-determined body weight-normalized LV mass were similarly increased in ISO and ANG treated E2F1+/+ and E2F1−/− mice, supporting the echocardiographic data. These data indicate that E2F1 is not necessary for the development of cardiac hypertrophy although studies using an overexpression approach suggest a causal role of E2F1. The reason for this discrepancy is unclear, although it is possible that other E2F-family members (e.g., E2F2) may play a compensatory role. In conclusion, our data demonstrate that cardiac hypertrophy can be induced in an E2F1-independent fashion and suggest that in contrast to previous reports, targeting E2F1 is not a good therapeutic approach.

| E2F1+/+ (Pro-ISO) | E2F1+/− (ISO) | E2F1−/− (Pro-ISO) | E2F1−/− (Post-ISO) | E2F2+/− (Pro-ISO) | E2F2−/− (Pro-ISO) | E2F2−/− (Post-ISO) | LVMi | 6.46±0.5 | 6.81±1.4 | 5.4±1.3 | 6.3±1.3 | 3.0±0.5 | 5.9±0.7 | 5.1±1.0 | 6.6±1.1 | *p<0.05 LVMi–LV mass indexed for weight; RWTT–relative wall thickness; MPI–myocardial performance index

94 PBEF GENE POLYMORPHISMS ARE ASSOCIATED WITH ACUTE LUNG INJURY IN US- AND SPANISH-BASED COHORTS

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Rationale: Acute lung injury (ALI) is a severe, often fatal inflammatory lung disorder that affects 150,000 Americans each year. We previously reported pro-B-cell colony-enhancing factor (PBEF, also called nicotinamide phosphoribosyl transferase, or NAMPT) to be a pro-inflammatory cytokine that is highly expressed in ALI patients and preclinical models of ALI and VILI. In addition, we reported the association of four PBEF promoter SNPs with ALI (−3185G>A, −1535C>T, −1001T>G and −948G>T), implicating it as a novel candidate for the development of therapeutic targets for ALI.

Methods: Patients were selected from two cohorts: a cohort of major trauma-induced ALI patients (47 EA, 41 AA) from the University of Pennsylvania with 101 unequivocal controls (42 EA, 59 AA), and a case-control study from the Spanish Canary Islands with 66 ALI patients and 96 controls. Genotyping of 30 promoter and intragenic SNPs was performed using iPLEX Gold reactions. Departures from Hardy-Weinberg equilibrium (HWE) were tested by means of Pearson goodness-of-fit χ2-test, and associations of individual SNPs were evaluated via trend tests.

Results: Carriers of the −948T allele in trauma cohort EAs exhibited higher susceptibility to ALI than non-carriers (p=0.014). In the Spanish study, a novel association of promoter SNP rs7789066 (−2422T>C) with ALI was observed (OR=2.73, 95% CI=1.18–6.30, p=0.015).

Conclusions: These results replicate the previously observed association of PBEF promoter SNP –948G>T with ALI and highlight the novel association of an additional promoter SNP with ALI. Functional studies are now needed to further investigate the full impact of NAMPT variants in ALI as well as other inflammatory lung diseases.

95 NOX4-DERIVED REACTIVE OXYGEN SPECIES MODIFY CELLULAR REDOX STATE AND PROGRESSION TO APOPTOSIS

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PBEF GENE POLYMORPHISMS ARE ASSOCIATED WITH ACUTE LUNG INJURY IN US- AND SPANISH-BASED COHORTS

96 CHARACTERIZATION OF PAXILIN INTERACTION WITH β-CATENIN: IMPLICATION IN ENDOTHELIAL BARRIER PROTECTION

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Rationale: Increase in lung endothelial permeability observed in ventilation induced lung injury (VILI) is accompanied by compromised cell-cell and cell-matrix contacts. We have previously demonstrated co-localization of focal adhesion (FA) and adherens junction (AJ) complexes in pulmonary endothelial cells (EC) and described increased interactions between a scaffold FA protein paxillin and multifunctional AJ adapter protein β-catenin induced by barrier protective oxidized phospholipids. Paxillin interacts with numerous binding partners via amphipathic helices of five LD-domains in N-terminal and four double zinc-finger LIM-domains in C-terminal part of the protein.

Objective: Whether paxillin - β-catenin interaction is direct and what paxillin domain(s) interact with β-catenin remains unknown, and this study addressed this question.

Method: To characterize interactions between paxillin and β-catenin directly, we generated series of plasmids encoding full-length paxillin and its domains LD-1,2,3,4,5 and LIM-1,2,3,4 and LIM-3,4. The full-length and truncated proteins were expressed as GST-fusion proteins in human HEK293 cell line, and in E. coli, partially purified, immobilized on GSH-sepharose, and used in pull-down assays with endothelial cell lysates or HEK293 cell lysates containing overexpressed recombinant β-catenin.

Results: No binding was observed between bacterially expressed β-catenin and full-length paxillin or its domains. In contrast, bacterially expressed paxillin was able to bind endogenous β-catenin from endothelial cell lysates. This interaction is significantly increased by stimulation with barrier-protective oxidized phospholipids. The β-catenin binds LIM-3 and 4 and to a lesser degree LIM-1,2 domains, however, this binding is significantly lower as compared to full-length paxillin. Moreover, interaction of LIM-3 and 4 and LIM-1,2 domains with β-catenin is no more upregulated by cell stimulation with barrier protective agonists. Specific binding of β-catenin to LD1-2 or LD3-4 domains was not detected.

Conclusions: These data suggest that paxillin binds β-catenin via LIM domains (mainly LIM-3,4), and enhancement of this interaction by barrier protective oxidized phospholipids in pulmonary EC may be additionally regulated by LD domains. This interaction, probably, is indirect. We speculate that β-catenin binding to paxillin may be important for paxillin targeting to cell junctions and formation of new FA complexes leading to further endothelial barrier enhancement.

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98 PHARMA-CO-PROTEOMICS AT THE BEDSIDE: A STUDY OF tPA TREATMENT IN ACUTE STROKE PATIENTS

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Thrombolysis with tissue plasminogen activator (tPA) is currently the only FDA-approved medical therapy for acute ischemic stroke. Although highly efficacious improving good neurologic outcome by 30−35%, tPA is only given to less than 5% of all stroke patients, due to its side-effects of intracranial hemorrhage (ICH), with more than 50% of patients given to less than 5% of all stroke patients, due to its dreaded side-effect of intracranial hemorrhage (ICH). We use high-throughput proteomic technology to study the thrombolysis affects clinical outcomes. tPA, a serine peptidase, affects the plasma without strokes had stable protease substrate patterns over time. In order to determine the benefit of thrombolysis by further widening the therapeutic window, it is imperative to better understand the mechanisms by which thrombolysis affects clinical outcomes. tPA, a serine peptidase, affects the expression of matrix metalloproteinases (MMPs), which are implicated in tPA-related ICH. We use high-throughput proteomic technology to study the effects of tPA at the bedside with respect to MMPs. We hypothesize that proteomic profiling of the global tPA-MMP axis may help to elucidate the mechanisms of thrombolysis-related therapeutic efficacy, and may discover new biomarkers to triage treatment.

Methods: Plasma was sampled from tPA-treated acute stroke patients, stroke patients who did not receive tPA, and from healthy controls with similar risk factors, at T1= hyperacute, <3 hrs(pre-tPA), T2= acute, 12−24 hrs(post-tPA), and T3= subacute, 48−72 hours, post stroke onset. All subjects were matched in clinical characteristics to adjust for confounders. Plasma was analyzed using protein microarrays to quantify expression of MMP-1, 2, 3, 7, 8, 9, 10, 13, and inhibitors TIMP-1, TIMP-2, and also fractionated by HPLC and analyzed on Mass Spectrometer.

Results: 1. Ranked levels of multiple MMPs and inhibitors demonstrate coordinated changes in non-stroke controls, non-tPA-treated and tPA-treated stroke patients, at T1, T2 and T3. Of note, post-tPA plasma demonstrated upregulation of multiple MMPs in comparison to pre-tPA levels. 2. Control plasma without strokes had stable protease substrate patterns over time. In acute stroke patients, there are differential expression patterns of protease substrates as the stroke progresses over time. Compared to controls and non-tPA strokes, tPA-treated stroke patients demonstrated increased protease substrate patterns over time from those of untreated patients. 3. Substrate profiles of post-tPA plasma revealed increased MMP substrates in comparison to pre-tPA plasma and non-tPA treated patients. Patients who had tPA-related hemorrhagic events had the highest levels of multiple MMPs and the most active protease substrate profiles. 4. Principle component analysis of degraded substrates of MMPs demonstrates global differences in non-tPA vs tPA-treated samples, suggesting differential expression within the proteolytic cascade.

Conclusion: By studying the proteomic substrate profiles of tPA treatment in acute stroke, we demonstrate the feasibility of using proteomic proteomics to the bedside. We found different protease substrate patterns, progressing differently over time, in tPA-treated and untreated stroke patients, suggesting a view into the therapeutic efficacy of tPA. These early results are a step toward the elucidation of the mechanisms of tPA’s effects in acute stroke. These findings also lend support to the use of broad-spectrum exogenous blockade of MMPs to improve the safety profile of tPA and the utility of MMPs and their protease substrates as biomarkers to help triage tPA administration. Utilizing proteomic technology, our ultimate goals are to improve and individualize stroke treatment by better selecting patients for tPA.

99 EXAMINING THE ROLE OF CHROMOSOMAL INSTABILITY (CIN) IN TUMOR GROWTH

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The majority of human solid tumors are characterized by the presence of aneuploid cellular karyotypes. Aneuploidy largely results form whole chromosome mis-segregation during tumor cell division, a process called Chromosomal Instability (CIN). Despite its widespread prevalence, the origin and contribution of CIN in cancer have long been a mystery. CIN positively correlates with poor prognosis and drug resistance presumably by promoting genetic plasticity and tumor cell heterogeneity. We have previously shown that CIN mostly arises from improperly regulated attachments of microtubules to chromosomes during cell division and we found that the stability of these attachments is inherently increased in cancer cells with CIN. By selectively destabilizing the attachments of microtubules to chromosomes, we were able to successfully restore chromosomal stability to otherwise chromosomally unstable cancer cells. Using this unique approach, we sought to study the contribution of CIN towards tumor growth using a xenograft mouse-model. By overexpressing proteins that destabilize microtubule attachments to chromosomes, we suppressed CIN in two cell lines derived from human glioblastomas (U251 and U118) thereby gaining the ability to selectively examine the role of CIN in otherwise isogenic conditions. Surprisingly, immuno-competent immunocompromised mice injected with cells where CIN was suppressed developed larger tumors than mice injected with chromosomally unstable cells. Furthermore, tumors where CIN was suppressed had much larger tumor margins and smaller necrotic cores compared to tumors that originated from chromosomally unstable cells. We then examined the behavior of sphere forming cells, which are characteristic features of glioblastomas and are considered to be enriched in tumor initiating ‘stem’ cells. In many cases, the growth of tumor initiating cells more closely resembles the growth characteristics of the tumor in vivo. In accordance with the xenograft experiments, suppressing CIN lead to an increase in sphere size and cell number when cells where cultured under normal adherent conditions. This suggests that tumor initiating cells are also chromosomally unstable and that this instability may curtail their growth once they have achieved tumorigenic potential. Current studies aim to identify the specific mechanism by which CIN affects the proliferation of tumor initiating cells in vitro and in vivo. Our results unexpectedly indicate that CIN may lead to suppression of tumor growth, in part, by limiting the proliferation of tumor initiating cells. Previous studies show that inducing CIN can lead to tumorogenesis yet our work shows that once cells have achieved a favorable aneuploid karyotype, suppressing CIN may, in fact, aid their proliferation by stabilizing a tumorigenic karyotype. A concept thus emerges where cancer cells must balance their ability to evolve by shuffling chromosomes during cell division with their need to stably maintain aneuploid karyotypes that confer growth advantage and rapid growth.
100 MODULATION OF MITOCHONDRIAL RESPIRATION BEFORE ISCHEMIA PREVENTS MITOCHONDRIAL PERMEABILITY TRANSITION PORE OPENING DURING REPERFUSION IN ISOLATED RAT HEARTS

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Blockade of electron transport during ischemia (ISC) with the reversible inhibitor amobarbital (AMO) protects the electron transport chain (ETC) against damage during ISC. Inhibition of respiration by AMO is rapidly reversed during reperfusion (REP) (< 30 sec). Despite treatment only during ISC, cardiac injury measured following REP is decreased. Mitochondrial permeability transition pore opening (MPTP) is a major mechanism of cardiac injury during REP. We asked if protection of the ETC by AMO inhibition during ISC decreases cardiac injury by preventing MPTP during REP. Langendorff-perfused Fischer 344 adult (6 mo.) rat hearts underwent 25 min. global ISC (37°C) and 30 min REP. In AMO treated hearts, AMO (2.5 mM) was given for 1 min. immediately before ISC. Mitochondria were isolated following 30 min REP, and mitochondrial oxidative phosphorylation (OXPHOS) and calcium tolerance were measured. Compared to untreated ISC-REP hearts, AMO given only before ISC preserved OXPHOS (glutamate as a complex I substrate). LDH release during REP as an index of cardiac injury was decreased. AMO treatment markedly improved mitochondrial calcium tolerance assessed following REP. Thus, blockade of electron transport with AMO during ISC protects mitochondria, with preserved OXPHOS and calcium tolerance evident during the critical REP period. When the heart is reperfused with protected, rather than ISC-damaged mitochondrial cardiac injury is decreased, indicated by less release of LDH during REP In sum, blockade of electron transport during ISC prevents mitochondrial-driven ETC damage during ISC that leads to mitochondrial-mediated cardiac injury during REP. AMO treatment improved calcium tolerance in mitochondria following ISC-REP, suggesting that ISC damage to the ETC leads to mitochondrial-driven cardiac injury during REP. Manipulation of mitochondrial respiration provides an opportunity to decrease cardiac injury by modulating MPTP.

<table>
<thead>
<tr>
<th>Time control (n=5)</th>
<th>ISC-REP (n=8)</th>
<th>AMO-ISC-REP (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH (µm/mg tissue)</td>
<td>12 ± 6</td>
<td>437 ± 60*</td>
</tr>
<tr>
<td>OXPHOS (aAO/mg/min)</td>
<td>205 ± 24</td>
<td>89 ± 6*</td>
</tr>
<tr>
<td>Ca2+-tolerance (µM/mg)</td>
<td>700 ± 62</td>
<td>350 ± 50*</td>
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Mean ± SE, *P<0.05 vs. Time control, #P<0.05 vs ISC-REP. Ca2+ calcium

101 ALVEOLAR EPITHELIAL CELL MIGRATION AND WOUND REPAIR BY AUTO TAXIN RELEASE AND LY SOPHOSPHATIDIC ACID GENERATION

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Rationale: Re-epithelialization by alveolar cell migration plays a critical step in lung remodeling after acute lung injury. Autotaxin (ATX) is a cell motility-stimulating factor, which exhibits lysophosphatidase D (lysoPLD) activity. The present study demonstrates that ATX stimulates lung alveolar cell line (A549) migration through LPA generation and LPA-LPA receptors-mediated signal transduction.

Methods/Results: ATX protein and activity were detected in media collected of A549 cells. To further investigate mechanism(s) of ATX release and role of ATX in lung alveolar cells, ATX cDNA was cloned, tagged with V5 and inserted into adenoviral expression vector. ATX-V5 was detected in media of A549 cells after 24 and 48 h post-infection with ATX-V5 and pretreatment with cytochalasin D attenuated ATX-V5 release. Downregula tion of ATX by siRNA or pretreatment with ATX inhibitor attenuated A549 cells migration, while, overexpression of ATX-V5 wild type (WT) enhanced A549 cells migration. Further, addition of ATX-V5 WT overexpression condition medium (ATX-V5 WT CM) enhanced LPA generation and A549 cells migration. Knockdown of LPA receptor1 by siRNA or pretreatment with LPA receptor1 and 3 antagonist (K16425) attenuated ATX-V5 WT CM-induced A549 cells migration. Additionally, ATX-V5 WT CM induced phosphorylation of PKC ε and cortactin at leading edge of migratory cells, while inhibition of PKC ε by overexpression of dominant negative (dn)PKC ε (10 MOI, 24h) attenuated cortactin phosphorylation and migration.

Conclusions: ATX released from A549 cells is involved in migration through LPA generation, and LPA receptors-mediated phosphorylation of PKC ε and cortactin. This study suggests a role of ATX and LPA in lung alveolar epithelial remodeling. Supported by NIH091916 grant to Y.Z.

102 T-786 C POLYMORPHISM OF THE ENDOTHELIAL NITRIC OXIDE SYNTHASE GENE AND NEURALGIA-INDUCING CA VITATIONAL OSTEONECROSIS OF THE JAWS

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Introduction: Neuralgia-inducing cavitation osteonecrosis of the jaws (NICO) is commonly multifactorial involving local trauma and infection and systemic disorders including thrombophilia and hypofibrinolysis.

Objective: We hypothesized that similar to idiopathic hip osteonecrosis, the T-786C mutation of the endothelial nitric oxide synthase (eNOS) gene affecting nitric oxide (NO) production was associated with NICO.

Methods: In 22 NICO patients, not having taken bisphosphonates, mutations affecting NO production (eNOS T-786C, stromelysin 5A6A) were measured by polymerase chain reaction (PCR). Two healthy normal controls were matched per case by age, race and gender.

Results: Homozygosity for the mutant eNOS allele (TT) was present in 6 of 22 (27%) patients with NICO compared to 0 of 44 (0%) race, gender-matched controls; heterozygosity (TC) was present in 8 patients (36%) vs 15 controls (34%); and the wild-type normal genotype (CC) was present in 8 patients (36%) vs 29 controls (66%) (p = 0.0008). The mutant eNOS T-786C allele was more common in cases (20/44 [45%]) than in controls (15/88 [17%]), p = 0.0008. The distribution of the stromelysin 5A6A genotype in cases did not differ from controls (p=13).

Conclusions: The eNOS T-786C polymorphism and resultant reduction of NO production associated with NICO, may contribute to the pathogenesis of NICO and, speculative, may open therapeutic medical approaches to treatment of NICO through provision of L-arginine, the amino-acid precursor of NO.

103 RELIGIOSITY AND SPIRITUALITY AS PREDICTORS OF MEDICAL INPATIENTS’ END-OF-LIFE CARE PREFERENCES

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Background: Prior studies suggest that terminally ill patients who use religious coping are less likely to have advance directives and more likely to opt for heroic end-of-life measures. Yet, no study to date has examined whether end-of-life practices are associated with general measures of religiosity and spirituality.

Methods: We examined data from the University of Chicago Hospitalist Study, which gathers sociodemographic and clinical information from all consenting general internal medicine patients at the University of Chicago Medical Center. Primary outcomes were the prevalence of having an advanced directive or DNR order, a durable power of attorney for health care, and an informally designated decision-maker. Primary predictors were religious attendance, intrinsic religiosity, and self-rated spirituality.

Results: Our sample population (N=8,388) was predominantly African-American (73%) and female (60%). The prevalence of advance directives in this population was 1.5% and of DNR orders was 10.4%. Half (51%) of patients had specified a decision-maker. White patients were more likely than African-American patients to have an advance directive (adjusted odds ratio [OR], 2.1; 95% confidence interval [CI], 1.1 to 4.0) and a DNR order (OR, 1.7; CI, 1.0 to 2.9). Patients reporting high intrinsic religiosity (OR, 1.3; CI, 1.1 to 1.6) and high spirituality (OR, 1.3; CI, 1.1 to 1.5) were more likely to have specified a decision-maker than those with low religiosity and low spirituality, respectively. Religious characteristics were not significantly associated with having an advance directive or DNR order.

Conclusions: Among general medicine inpatients at an urban academic medical center, those who were highly religious and/or spiritual are more likely to have a designated decision-maker to execute their end-of-life care, but they did not differ from other patients in their likelihood of having an advanced directive or a DNR order. Further study is needed to understand the reasons for these findings.
104 IMPACT OF FDA BLACK BOX ADVISORY ON ANTIPSYCHOTIC MEDICATION USE
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Background: The Food and Drug Administration (FDA) is responsible for communicating prescription drug risks to providers and the public, and the Sentinel Initiative will substantially increase the number of safety signals that the FDA must evaluate. We evaluated the effect of a regulatory warning regarding atypical antipsychotics, which represent some of the most widely prescribed psychotropic medicines. This warning, issued in April 2005, included a Public Health Advisory and subsequent black box warning regarding the risks of atypical antipsychotics among elderly patients with dementia.

Methods: We used time-series analyses to examine nationally representative data from IMS Health’s National Disease and Therapeutic Index from January 2003 through December 2008. The primary measurement from this audit of office-based physicians was the use of an atypical antipsychotic agent. We quantified the impact of the advisory on atypical antipsychotic use among all individuals and those over 65 years of age with dementia. We also quantified unintended advisory effects, such as decreases in atypical use for FDA approved indications.

Results: From January 2003 through March 2005, atypical antipsychotic mentions increased at an annual rate of 32% (95% confidence interval [CI], 2% to 65%), increasing from 1.0 million to 1.4 million monthly drug mentions. For those over 65 with dementia, the annual growth rate was 16% (95% CI, 7% to 25%), and the number of monthly mentions increased from 69,000 (January 2003) to 65,000 (March 2005). In the year prior to the FDA advisory, there were approximately 13.6 million atypical mentions, including 0.8 million among those with dementia. In the year following the advisory, atypical mentions fell 2% overall and 19% among those with dementia. In 2004, 19% (0.8 of 4.1 million) of drug mentions for dementia were for an atypical agent. By 2008, this proportion decreased to 9% (0.4 of 4.3 million). Atypical use slowed for both FDA approved and off-label indications, and declined through 2008 for all populations examined.

Conclusion: The April 2005 FDA advisory was associated with a significant decrease in the use of atypical antipsychotics among the elderly with dementia that occurred soon after the advisory was issued. These findings are important because atypical antipsychotics are a widely used drug class accompanied by significant safety concerns and the impact of the FDA advisory has not been clear. Despite the decrease, atypical antipsychotics still comprised 9% of prescription drug uses for dementia among the elderly at the end of 2008. The residual use in the population at risk and the decrease in the use of atypical antipsychotics in the general population, who were not targeted by the warning, raise the question as to how the effect and specificity of FDA regulatory actions can be enhanced. Targeting specific segments of patients and physicians (e.g., high prescribers) and further customizing the impact of regulatory actions may improve their impact at minimizing the risks associated with select prescription medications. Developing evaluations of such efforts is highly important, and consistent with recent comments by the new FDA commissioners that, “The FDA must make difficult decisions in the absence of ideal information . . . . For [FDA] communications to have credibility, the public must trust the agency to base its decisions on science.”

105 ASEPTIC MENINGITIS SYNDROME IN ADULTS: RISK CLASSIFICATION FOR AN URGENT TREATABLE ETIOLOGY
R Hashbun, C Hadi, M Hussein Houston, TX and New Orleans, LA.UTHSC.

Background: The management of adults with the aseptic meningitis syndrome is problematic due to a diverse spectrum of potentially treatable etiologies.

Methods: We prospectively studied 193 patients with community-acquired meningitis and a negative Gram stain. Baseline characteristics were analyzed to create a clinical predictive model to classify the risk of an urgent treatable cause of aseptic meningitis. Prediction accuracy of the model was determined using the concordance index (c-index) and validated internally using bootstrapping.

Results: Of the 193 patients, 46 (24%) had an urgent treatable cause of aseptic meningitis (i.e., bacterial, mycobacterial or fungal meningitis, bacteremia, herpetic meningoencephalitis, meningeval carcinomatosis, parameningeval mass lesions, intracranial mass lesions or hemorrhages). A predictive model was created using three composite baseline variables that were independently associated with an urgent treatable cause (p < 0.05): abnormal serum creatinine (> 1.0 mg/dl), history of cerebrovascular disease (e.g., stroke, 60 years or immunocompromised or history of injecting drug use), abnormal exam (abnormal mental status or focal neurological exam or recent seizure or vesicular or petechial rash), and abnormal labs (CSF glucose < 45 mg/dl or CSF protein >100 mg/dl or serum white cell count > 12,000 cells/mm3). The model classified patients into three risk categories for an urgent treatable cause: low risk (0%), intermediate risk (15%), and high risk (40%) (p < 0.001). The model predicted an urgent treatable cause significantly better than chance (c-index=0.84) and was validated with the bootstrap model.

Conclusion: Adults with the aseptic meningitis syndrome can be accurately stratified for risk of an urgent treatable cause using clinical variables at presentation.

106 COMPARISON OF NOVEL EARLY BIOMARKERS OF ACUTE KIDNEY INJURY (AKI) TO PREDICT THE COURSE AND SEVERITY OF AKI DEVELOPING AFTER CARDIAC SURGERY

To evaluate the utility of multiple novel urinary biomarkers of AKI (Neutrophil Gelatinase Associated Lipocalin (NGAL), Cystatin C (CyC), Kidney Injury Molecule-1 (KIM1), Hepatocyte Growth Factor (HGF), - Glutathione S-Transferase (GST) and -GST in the diagnostic and prognostic evaluation of AKI, we prospectively studied 115 adults undergoing cardiac surgery. Perioperatively blood and urine were sampled for fractional excretions of sodium and urea (FENa and FEUrea) and urine for CyC, NGAL, KIM1, HGF, and -GST. AKI (AKIN definitions) developed in 46 subjects (40.0%); of these 37 (32.2%) only developed Stage 1 AKI within 72 hours of the surgery. All remaining 9 AKI subjects progressed to Stage 3 AKI. We performed Receiver Operator Characteristic Curves at the time of AKI diagnosis (creatinine elevation - Stage 1 AKI) for the progression to Stage 3; AUCs in Table. At the time of serum creatinine increase FEUrea and KIM1 are unable to predict the progression to more severe AKI and HGF and FENa are potentially useful. There was a difference between those who remained at Stage 1 compared to those who progressed to Stage 3 AKI for NGAL (median, 25.75%) (4612-133) vs. 571(250-6273); p=0.03), CyC (0.009(0.04-0.32) vs. 0.62(0.26-1.05); p=0.03), and -GST (0.033(0.02-0.12) vs 0.92(0.17-13); p=0.003). NGAL, CyC and -GST are useful in the diagnostic and prognostic evaluation of AKI following adult cardiac surgery and can predict progression to severe AKI.

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Midwestern Regional Program Abstracts
represent a novel modulatory mechanism of TGF-
A
remains unknown. However, it was demonstrated that nuclear EGFR controls
localization of these receptors as well as of the T
A
very few examples of cell transmembrane receptors that are found also, in
the cell membrane into the cytoplasm compartment after ligand binding, there are
signal transduction systems involves the mobilization of receptors from the
receptors in human airway smooth muscle cells. Although the majority of cell
our knowledge, this is the first demonstration of nuclear localization of TGF
receptors were also found in the
NE. Interestingly, Smad2/3 was observed in the CE and in the NE of
untreated cells, which is consistent with our previous observation in canine
ASM cells of a basal stimulation of the TGF
A
expression.

109
THE EFFECT OF TUMOR NECROSIS FACTOR-ALPHA
BLOCKADE ON CARdiovascular OUTCOMES AND
SURVIVAL IN PATIENTS WITH RHEUMATOID ARTHRITIS
Z. Al-Aly, H Pan, A Zeringue, H Xian, M Jay, S Eisen Saint Louis, MO. SAINT Louis Veterans Affairs Medical Center.

Background: TNF-alpha is implicated in the biology of rheumatoid arthritis (RA). TNF-alpha antagonists have been shown to ameliorate the course of RA. However, beyond its role in RA, TNF-alpha has numerous other pleiotropic effects on endothelial, immune, and vascular cells, immune system, and cancer biology, and the effect of TNF-alpha blockade on cardiovascular outcomes and long term survival in RA patients is not known.

Objective: To examine the effect of TNF-alpha blockade on the risk of cardiovascular events and survival in patients with RA.

Methods: Cox survival models were built to examine the effect of TNF-alpha antagonist treatment on the risk of cardiovascular outcomes and all-cause mortality.

Results: In a cohort of 20,811 patients with RA (49,756 person years), fully adjusted Cox regression survival models showed that treatment with TNF-alpha antagonists was not associated with significant effect on the composite end-point of cardiovascular outcomes (defined as the occurrence of atherothrombotic heart disease, congestive heart failure, peripheral artery disease, or cerebrovascular disease). When each outcome was examined separately, treatment with TNF-alpha antagonists did not associated with increased risk of atherothrombotic heart disease, congestive heart failure, or peripheral artery disease but was associated with decreased risk of cerebrovascular disease (HR=0.836; CI=0.710–0.948; P=0.0315). Treatment with TNF-alpha antagonists did not affect the risk of death (HR=0.946; CI=0.825–1.084; P=0.4256). In subgroup analyses, TNF-alpha antagonists use was associated with reduced risk of cardiovascular outcomes (HR=0.876, CI=0.786–0.977; P=0.0177) and a trend toward decreased risk of death (HR=0.814, CI=0.655–1.012; P=0.063) in patients who are younger than 63 years old.

Conclusion: The risk of cardiovascular events and survival in patients receiving TNF-alpha antagonists is not different than those receiving other disease-modifying anti-rheumatic drugs.

108
PREDICTORS OF SURVIVAL IN PATIENTS WITH PROSTATE CANCER AND SPINAL METASTASIS
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Background: Prostate cancer is the second most common malignancy to
cause death in men, with metastases to the spine being the most common site
of metastatic burden.

Objective: An observational study was performed to determine predictive
factors of mortality in patients with prostate cancer who were just diagnosed
with spinal metastasis.

Methods: The patient population was extracted from the Prostate Clinical
Research Information System (PCRIS) at the Dana-Farber Cancer Institute.
Patients were observed over 19 years from June 1990 to April 2009. Baseline
demographic information for the patients and characteristics of the tumor were collected. The following tumor data were collected at the time of
diagnosis of spinal metastasis: duration from diagnosis of prostate cancer,
location of concurrent metastasis (lung, liver, bone [non-spinal], lymph node,
bladder, brain, soft tissue), presence of local prostate cancer recurrence,
number of different sites of metastasis, prostate-specific antigen, and
presence of cord compression. Though the data was collected retrospectively,
the outcome of interest (mortality) was determined prospectively (as patients
were included based on Current Procedural Terminology code of metastasis rather than outcome of progression). Kaplan-Meier survival estimates were used to determine overall survival of patients. Univariate analysis was performed using log-rank test and multivariable analysis included use of the Cox regression model to determine the significance of covariates in predicting death. Hazard ratios, 95% Confidence Intervals (CI) and
probability values were calculated.

Results: There were a total of 9010 patients in the PCRIS database, of which
333 met criteria for inclusion in our analysis. A total of 235 patients died,
with a median survival of 1.92 years (95% CI = 1.65–2.18). Evaluation of
predictors of death revealed a statistically significant effect of age at diagnosis
of spinal metastasis (HR = 1.02, P = 0.018, 95% CI = 1.00–1.04), presence of
bony (non-spinal) metastasis (HR = 2.60, P = 0.009, 95% CI = 1.27–5.30),
and higher number of different sites of metastasis (HR = 1.44, P = 0.001, 95% CI = 1.15–1.81).

Conclusion: The results of our study are important for oncologists,
neuropres, and primary care physicians who have patients with prostate
cancer metastatic to the spine because they can be used to predict prognosis
and guide the physician in making appropriate decisions regarding the
patient’s treatment. Future work should include building a predictive model
that accurately determines survival in patients with metastatic disease, as this
would guide the physician in devising the most appropriate treatment plan for
each individual patient.
bleeding vs the risk of thrombosis. Only one study has assessed the use of TEG-PM in the surgical population and with ambiguous results. Therefore, our aim is to assess the ability of the TEG-PM to detect platelet inhibition secondary to clopidogrel and/or aspirin therapy in patients scheduled for general surgery. We collected data on 10 patients. The average age is 60 years old, min 25 and max 87. 7 out of the 10 patients suspended the daily therapy for less than 3 days (3 patients last dose the day before surgery and 3 patients last dose two days before surgery). The three that suspended the therapy for more than 3 days (4 min and max 8 days for aspirin and min 4 and max 18 days for clopidogrel) showed preoperatively an aspirin pathway inhibition (AA) of 65% (vs 61% less than 3 days) and a clopidogrel inhibition (ADP) of 52% (vs 60% less than 3 days) with no significance difference (t-test p= 0.3803 for ADP and p=0.5778 for AA). In four patients out of the 10, we were able to collect data after the surgical procedure. Two relevant results are important to be discussed. First that patients resistance to dual therapy occurs more frequently than thought, and besides it is claimed to be related to the accuracy and precision of the platelet mapping test, it is not reflected in an increased risk of intraoperative bleeding and transfusion. Second the inhibition effects is further decrease after surgery, and even in patient under therapy the effect is up to 50% reduction. These preliminary data support for a prospective cross sectional study. Practice Alert for the Perioperative Management of Patients with Coronary Stents.” Anesthesiology 2009; 110(1):22-23: King SB, Smith SC, Himmelfeld JW, et al. “2007 Focused Schouten Q, van Domburg RT, et al. “Noncardiac Surgery After Coronary Stenting: Early Surgery and Interruption of Antplatelet Therapy are Associated with an Increase in Major Cardiac Events.” J Am Coll Cardiol 2007; 49(1):122-124. Iakovou I, Schmidt T, et al. “Incidence, Predictors, and Outcome of Thrombosis After Successful Implantation of Drug-Eluting Stents.” JAMA 2005; 293(17):2126-2130. Collyer TC, Gray DJ, Sandhu R, Berridge J, Lyons G. Assessment of platelet inhibition secondary to clopidogrel and aspirin therapy in the preoperative acute surgical patients measured by Thrombelastography Platelet Mapping. Brit J Anaesth 2009; 102 (4):492-8.

111 INTRAVENOUS SOTALOL INDUCED QT PROLONGATION PREDICTS SERUM SOTALOL CONCENTRATION

Introduction: Sotalol is an antiarrhythmic agent that prolongs the cardiac action potential that can be observed as QT interval (QT) prolongation on the surface EKG. It is known that sotalol causes Torsade de Pointes ventricular tachycardia (TdP) that is due to dose related QT prolongation. We were able to develop a correlation between QT prolongation and sotalol serum concentration.

Methods: 15 healthy volunteers (age: 32±8 y) received 75 mg intravenous sotalol over 2.5 hr at a constant infusion rate. Serum sotalol concentrations were determined and a 12-lead EKG was recorded at baseline, 0.5, 1, 2, 3, 4, and 5 hrs following dosing. The QT and RR intervals were measured by two blinded investigators. Heart rate corrected QT (QTc) was calculated by the Bazett, Fridericia, and Framingham formulas. Linear regression analysis was performed between S concentration and QT measurements.

Results: The analysis showed close correlation between sotalol serum concentration and QT (r=0.938, p=0.02), Bazett QTc (r=0.860, p=0.02), Framingham QTc (r=0.966, p<0.001), and Fridericia QTc (r=0.967, p<0.001). The equation of the regression line was: QTc= 0.3838 x (sotalol concentration) + 402.63, which closely predicted actual QTc at any sotalol concentration. Conversely any degree of QT prolongation can predict the measured serum sotalol concentration.

Conclusion: A simple equation was found that predicts serum sotalol concentration from QT prolongation. Measuring QT prolongation can serve as a quick method of estimating serum sotalol concentration, as well as the electrophysiology effects of serum sotalol. Excessive serum sotalol levels that causes excessive QT prolongation can be avoided, possibly reducing the risk of TdP.

112 NON-MUSCLE MLCK IS A KEY REGULATOR OF HYPEROXIA-INDUCED NADPH OXIDASE ACTIVATION AND ROS GENERATION IN CAVEOLIN-ENRICHED MICRODOMAINS OF LUNG ENDOTHELIUM

Background: Generation of reactive oxygen species (ROS), particularly by the endothelial NADPH oxidase, plays a major role in the pathophysiology of hyperoxia-induced lung injury. We have earlier demonstrated that hyperoxia-mediated activation of NADPH Oxidase in human pulmonary artery endothelial cells (HPAECs) is regulated by Src-dependent tyrosine phosphorylation of cortactin and p47(phox) as well as dynamin2 and c-Abl. Here we examined the potential role of non-muscle myosin light chain kinase (nmMLCK) as a novel regulator of NADPH Oxidase-dependent ROS production by hyperoxia in caveolin-enriched microdomains (CEM) of HPAECs.

Methods and Results: Exposure of HPAECs to hyperoxia (95 % O2/5 % CO2 for 3 h) resulted in superoxide/ROS generation that was independent of early endosomes formation. However, co-staining of ROS, asDCFDA fluorescence, with chola toxin-B showed localization in the cytosol and caveolin-enriched microdomains (CEM) after exposure to hyperoxia. Hyperoxia stimulated phosphorylation of myosin light chain (MLC), and recruitment of phosphorylated and non-phosphorylated cortactin, MLC, Src and p47(phox) to CEM. Silencing nmMLCK with siRNA attenuated hyperoxia-mediated phosphorylation of MLC, cortactin, Src and p47(phox), recruitment of these components to CEM and ROS generation. Furthermore, nmMLCK−/− mice exposed to hyperoxia (72 h) exhibited reduced ROS production, lung inflammation and pulmonary leak compared to control mice.

Conclusion: These results suggest a novel role for nmMLCK-dependent phosphorylation of MLC in hyperoxia-induced recruitment of cytoskeletal proteins and NADPH Oxidase components to CEM and ROS production. This work was supported by NIH grant P01 HL58064 to VN, J.G.N. and P.A.S.

113 ESSENTIAL OIL ATTENUATES INFLUENZA VIRUS INFECTION VIA INHIBITION OF MEMBRANE FUSION IN VITRO
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Influenza is a significant cause of morbidity and mortality. The recent pandemic of a novel H1N1 influenza A virus has stressed the importance of the search for effective treatment for this disease. Originated in ancient Egypt, essential oils from aromatic plants have been used for a wide variety of applications, such as personal hygiene, therapeutic massage and even medical practice. Here, we studied a commercially available essential oil blend, OnGuard(TM) protective blend oil (doTERRA International, LLC, Orem,UT), and evaluated its ability to control influenza virus A/PR8/34 infection in Madin-Darby canine kidney (MDCK) cells. We first determined the effect of the oil on viability of the cells using trypan blue staining. The data showed there was no alteration of cell viability by the oil at dilutions greater than 1 to 3000. Next, fluorescent focus assay (FFA) showed that virus treated with a 1 to 4000 dilution of protective oil decreased the number of infected MDCK cells by 90%, and by 40% at a dilution of 1 to 6000. In order to further elucidate the mechanism of inhibition, we investigated hemagglutination (HA) activity, binding and internalization of the oil-treated virus by HA assay, flow cytometry and immunofluorescence microscopy. There was no decrease in HA activity in oil treated viral particles even after 72 h oil treatment at a dilution of 1 to 3000. Also, oil treated virus had the same ability to bind to and internalize in MDCK cells compared with untreated virus, suggesting that the inhibition did not occur at early stages of infection. Confocal microscope result suggested that the inhibition occurred at the viral membrane fusion. Taken together, we found an essential oil blend notably attenuates influenza virus PR8 infection in vitro via inhibition of membrane fusion.

114 A COMPARISON OF MORTALITY AND MORBIDITY IN MULTIPLE SINGLETONG VERY LOW BIRTH WEIGHT INFANTS
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Objective: To compare mortality and morbidity of singleton and multiple gestations weighing 500g to 1500g admitted to the Neonatal Intensive Care Unit (NICU). Thirty-five percent of admissions at the Good Samaritan Hospital NICU are very low birth weight multiple gestations.

Methods: Data were retrieved from the Good Samaritan Hospital Vermont Oxford Network database which includes infants admitted to the Good
Summaritan Regional NICU weighing 500–1500g. Infants from 1999 through 2008 were included in this study with a total sample size of 2,144 infants: 1,389 of singleton gestations and 755 multiple gestations. A chi-square test of association was used to determine if there was a significant difference in mortality and secondary outcomes between singleton and multiple gestations.

**Results:** Very low birth weight singleton and multiple infants have similar mortality rates, 14.9% versus 14.6%, respectively. Multiple gestations were more likely to have severe intraventricular hemorrhage (IVH) (7.8% versus 5.0%, p < 0.05), have been administered antenatal steroids (90.6% versus 82.0%, p < 0.1), and have a cesarean delivery (74.7% versus 60.7%, p < 0.001). Conventional ventilation (54.5% versus 49.8%, p = 0.05) was more common among singletons. Congenital malformations, respiratory distress syndrome, IVH, necrotizing enterocolitis, retinopathy of prematurity, late bacterial infection, pneumothorax and high frequency ventilation were similar between singleton and multiple gestations.

**Conclusion:** Findings demonstrate that very low birthweight singleton and multiple gestations have similar outcomes with respect to mortality and morbidity, however, severe IVH is higher in multiple gestations. A larger sample size is needed to determine if statistically significant differences exist between singletons, twins and triplets or greater gestations and to determine if there are differences between singletons and multiples within birthweight categories (e.g., 501–750 grams, 751–1000 grams, 1001–1250 grams and 1251–1500 grams).

### 115 IS CIRCADIAN TYPE ASSOCIATED WITH SLEEP DURATION IN TWINS?


**Introduction:** The genetic underpinnings of circadian rhythms have been described, but the genetics of sleep duration remain largely unresearched. We used the population based University of Washington Twin Registry to investigate the genetic association between circadian type and sleep duration.

**Methods:** Habitual sleep duration was obtained by self-reported length of sleep per night and circadian type was ascertained using a reduced 5-item Horne-Ostberg Morningness-Eveningness questionnaire. Univariate and bivariate genetic analyses were fit using structural equation models. We described, but the genetics of sleep duration remain largely unresearched. We used the population based University of Washington Twin Registry to investigate the genetic association between circadian type and sleep duration.

**Results:** We surveyed 1,136 twins from same-sex pairs (854 monzygotic, 282 dizygotic), 69% female, mean age 37 years (SD=15). Twenty-four percent of the total sample regularly slept < 7 hours, 67% 7–8 hours, 9% ≥9 hours per night. Thirty-four percent were morning-type, 51% neither, and 15% evening-type. The heritability of sleep duration was 33% (p=0.001) and circadian type 40% (p=0.001). The bivariate analysis revealed shared genetics of 10% (≈ 0.06, 0.37; p=0.50). Using morning-type twins as the baseline, evening-type twins had a higher relative risk of being a short sleeper (< 7 hours/night; RR=1.7; 1.1, 2.7; p=0.05) and long sleeper (≥ 9 hours/night; RR=2.5; 1.1,6.1; p=0.05). The within-pair analysis attenuated these associations.

**Conclusions:** Sleep duration and circadian type do not have substantial genetic overlap in our twin sample. Circadian evening-type was associated with both shorter and longer habitual sleep duration than morning-type twins. These findings are attenuated in the within-pair analysis, suggesting that familial factors (i.e., genetics and common environment) may confound the associations.

### 116 FREQUENCY OF CD4+CD25HIFOXP3+ REGULATORY T CELLS HAS PROGNOSTIC VALUE AS A BIOMARKER FOR ACUTE GRAFT-VERSUS-HOST DISEASE


Regulatory T cells (Treg) play an important role in the maintenance of tolerance after bone marrow transplantation (BMT) in experimental models. However, the relationship between Treg and acute graft-versus-host disease (GVHD) in allogeneic BMT recipients is not well established. We conducted a prospective analysis of Treg frequency in 215 BMT patients (125 allogeneic, 90 autologous) at the University of Michigan. Fresh peripheral blood samples were acquired prior to GVHD onset within 24 hours and at median day of sample acquisition. Frequencies of CD4+CD25HIFOXP3+ cells within total lymphocytes were used to determine if there was a significant difference between patients with and without GVHD for median age, nonmalignant disease, conditioning intensity, and median day of sample acquisition. Recipients of grafts from donors who were not family members or who were not HLA-matched were overrepresented in the GVHD group. Autologous BMT patients (N=90) and allogeneic BMT patients without GVHD (N=65) had the same mean Treg frequency (1.09%±0.11 vs. 1.06±0.10, p=0.84). The absolute Treg counts (CD4+CD25HIFOXP3+ frequency multiply by the absolute lymphocyte count (ALC)) were significantly less in allogeneic patients without GVHD compared to autologous BMT patients suggesting that the use of calcineurin inhibitors did not affect Treg frequency while absolute Treg numbers were lower in allogeneic BMT patients due to decreases in the absolute lymphocyte count. Patients with GVHD (N=60) had 40% fewer Treg (0.66±0.07, p=0.001) than those without GVHD. Absolute Tregs in allogeneic BMT patients with GVHD remained significantly less than allogeneic patients without GVHD (p=0.02). The calculated Receiver Operating Characteristic (ROC) curve for Treg frequency as an independent biomarker of GVHD was 0.69 (95%CI, 0.55–0.83). Treg frequencies decreased prior (3 to 14 days) to GVHD onset (p<0.003), suggesting an active loss of Tregs occurred before clinical GVHD for some patients. Frequencies of Tregs decreased in a linear fashion with each increasing grade of GVHD at onset, and were significantly reduced in patients with grade III compared to patients without GVHD (p=0.004). The Treg frequency also correlated with the eventual maximum overall grade of GVHD (p=0.001), suggesting a prognostic value for this measurement. Therefore, we evaluated whether Treg frequency would correlate with non relapse mortality (NRM) in the 60 patients with GVHD. Patients with low Treg frequency (<0.5%, N=30) had a significantly greater NRM (41% vs. 7%, p=0.01) than patients with high Treg frequency (≥0.5%, N=30), which resulted in an inferior survival at two years (38% vs. 63%, p = 0.03) (Table 1). Acute GVHD accounted for the majority of NRM in the low Treg frequency group. Relapse mortality was similar between groups (p=0.86) (Table 1). This difference in survival remained significant after adjusting for other important prognostic factors such as age, degree of HLA-match, donor source (related or unrelated), conditioning intensity and ALC (Hazard Ratio 2.65, p=0.04). In addition, frequency of Tregs at onset of GVHD predicted the response to GVHD treatment (p=0.003). In this set of sixty patients, frequency of CD4+CD25HIFOXP3+ Treg at onset of GVHD correlates with GVHD severity, eventual maximum grade, NRM, response to GVHD intervention and OS. Treg frequency thus has important prognostic value as biomarker for acute GVHD.

### 117 POSTOPERATIVE PULMONARY INSPIRATORY RESERVE VOLUME AND INCENTIVE SPIROMETRY IN MORBIDLY OBSESE PATIENTS UNDERGOING BARIATRIC SURGERY

D Cattano, A Altamirano, V Melnikov, E Pivalizza, A Feldman, C Hagberg Houston, TX. University of Texas, HSC Houston.

Morbidly obese patients undergoing general anesthesia for laparoscopic bariatric surgery require special considerations due to an increased risk of postoperative pulmonary complications. Studies directed at combating pulmonary risk in the postoperative period demonstrated that lung volume expansion techniques, such as incentive spirometry (IS), are useful in preventing complications. Other studies, however, revealed IS to be of questionable benefit when used as a prophylactic measure postoperatively. The purpose of this study was to determine how incentive spirometry, prior to surgery, could improved respiratory mechanics be used to determine the amount of loss in pulmonary function postoperatively. Our hypothesis was that application of a standardized protocol of preoperative respiratory care

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teaching and exercise would improve lung performance that will subsequently result in improved inspiratory capacity. Morbidly obese patients, BMI > 40 kg/m2 undergoing bariatric surgery were enrolled in the study. Patients were blindly randomized into 2 groups. The control group was instructed to use the incentive spirometer for 3 breaths, once per day. The experimental group was instructed to use the incentive spirometer for 10 breaths, 5 times per day. Each patient’s greatest inspiratory volume achieved with IS (best of 3 attempts) was recorded during their preoperative consultation, the day of surgery, and postoperative day 1. 26 patients were enrolled (12 experimental/14 control). No significant differences were found preoperatively between the 2 groups. The average inspiratory capacity (IC) was respectively 2083 ± 733.4 cc and 1946 ± 754.3 in the experimental and control group. On postoperative day one the volume were respectively 1479 ± 669.6 and 1417 ± 842.5 in the experimental group and control group. The results of this study were unexpected for our hypothesis but confirmatory of the postoperative lung volume loss. It appears that preoperative use of incentive spirometry does not significantly improve respiratory function between the initial preoperative consultation and the day of surgery. Of particular importance, it appears to be a significant decrease in lung function postoperatively in both the experimental group and the control group. The use of the IS preoperatively does not prevent the reduction in pulmonary function; patients tend to have poor compliance in doing IS, and IS seems a precise and accurate but inexpensive method of measuring postoperative volume capacity. Lawrence VA, Cornell JE, Smetana GW. “Strategies to reduce postoperative pulmonary complications after noncardiothoracic surgery: systematic review for the American College of Physicians.” Ann Intern Med 2006; 144(8): 596-608. Pasquini P, Tramer MR, et al. “Respiratory physiotherapy to prevent pulmonary complications after abdominal surgery: a systematic review.” Chest 2006; 130: 1887–1899. Jensen C, Teijiran T, Lewis C, Yadeger J, Dutson E, Mehran A. Postoperative CPAP and BiPAP use can be safely omitted after laparoscopic Roux-en-Y gastric bypass. Surg Obes Relat Dis. 2008; 4(4): 512–14. Thomas JA, McIntosh JM. “Are incentive spirometry, intermittent positive pressure breathing, and deep breathing exercises effective in the prevention of postoperative pulmonary complications after upper abdominal surgery?” A systematic review and meta-analysis.” Phys Ther 1994; 74(1): 3–16.

118 PREVALENCE AND CHARACTERISTICS OF PSYCHIATRIC ILLNESS IN A LARGE COMMUNITY EMERGENCY DEPARTMENT

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Objectives: Establish the prevalence and characteristics of psychiatric illness among a large community hospital emergency department (ED).

Methods: Retrospective chart review was performed for all patients presenting to a community hospital Emergency Department during two randomly selected months in 2006. Charts were screened for the following: chief complaint, current psychiatric medications, past psychiatric history, disposition, and prescription of psychiatric medications on discharge.

Results: Of the 11,509 patient charts reviewed 28% (3189) screened positive for psychiatric illness. Average age of patients that screen positive was 46.5 (±19.6) and 64% were women. The most common presenting chief complaints were musculoskeletal, gastrointestinal and psychiatric respectively. Seventy-four percent had positive past medical history of one or more psychiatric illnesses, with mood and anxiety disorders being the most common. Benzodiazepines and selective serotonin reuptake inhibitors were the most common current psychiatric medications. While 62% of patients screening positive for psychiatric illness are discharged from the ED, they were more likely to be admitted medically than those screening negative. Males, compared to females that screen positive are younger, more likely to have a psychiatric chief complaint, less likely to be taking psychiatric medication, less likely to be discharged and more likely to require psychiatric admission.

Conclusions: Mental health and physical health have a complex interaction that must be considered in the ED patient population. More than a quarter of patients screened positive for psychiatric illness. Due to lack of availability of outpatient psychiatric services more patients are presenting to the ED with psychiatric illness. It is important to understand the characteristics of this population to properly treat and refer patients.
transported to regional lymph nodes, from which vegetative bacterial forms disseminate to cause systemic disease. Of the cell types resident in the lung, three have been reported to take up B. anthracis: alveolar macrophages (AM), dendritic cells (DC) and alveolar epithelial cells (AEC); however, the major cell type responsible for dissemination of B. anthracis to the peripheral blood is unknown. Originally, AM were thought to be the most important cell for dissemination, although recent evidence suggests a more prominent role for DC and possible involvement of AEC. We hypothesize that B. anthracis spores will be engulfed by, and survive within, cells that play a major role in dissemination, but will either not be taken up or not survive in cells that do not participate in dissemination. We used a human lung organ culture model and fluorescent confocal imaging to quantify the relative uptake and distribution of spores in AM, DC and AEC over time. We distinguished among cell types by location in the tissue, morphology, and positive staining for HLA-DR (AM and DC), cavolin (AEC) and cytokeratin (AEC). We found that spore ingestion was most significant for HLA-DR positive intraalveolar cells with spherical morphology (AM), though HLA-DR positive interstitial cells with dendritic morphology (DC) also ingested a lesser number of spores. Minimal spore uptake was observed in AEC. We also found that spore density declined over time in HLA-DR positive spherical cells. In contrast, HLA-DR positive cells with dendritic morphology maintained spore density over time. These findings are consistent with a rapid clearance of the pathogen from the alveolar space, either through migration out of the lung in AM, or more likely destruction of the pathogen in situ by AM. The findings are also consistent with a purported role for DC as a cell by which the pathogen escapes the human lung.

121
APOCYNIN BLOCKS DELAYED AFTERDEPOLARIZATIONS IN HUMAN ENDOCARDIUM
AK Chaudhary, FL Johnson, JE Davis, Y Suzuki, JB Martins Iowa City, IA. University of Iowa Hospitals and clinic.

Background: Ventricular tachycardia (VT) of focal endocardial origin occurs in humans with coronary artery disease. Triggered activity (TA) due to delayed afterdepolarizations (DADs) is a mechanism of focal VT in dogs with ischemia; both TA and VT may be prevented by blocking reactive oxygen species (ROS). Here, we tested the effects of Apocynin (APO), an NADPH oxidase inhibitor (10-6 M) in diseased human left ventricular endocardium, suggesting APO had little or no direct effect on standard ventricular morphology maintained spore density over time. These findings are consistent with a rapid clearance of the pathogen from the alveolar space, either through migration out of the lung in AM, or more likely destruction of the pathogen in situ by AM. The findings are also consistent with a purported role for DC as a cell by which the pathogen escapes the human lung.

122
THE EFFECT OF QUADRICEPS STRENGTH AND PROPRIOCEPTION ON RISK FOR KNEE OSTEOARTHRITIS
NA Segal, N Glass, DT Felson, M Hurley, M Yang, M Nevitt, CE Lewis, JC Torner Iowa City, IA, Boston, MA, London, United Kingdom, San Francisco, CA and Birmingham, AL. The University of Iowa.

Purpose: Impaired quadriceps strength and knee joint position sense (JPS) have been linked with knee osteoarthritis (OA) cross-sectionally. While neither has been independently associated with incident radiographic knee OA, their combination may mediate risk. The purpose of this study was to determine whether better sensormotor function protects against the development of incident radiographic or incident symptomatic knee OA.

Methods: Participants in the Multicenter Osteoarthritis (MOST) Study, a longitudinal study of adults age 50-79 years at high risk for knee OA underwent bilateral, weight bearing, fixed-flexion radiographs, JPS acuity tests, and isokinetic quadriceps strength tests. The relationships between combinations of the tertiles of sex-specific baseline peak quadriceps strength and mean JPS acuity, and development of incident radiographic (Kellgren-Lawrence grade ≥2) or incident symptomatic knee OA (combination of Kellgren-Lawrence grade ≥2 and daily knee pain or stiffness) at 30-month follow-up were evaluated. Secondary analyses defined JPS as the continuous variance in JPS acuity over the 10 JPS trials, and also assessed the interaction of strength and JPS in predicting each outcome.

Results: The study of incident radiographic knee OA included 1103 participants (age 60.9±7.8 years and BMI 29.3±5.0 kg/m2) and the study of incident symptomatic knee OA included 1551 participants (age 62.1±8.0 years and BMI 29.8±5.4 kg/m2). Greater strength protected against incident symptomatic but not incident radiographic knee OA regardless of JPS tertile (Table I). When JPS and strength were examined separately, high compared with low quadriceps strength was associated with a decreased risk for developing incident symptomatic knee OA (OR=0.43, 95% CI=0.31-0.61, p<0.001) but not incident radiographic knee OA (OR=0.73, 95% CI=0.48-1.14, p=0.17). JPS did not predict risk of incident radiographic or incident symptomatic knee OA. There was no significant relationship between the strength-JPS interaction and the development of incident symptomatic or incident radiographic knee OA.

Conclusion: The finding that quadriceps strength protected against incident symptomatic but not incident radiographic knee OA regardless of JPS tertile suggests that quadriceps strength may be more important than knee joint position sense in mediating risk for development of symptomatic knee OA.

The effect of combinations of baseline quadriceps strength and mean knee joint position sense (JPS) on risk for incident symptomatic knee osteoarthritis

<table>
<thead>
<tr>
<th>Knee Osteoarthritis</th>
<th>JPS Categories (High/Middle/Low)</th>
<th>N of cases/total (%)</th>
<th>OR (95% CI) for outcome, adjusted for baseline age, BMI, knee injury, knee surgery, PASE, and sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>High/High</td>
<td>26/388 (6.7)</td>
<td>0.42 (0.25, 0.72)*</td>
<td></td>
</tr>
<tr>
<td>High/Middle</td>
<td>21/344 (6.1)</td>
<td>0.36 (0.20, 0.66)*</td>
<td></td>
</tr>
<tr>
<td>High/Low</td>
<td>21/320 (6.6)</td>
<td>0.46 (0.26, 0.82)*</td>
<td></td>
</tr>
<tr>
<td>Middle/High</td>
<td>27/331 (8.2)</td>
<td>0.53 (0.32, 0.89)*</td>
<td></td>
</tr>
<tr>
<td>Middle/Middle</td>
<td>20/364 (5.2)</td>
<td>0.57 (0.32, 0.96)*</td>
<td></td>
</tr>
<tr>
<td>Middle/Low</td>
<td>29/345 (8.4)</td>
<td>0.50 (0.29, 0.86)*</td>
<td></td>
</tr>
<tr>
<td>Low/Low</td>
<td>47/544 (13.7)</td>
<td>0.87 (0.55, 1.38)</td>
<td></td>
</tr>
<tr>
<td>Low/Middle</td>
<td>52/337 (15.6)</td>
<td>1.01 (0.64, 1.58)</td>
<td></td>
</tr>
<tr>
<td>Low/Low</td>
<td>55/373 (14.8)</td>
<td>Referent</td>
<td></td>
</tr>
</tbody>
</table>

* Significant predictor of incident symptomatic knee osteoarthritis

123
FACTORs CAUSING THROMBOCYTOPENIA FOLLOWING ON-PUMP CARDIOPULMONARY BYPASS SURGERY AND INCIDENCE OF HEPARIN INDUCED THROMBOCYTOPENIA (HIT) IN POST-OPERATIVE PERIOD
AB Chandra, N Mittal, S Pathak, H Pathak, S Sambidi, A Belur, Y Xu Brooklyn, NY. Maimonides Medical Center.

Introduction: Cardiopulmonary bypass (CPB) surgery is known to be associated with transient thrombocytopenia after the procedure, secondary to platelet consumption. Also, patients are at high risk for Heparin induced thrombocytopenia (HIT) secondary to marked platelet activation, massive Platelet Factor-4 (PF4) release and high dose of heparin use during the procedure. Our aim was to analyze the incidence of thrombocytopenia and HIT in patients undergoing CPB surgery and to evaluate the etiology, characteristics, duration till recovery of thrombocytopenia after CPB.

Methods: The study was a retrospective study that included patients who underwent on-pump CPB (valve surgery or Coronary Artery Bypass Graft surgery). Eligible patients should have platelet count drop of one-third and have at least 1 platelet count equal to or less than 100,000. Recovery after platelet nadir was defined as platelet count increase by 50% from lowest surgery). Eligible patients should have platelet count drop of one-third and have at least 1 platelet count equal to or less than 100,000. Recovery after platelet nadir was defined as platelet count increase by 50% from lowest

Results: 303 consecutive patients were identified from database, and 97 had platelet drop that fit our eligibility criteria and charts of these patients were reviewed. Of 97 patients, 61 (63%) were male and 36 (37%) were female. The
range of age is 41 to 89 years with median age of 69 years. 32% of patients were found to have platelet drop after surgery, starting on day 6, and lasts about 14 days. Treatment salvage Platelet transfusion of 34 to 97%, with median drop of 61%. Platelet recovery starts on day 0 and is completed by day 10, with median of 5 days. All patients received heparin during surgery, and 32 (33%) patients also received platelet transfusion. 30 (31%) patients were suspected of having HIT, and had HIT-PF4 antibody sent, with 3 (3%) being positive. Of these 3 patients, one had negative Serotonin Release Assay (SRA). 7 patients had a second drop in platelet count, starting from days 2–13. Recovery of platelet count after second drop started after 1 day and was completed by 6th day. All 30 patients worked up for HIT had initial platelet nadir but only 3 patients had second platelet nadir. None of HIT positive patients fell into second nadir duration. The patients diagnosed with HIT showed platelet nadir on days 1–3. Confound by postop low platelet count After CPB, with 32% identified in our series. The mean time to platelet drop after CPB is 1.4 days, with full recovery by day 10. Incidence of HIT was lower than that reported in previous studies. Previous studies had shown that incidence of HIT was more common in patients with second drop in platelet count but our study did not confirm that. Diagnosis of HIT should be pursued in patients even if drop in platelet count occurs within first 4 days of CPB.

124 EPIDEMIOLOGY OF VITAMIN D DEFICIENCY IN PATIENTS REFERRED FOR MANAGEMENT OF HYPERLIPIDEMIA & ITS ASSOCIATION WITH STATIN INDUCED MYALGIAS

W Ahmed, N Khan, M Gowda, M Maddipati, N Goldenberg, A Khan, C Glaueck Cincinnati, OH. Jewish Hospital Cincinnati.
We have previously reported that low serum 25 (OH) vitamin D (D2+D3) (≤32 ng/ml) was associated with myalgia in statin-treated patients, and that myalgia was reversed by vitamin D supplementation. However, in we have also found vitamin D deficiency to be very common in asymptomatic patients referred for treatment of hyperlipidemia. Our specific aim was to assess the epidemiology of vitamin D deficiency in patients referred to regional lipid center for the diagnosis and management of hyperlipidemia. Of 942 statin treated patients, 221 (23%) had myalgia at entry and 721 (77%) were asymptomatic. Of the 221 asymptomatic patients, 165 (75%) had low vitamin D <32 ng/ml. Of 721 asymptomatic patients, 439 (61%) had vitamin D <32 ng/ml, x2=13.9, p=0.0002. Thus, nearly 2/3rds of asymptomatic hyperlipidemic patients had low serum vitamin D. Of the 165 vitamin D deficient, myalgic patients, while continuing statins, 88 were given vitamin D (50,000 units/week for 4.3±2.5 months). In these 88 patients, 84 (95%) had no myalgia at their last visit, and 67 (76%) had concurrently normalized vitamin D. We speculate that those asymptomatic hyperlipidemic patients with low pre-treatment vitamin D (<32 ng/ml) will be likely to develop myalgias later during treatment with statins, and should concurrently be treated with vitamin D therapy, to prevent development of myalgia-myositis arising from the synergism of low serum vitamin D and statin therapy. We suggest that all patients with high LDL cholesterol either currently on statins or about to receive statins should be counseled for vitamin D deficiency and those who are deficient should be treated to normalize serum vitamin D to avoid myalgia-myositis.

125 DOES THE PATIENT REALLY NEED BLOOD CULTURES?: ASSESSING PREDICTORS OF BACTEREMIA

B Coburn, A Detsky Toronto, ON, Canada. University of Toronto.
The diagnosis of bloodstream infection (BSI) has important treatment and prognostic implications. Most cultures are negative and a significant proportion of positive cultures grow only contaminants, resulting in increased costs and burden of care. Our objective was to test the accuracy of easily obtained historical, physical examination and laboratory information in the diagnosis of BSI. A literature search produced 14 studies specifically designed to assess the accuracy of clinical variables for the prediction of BSI. Eleven studies were included in the final analysis representing 1545 BSI and 10321 negative blood culture episodes. Individual clinical variables have limited utility for the detection of BSI. Rather, multivariable scores are required in order to produce sufficient accuracy to increase or decrease the likelihood of BSI. The presence of the systemic inflammatory response syndrome (SIRS) is a sensitive indicator of BSI (sensitivity 96%, specificity 47%, positive LR 1.80, negative LR 0.09). There are no published individual variables or multivariable scores that significantly improve on this widely recognized syndrome, however, SIRS may be less sensitive for infective endocarditis than for BSI of other types. Several clinical variables are specific but insensitive for BSI, including hypothermia (sensitivity 13%, specificity 97%, positive LR 4.9), hypotension or shock (sensitivity 27%, specificity 89%, positive LR 2.5). These are only present in rare instances. The presence of SIRS is a sensitive indicator of BSI and the lack of SIRS criteria is sufficient to preclude the need for blood cultures in the absence of an additional indication. Few single variables from patient history, physical examination or laboratory studies provide useful information for the detection of BSI. Until new information arises, blood cultures should be drawn when there is an indication of sepsis or severe infection, or when infective endocarditis is suspected.
Conclusions: Exercise induces KATP expression up-regulation through an AMPK-dependent mechanism. This KATP channel expression regulation is critical for cardiac action potential duration adaptation to increased workload.

127 CLINICAL AND ANGIOGRAPHIC OUTCOMES OF CORONARY PERFORATIONS IN THE CONTEMPORARY INTERVENTIONAL ERA - A SINGLE CENTER EXPERIENCE OVER A DECADE
PA Chandra, N Koradia, D Thekkoott, B Malik, R Frankel, J Shani New York, NY. Maimonides Medical Center.
Background: Coronary perforation during percutaneous coronary intervention is a rare but dreaded complication. The risk factors, optimal management and outcome remain obscure. The objectives of the study were to determine the predisposing factors, optimal management and preventive strategies.
Methods: We retrospectively looked at coronary perforations at our catheterization laboratory over the last 10 years. We reviewed patient charts and reports. Two independent operators in blinded approach reviewed all procedural cineangiograms. Data was analyzed by simple statistical methodology.
Results: A total of 22 patients were found eligible for the study. Their demographic and angiographic features are as follows: PLEASE REFER TABLE Management: 9 patients were treated conservatively and 6 patients were treated with prolonged balloon inflation. 6 patients were treated with polytetrafluoroethylene (PTFE) - covered stents and 3 patients required emergency pericardiocentesis. 1 patient required emergency CABG. No deaths were reported.
Conclusions: 1. Coronary perforations are rare but potentially fatal events. 2. Male sex, hypertension, small vessel diameter, high balloon-artery ratio, vessel calcification and presence of myocardial bridging appear to be possible risk factors. 3. Most perforations can be treated conservatively or with prolonged balloon inflations using perfusion balloons. 4. Use of PTFE-covered stents could be a life-saving measure in cases of large perforations.

Demographic and angiographic features of patients who developed coronary perforations over last 10 years at our cardiac catheterization laboratory

<table>
<thead>
<tr>
<th></th>
<th>Age (Mean)</th>
<th>Age (Range 32-82)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male Sex %</td>
<td>72.72</td>
<td></td>
</tr>
<tr>
<td>DM %</td>
<td>36.36</td>
<td></td>
</tr>
<tr>
<td>HTN %</td>
<td>81.81</td>
<td></td>
</tr>
<tr>
<td>Previous PCI %</td>
<td>36.36</td>
<td></td>
</tr>
<tr>
<td>Previous CABG %</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Proximal LAD</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Mid LAD</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Distal LAD</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Left Circumflex</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Proximal RCA</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Mid RCA</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Distal RCA</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Diagonal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre treatment stenosis range</td>
<td>65% - 99%</td>
<td></td>
</tr>
<tr>
<td>Pre treatment lesion severity A</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Pre treatment lesion severity B</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Pre treatment lesion severity C</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Reference vessel diameter (mm)</td>
<td>1.5 - 4.0</td>
<td></td>
</tr>
<tr>
<td>Hydrophilic wire</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Hydrophobic wire</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Balloon inflation related perforation</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Stent related perforation</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Mean Balloon to Artery Ratio</td>
<td>1.23</td>
<td></td>
</tr>
<tr>
<td>Mean Inflation Pressure in mm Hg</td>
<td>13.07</td>
<td></td>
</tr>
<tr>
<td>Mean Number of inflations</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Mean Balloon length in mm</td>
<td>16.714</td>
<td></td>
</tr>
<tr>
<td>Calcification</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Myocardial Bridging</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Death</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Note: 8 perforations were wire related and 14 perforations were related to balloon inflation (Total number 22)

128 INTEGRIN β4 IS ESSENTIALLY INVOLVED IN SIMVASTATIN-MEDIATED ATTENUATION OF MURINE ACUTE LUNG INJURY
W Chen, S Samman, B Mathew, JR Jacobson, JG Garcia Chicago, IL. University of Chicago.
Rationale: We previously characterized the protective effects of simvastatin, an HMG CoA-reductase inhibitor, in a murine model of lipopolysaccharide (LPS)-induced acute lung injury (ALI). In addition, we have conducted gene expression studies which confirmed the marked upregulation of integrin β4 in simvastatin-treated endothelial cells (EC), a class of proteins recognized to be involved in EC inflammatory responses. Although little is known about integrin β4 in EC signaling, we hypothesized that integrin β4 may be critical to vascular protection conferred by simvastatin in ALI and therefore represents a potentially novel ALI target.
Methods/Results: To investigate the potential anti-inflammatory role of integrin β4, IL-6 and IL-8 were measured in the media of EC treated with simvastatin (5 μM, 16 h) and either an integrin β4 neutralizing antibody (20 ng/ml, 2h) or a control (IgG) antibody prior to the administration of LPS (500 ng/ml, 4 h). Simvastatin alone significantly attenuated LPS-induced IL-6 and IL-8 expression which was reversed by pretreatment with the integrin β4 neutralizing antibody. Further, the integrin β4 neutralizing antibody (1 mg/kg, 4 h, IV) abrogated the protection by simvastatin (20 mg/kg, 16 h, intraperitoneal) in a murine model of LPS-induced ALI (1.25 mg/kg, 24 h, intratracheal) reflected in bronchoalveolar lavage (BAL) protein, total cell counts and BAL inflammatory cytokines. Our data suggest integrin β4 is a critical component in the anti-inflammatory effects of statins in murine ALI and implicate integrin β4 as a novel therapeutic target in ALI.

129 NICOTINE EXPOSURE DOES NOT CONFER SURVIVAL ADVANTAGE IN PATIENTS WITH SEPSIS SETH M GREGORY MD, JIO JOHN MD, GARY T KINASEWITZ MD
SM Gregory, J John, GT Kinasewitz Oklahoma City, OK. University of Oklahoma Health Sciences Center
Purpose: Sepsis results from the complex interaction between the microorganism and the host inflammation and coagulation cascade. Nicotine has been shown to interfere with production of inflammatory mediators through its interaction on the nicotinic and muscarinic receptors on peripheral blood cells. In vitro studies have shown that nicotine inhibits NF-κb and TNF production. We hypothesized that cigarette smoking would confer a survival advantage in patients who present with a diagnosis of sepsis.
Methods: The smoking status of patients with severe sepsis was determined by retrospective review of our database. The clinical characteristics and outcomes of smokers (current & previous) were compared to non smokers.
Results: A history of smoking was available in 61 patients. Of these 61 patients 33 were grouped as smokers and 28 as non smokers. There was no significant difference in their age 56±12.1 vs. 59.3 ± 16.9, APACHE II 21 ± 6.3 vs. 24 ± 7.3, white cell count 14.7 ± 11.5 vs. 15.4 ± 11.3 and need for mechanical ventilation 58% vs. 61% between smokers and non smokers respectively. Pneumonia as the etiology of sepsis was slightly higher in smokers at 52% compared to 32% in non smokers (p=0.2). The ICU mortality was similar in the smokers (36.4%) and non smokers (35.7%) and the length of stay in the ICU between the groups was not significantly different.
Conclusion: Smoking and thereby exposure to nicotine prior to admission with a diagnosis of sepsis did not confer a survival advantage.

130 RARE GENOMIC VARIANTS CONTRIBUTE TO SYSTOLIC BLOOD PRESSURE VARIATION IN THE FRAMINGHAM HEART STUDY
B Kerner, BO Muthien Los Angeles, CA. UCLA
The physiology and patho-physiology of blood pressure (BP) regulation is highly complex. The identification of genomic variants associated with variation in BP would likely enhance the understanding of this regulatory...
network. Genome-wide association studies in ten thousands of individuals have already identified some genomic variants that might play a role. However, given the limited genetic variation it is highly likely that populations have different patterns of SNPs and heterogeneous. Genetic factors that carry risk for BP elevation in early life might be different from those influencing high BP later in life. Physiologic BP variation in normal individuals might have different genetic contributions than those found in high risk individuals and risk factors might differ in men and women. Growth mixture modeling is a less explored method in genetic research to address unobserved heterogeneity in population samples. Here, we applied this technique to longitudinal data of the Framingham Heart Study. We examined systolic BP measures in 1060 males from 692 families at four different time points spanning thirty years of observation. We detected three subclusters, which varied significantly in their developmental trajectories over time. The first class consisted of 60 high-risk individuals characterized by elevated BP early in life and a steep increase over time. The second group of 131 individuals displayed first normal BP, but reached high BP values late in their life time. The largest group of 869 individuals could be considered a normative group with normal BP on all exams. In order to identify genetic modulators for this phenotype we tested the genome-wide association between class membership probability and single nucleotide polymorphisms using the Affymetrix 500k array. In unrelated individuals (one member per family) Class 1 membership probability was significantly associated with the C allele of the SNP rs10449067 located in the gene Netrin-G1 Precursor (NTNG1) on chromosome 1p13.3 under the recessive model (correlation trend p=1.22×10^-10). All individuals homozygote for this SNP allele were found to be members of Class 1 and homozygotes for the C allele were absent in all other A, C, T, G SNPs in the non-coding regions, and rare SNPs were present only in members of one latent class and absent in all others. The rare C allele of SNP rs1445404 located in the third exon of the gene EYA (eyes absent homolog 1 in Drosophila) was present in only four individuals, one homozygote and three heterozygotes from four different families (6.6% of the individuals in Class 1) (correlation trend p=1.39×10^-13; OR=8.1). This miss-sense mutation in exon 3 changes an Alanine to a Proline at amino acid position 20 of the protein with likely consequences for the protein structure and folding of the protein. Mutations in EYA4 were found in patients with Brachio-Oto-Renal syndrome, as well as in individuals with isolated renal malformations (Hoskins BE, 2008; Orten DJ, 2008). We demonstrate that stratification of population samples in high risk and low risk groups based on longitudinal development over time can facilitate the identification of rare genetic risk factors for common complex disorders.

### 131 STEM CELLS CULTURED FROM OMENTUM-INDUCED REGENERATING LIVER CAN BE DIFFERENTIATED TO HEPATOCYTES

N Pancholi, KP Gudeethula, J Patel, M Kraus, JA Arruda, G Dunea, AK Singh

Chicago, IL. Hektoen Institute of Medicine.

Hepatocytes from adult liver are difficult to maintain in culture and therefore there is need for a stem cell that can be easily maintained in culture and differentiated to hepatocytes for use in cell transplantation therapies. In earlier studies we have shown that making a small wedge cut (resection injury) in the liver and activating the omentum caused fusion of the omentum to the liver resulting in a vigorous regeneration of the liver to a mass 150% of the original liver mass (Singh et al., 2009). We have also shown that culture of such a regenerating liver yielded cells with a mesenchymal stem cell phenotype (CD90+, CD59+, CD45-) that could be maintained in culture indefinitely. These cells, called regenerating liver stem cells (RLSC), expressed both adult (WT-1, CXCR4) and embryonic (Oct-4, Nanog) stem cell markers and secreted high levels of growth factors (Pancholi et al., 2009). RLSC also expressed albumin (a marker of mature hepatocytes), and CD133, a marker of hepatic stellate cells; a liver stem cell. Further we wanted to study the ability of RLSC to differentiate into hepatocytes in culture. Here we show that when RLSC were layered on an extracellular matrix substratatum (Matrigel®) and incubated in medium containing hepatocyte growth factor (HGF) they differentiated into round hepatocyte-like cells within 2 weeks. These differentiated cells appeared to be attached to each other forming primitive hepatic cords in culture. Control (undifferentiated) cells cultured without matrigel and HGF did not differentiate to hepatocyte-like cells. On immunostaining, the differentiated hepatocyte-like cells were strongly positive for albumin as well as for α-fetoprotein. To assess whether these differentiated cells had gained hepatocyte-specific functions, we tested a) their ability to synthesize and secrete albumin in culture and b) their efficiency for nitrogen catabolism as judged by urea production. SDS-PAGE showed a prominent band of albumin in the media of the differentiated cells versus an undetectable band in the media of undifferentiated cells. Differentiated cells secreted albumin in the cell media at the rate of 19 ± 0.4 μg/hr/million cells, which was 50 times higher than that in the undifferentiated cells. Differentiated cells produced urea at a rate of 20 ± 0.9 μmoles/hr/million cells, which was twice than that in the undifferentified cells. When fluorescently labeled RLSC were systemically injected in rats immediately after liver resection injury, the cells localized to the injured liver at the edge of the resection site within 24 hours. Labeled cells were not visible in the uninjured areas of the liver showing that these cells specifically recognized and engrafted to an injured site in the liver. Further, from the shape of the individual labeled cells and their organization into cord-like structures at the injured site, these cells appeared to differentiate into liver cells and integrate into the native liver tissue. The RLSC were unipotent because attempts to differentiate them in-vitro along adipogetic, and osteogenic lineages were unsuccessful. The ability to maintain a hepatocytic stem cell in culture is clinically significant because it has the potential for use in cell transplantation therapies.

### 132 THE EPIDEMIOLOGY OF PULMONARY FIBROSIS IN THE VETERANS POPULATION

AK Gerke, GW Hunninghake

Iowa City, IA. University of Iowa.

Rationale: It is important to identify risk factors for decreased survival in patients with pulmonary fibrosis. Prior studies are limited by small sample size, variable controls, and study of subgroup populations. Further, although overall survival of patients with idiopathic pulmonary fibrosis is well established, co-morbidities that affect survival have not been defined. In addition, survival of a population inclusive of all types of pulmonary fibrosis has not been described. The objective of this study was to determine survival in a population inclusive of all pulmonary fibrosis patients and determine if certain comorbidities or patient characteristics contribute to prognosis.

Methods: A case control study was conducted using the Veterans Affairs (VA) National Patient Care claims database. ICD-9 codes (515, 516) were used to identify cases with pulmonary fibrosis and three control populations between 2001–2007. Cases were included if they had one or more primary care visits in the year prior to diagnosis of pulmonary fibrosis. Three control groups were selected: ‘typical VA users’ frequency matched for age, year of diagnosis, and gender (N=78,917), patients with lung cancer (N=136,579), and patients with rheumatoid arthritis (N=68,031). Survival analysis was done using Kaplan-Meier estimates. Log-rank tests were used to compare survival estimates. Comorbidities associated with survival were determined by Cox proportional hazards regression.

Results: Overall median survival is 3.9 years (confidence interval (CI) 3.8–4.1) in pulmonary fibrosis patients, 8.0 years (CI 7.9–8.1) in typical VA users matched for age and gender, 8.1 years (CI 8.0–8.1) in rheumatoid arthritis patients, and 1.1 years (CI 1.0–1.1) in lung cancer patients (Figure 1). Median survival in women is 6.3 years (CI 5.2–9.0). Pulmonary fibrosis patients have decreased survival as compared to typical VA users (p<0.0001) and rheumatoid arthritis patients (p<0.0001), but increased survival compared to lung cancer patients (p=0.0001). Adjusted for race and age, congestive heart failure (hazard ratio (HR) 1.6, CI 1.5–1.6), renal disease (HR 1.4, CI 1.3–1.5), weight loss (HR 1.4, CI 1.3–1.5), pulmonary hypertension (HR 1.3, CI 1.2–1.4), arteriosclerosis (HR1.3 CI 1.2–1.3), and hypertension (HR 1.2, CI 1.2–1.3) had the highest association with decreased survival in patients with pulmonary fibrosis. Conversely, female gender (HR 0.71, CI 0.61–0.84), obstructive sleep apnea (OSA) (HR 0.37, CI 0.29–0.46) and obesity (HR 0.65, CI 0.62–0.68) are associated with improved survival.

Conclusions: The mortality of patients with all types of pulmonary fibrosis is high in the Veterans population and very similar to the natural history of idiopathic pulmonary fibrosis. Females have improved survival even after adjusting for age and comorbidities. Comorbidities related to cardiovascular and coronary artery disease contribute to decreased survival. Additionally, weight loss is associated with poorer survival, consistent with other chronic lung diseases such as chronic obstructive pulmonary disease (COPD). Interestingly, OSA is markedly associated with improved survival.
Presumably, in the VA population, many of these patients are likely to have been treated, which may imply that treatment offers survival benefit in this population.

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**ABSOLUTE FLOW-MEDIATED DILATION (FMDmm) AND NITRIC OXIDE DEPENDENT VASODILATOR CAPACITY OF THE BRACHIAL ARTERY, BUT NOT PERCENT FLOW MEDIATED DILATION(FMD%), ARE INCREASED IN TRAINED ATHLETES**

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**Background:** Exercise training induces alteration in both muscular artery structure and dynamic function. Increased laminar shear stress with exercise training is known to induce adaptive arterial remodeling, causing an increase in luminal diameter while wall thickness remains normal. While short term exercise training improves endothelial function, recent data suggest exercise-induced improvements in endothelial function, measured as percent flow-mediated dilation (FMD%) in the brachial artery, are short-lived, with FMD% returning to pre-training levels within two months. These data support a hypothesis that exercise training initially induces improvements in endothelial function which regress to baseline as structural remodeling occurs. Given impaired endothelial function in the brachial artery predicts increased cardiovascular risk, the concept that chronic exercise training does not lead to long-term improvements in endothelial function is difficult to reconcile with the known cardiovascular benefits of exercise. Vessel diameter has a strong, inverse correlation with FMD%. Increased luminal diameter secondary to arterial remodeling in the setting of chronic exercise training may mask advantageous functional adaptations of the vascular endothelium if FMD% is used as the sole metric of endothelial function.

**Hypothesis:** Chronic exercise training is associated with favorable alterations in dynamic vessel function, including 1) an overall increase in flow-mediated dilation to shear stress with flow-mediated dilation expressed as an absolute diameter difference (FMDmm) rather than a percentage increase from baseline (FMD%) 2) an increase in absolute nitric oxide (NO)-dependent vasodilatory capacity to saturating doses of an NO donor.

**Methods:** We included 12 trained adult athletes (42% female) and 31 untrained, sedentary adult controls (37% female) for this cross-sectional study. Baseline brachial artery diameter, FMD%, and FMDmm were measured using standard brachial artery reactivity testing techniques using high resolution ultrasound. Briefly, the brachial artery was imaged in longitudinal cross-section at baseline, and blood flow to the forearm was interrupted for 5 minutes with a forearm cuff. Following cuff release, the maximal brachial artery diameter was measured using standard procedures. Following a rest period, overall vessel responsiveness was assessed by measuring the brachial artery diameter prior to and following administration of 0.4 mg of sublingual nitroglycerin.

**Results:** Brachial artery diameter was significantly larger in athletes relative to controls (5.3±0.8 cm vs. 3.7±0.7 cm, P<0.0001). FMD% did not differ between the two groups (6.2±4.5% for athletes vs. 6.6±1.9% for controls, P=0.68). However, FMDmm was significantly greater in the athletes relative to controls (0.35±0.12 mm vs. 0.24±0.05 mm, P=0.0001). Further, the absolute increase in brachial artery diameter following nitroglycerin was significantly higher in athletes relative to controls (1.21±0.22 mm vs. 0.94±0.22 mm, respectively, P=0.002). There were no significant differences in resting or peak shear stress.

**Conclusions:** Contrary to prior work using FMD% as a metric of vascular function, chronic exercise training is associated with favorable alterations in dynamic vascular function, including improved endothelium-dependent vasodilation to shear stress and increased maximal dilation capacity to NO. FMD% is not a reliable metric for following dynamic alterations in vascular function in the setting of chronic exercise training. FMDmm and NO-dependent vasodilator capacity are better indices of vascular function in the setting of chronic exercise training.

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**INVESTIGATING THE PATHOGENIC ROLE OF NOTCH SIGNALING IN T CELL-MEDIATED IMMUNE DISORDERS**

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Notch signaling is a well-conserved cell-to-cell communication pathway that plays multiple functions in development and tissue homeostasis. Notch ligands of the Jagged or Delta-like families interact with one of four mammalian Notch receptors (Notch1-4). Ligand-receptor interaction leads to cleavage of the receptor by the gamma secretase complex, followed by migration of the intracellular Notch domain into the nucleus and transcriptional activation of target genes. In the hematopoietic system, Notch was first identified for its essential function at early steps of T cell development in the thymus. However, Notch ligands and receptors are also engaged in mature T cells during antigen-driven immune responses, exerting an emerging spectrum of important functions.

Our laboratory is investigating the role of Notch signaling in alloimmune T cell responses against foreign tissue antigens, either in the setting of allogeneic hematopoietic stem cell transplantation (allo-HSCT) or after solid organ transplantation. Allo-HSCT is a successful cancer therapy that functions by eliminating malignant cells via donor T cells. However, its success is limited by life-threatening graft-versus-host disease (GVHD). Novel immunomodulatory approaches are needed to effectively control GVHD. Using genetic and pharmacological approaches to block Notch signaling, we found that Notch is a potent regulator of T cell activation, differentiation and function during acute GVHD. Inhibition of canonical Notch signaling in donor T cells markedly reduced GVHD severity and mortality in several mouse models of allogeneic HSCT. Notch-deprived T cells were able to proliferate and expand; however both their cytokine response and induction of selected effector molecules in CD4+ and CD8+ T cells were defective. Notably, Notch-deprived alloreactive T cells retained potent anti-leukemia activity, leading to improved overall survival of the recipients. These results differ from all past reports of Notch’s function in mature T cells. In addition, they identify Notch signaling as an essential regulator of pathogenic T cell responses and a promising therapeutic target following allogeneic HSCT.

We are currently investigating the cellular and molecular mechanisms of Notch’s action in alloreactive T cells and in other T cell-mediated disorders characterized by persistent exposure to systemic antigens. We anticipate that our work will identify novel ways to harness the therapeutic benefits of Notch manipulation in the immune system.