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ELASTIN HAPLOINSUFFICIENCY RESULTS IN PROGRESSIVE AORTIC VALVE MALFORMATION AND LATENT DISEASE IN A MOUSE MODEL

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Objective: Elastin is a ubiquitous extracellular matrix (ECM) protein that is highly organized in heart valves. Because elastic fiber abnormalities are a central feature of degenerative valve disease, we hypothesized that elastin deficient mice would manifest viable heart valve disease. The purpose of the study was to analyze valve structure and function in elastin insufficient mice (*Eln+/−*) at neonatal, juvenile, adult and aged adult stages. **Methods and Results:** At birth, histochemical analysis demonstrated normal ECM organization in contrast to the aorta. However, at juvenile and adult stages thin elongated valves with ECM disorganization, including elastin fragment infiltration of the annulus, were observed. The valve phenotype worsened by the aged adult stage with overgrowth and proteoglycan replacement of the valve annulus. Ultrastructural analysis identified early changes in the annulus region. Using immunohistochemistry, *Eln+/−* mice demonstrated increased valve interstitial cell (VIC) proliferation at the neonatal stage (anti phospho-Histone H3) and varied regional VIC activation at early and late stages (anti alpha-smooth muscle actin and anti nonmuscle myosin heavy chain, SMemb). Gene expression profile analysis (Affymetrix, MOE 430 2.0) identified decreased TGF-β mediated fibrogenesis signaling in *Eln+/−* valve tissue, and further immunohistochemistry demonstrated decreased TGF-β signaling (anti phospho-Smad2). Transthoracic echocardiography (VisualSonics Vevo 770) was used to assess in vivo valve function. Juvenile *Eln+/−* mice demonstrated normal valve function, but progressive valve disease (predominantly aortic regurgitation) was identified in 17% of adult and 70% of aged adult *Eln+/−* mice. **Conclusions:** These results identify the *Eln+/−* mouse as a model of latent aortic valve disease and establish a role for elastin dysregulation in valve pathogenesis.

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EFFECTS OF α-SMOOTH MUSCLE ACTIN MUTATIONS ASSOCIATED WITH CONGENITAL AND ACQUIRED HEART DISEASE

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Aneurysms and dissections of the thoracic aorta are the 15th leading cause of death in the United States. Recently, mutations in alpha-smooth muscle actin (*ACTA2*) were identified to cause autosomal dominant thoracic aortic aneurysms. The biochemical basis for the actin mediated aortopathy is unknown. We hypothesized that *ACTA2* mutations contribute to aortic disease by inducing actin filament instability. We engineered four of the known human mutations, N115T, R116Q, R147C, and R256C, into yeast actin, which is 94% similar to human alpha-smooth muscle actin to investigate their effect on *in vivo* and *in vitro* actin function. Cells expressing three of the four mutant actins as the sole actin in the cell were viable but R147C was lethal. In three of the four viable mutants tested, only N115T had growth problems which included slower doubling times and growth defects with elevated temperature, increased salt concentration, and utilization of glycerol as the sole carbon source. *In vitro*, we assessed biochemical changes in the actin monomer. N115T, R116Q and R256C mutations conferred decreased thermostability with lower melting temperatures than wild type actin. Mutations also had significantly slower nucleotide exchange rates. We then interrogated filament dynamics. Allele specific polymerization defects were seen in each of the three viable mutants, including abnormalities in nucleation, elongation and critical concentration. R116Q had the most dramatic polymerization defect. These data indicate filament instability which is supported by previously published findings of fewer and shorter alpha-smooth muscle actin filaments in aortic vascular smooth muscle cells from affected patients (Guo et al., 2007). To interrogate filament stability, we monitored filament response to cofilin, a major regulatory actin binding protein that severs filamentous actin and sequesters actin monomers. R116Q and R256C are both hypersensitive to cofilin, again with R116Q being the most susceptible. In contrast, N115T was hyposensitive to cofilin severing exposing a difference in regulation by actin binding proteins. These are the first studies describing the biochemical properties of human alpha-smooth muscle actin mutations. The allele specific defects indicate varied pathogenic mechanisms of aortic disease due to actin mutations. Our data highlight that the yeast model is an excellent system to investigate the role of alpha-smooth muscle actin in familial thoracic aortic aneurysm and dissection.

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EXPRESSION OF HUMAN cMyBP-C RESTORES CONTRACTILE FUNCTION IN MOUSE ECT DEFICIENT IN MOUSE cMyBP-C, WHILE EXPRESSION OF THE cMyBP-C^{E258K}HCM-CAUSING MUTATION IN ECT ACCELERATES CONTRACTILE KINETICS

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Mutations in cardiac myosin binding protein C (cMyBP-C), encoded by the *MYBPC3* gene, are common causes of hypertrophic cardiomyopathy (HCM) in humans. Even though the *MYBPC3* E258K missense mutation is among the most prevalent HCM-causing mutations, the mechanism through which it causes disease remains unclear. We developed a novel neonatal murine 3D engineered cardiac tissue (ECT) model and previously presented data showing that *Mybpc3* ablation (*Mybpc3*$^{-/-}$) accelerates the kinetics of contraction and relaxation in the absence of hypertrophic remodeling in ECT. In order to study the effect of HCM-causing mutations on contractile function of ECT, we prepared an adenoviral mediated gene delivery system that facilitates expression of wild type and mutant human cMyBP-C (HcMyBP-C^{WT} and HcMyBP-C^{E258K}) in *Mybpc3*$^{-/-}$ ECT. The expression level of HcMyBP-C^{WT} in *Mybpc3*$^{-/-}$ ECT is similar to that of endogenous mouse cMyBP-C in wild type ECT. Functionally, the kinetics of contraction and relaxation in HcMyBP-C^{WT} ECT were restored to levels similar to that observed in wild type ECT (rate of contraction: 50.9±4.2 ms vs. 50.7±2.2 ms time to F_{max}; rate of relaxation: 40.7±4.6 ms vs. 40.6±3.0 ms time to 50% force decay). These data indicate that adenoviral mediated expression of human wild type cMyBP-C in *Mybpc3*$^{-/-}$ ECT restores contractile function to levels indistinguishable from wild type ECT. Adenoviral mediated gene transfer of HcMyBP-C^{E258K} ECT, however, resulted in faster rate of contraction than in HcMyBP-C^{WT} ECT (42.6±1.1 ms vs. 50.9±4.2 ms time to F_{max}; $p<0.001$). The rate of relaxation was also faster in *MYBPC3*^{E258K} than in *MYBPC3*^{WT} ECT (26.0±1.6 ms vs. 40.7±4.6 ms time to 50% force decay; $p<0.001$). These findings indicate that the E258K mutation accelerates the rates of contraction and relaxation in ECT and that this mutation may primarily affect heart function by diminishing systolic ejection time in patients suffering from HCM. We conclude that adenoviral mediated gene transfer into ECT can be used to study the contractile effects of HCM-causing mutations. The ECT model allows the study of mutations in the absence of hypertrophic remodeling, and provides a faster and economical screening tool for defining the functional phenotype of important human HCM-causing mutaitons.

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ANGIOTENSIN II MEDIATES CARDIAC REMODELING IN THE LATE GESTATION FETAL HEART

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Background: Angiotensin II (ANG II) stimulates the ANG type 1 (AT1) receptor to exert multiple effects on the fetal cardiovascular system, including stimulating growth of the fetal heart. However, the mechanisms by which ANG II stimulates fetal heart growth, and whether this involves cardiomyocyte hypertrophy and/or hyperplasia, are not known. **Objective:** To determine whether ANG II stimulates cardiomyocyte hypertrophy and/or hyperplasia *in utero* and to identify molecular mechanisms mediating the response using microarray analysis and gene expression profiling. **Methods:** Twin gestation pregnant sheep were used for the study. For determination of cardiomyocyte response, one fetus from each ewe (n=4) was catheterized and received a continuous infusion of ANG II (50ug/kg/min), the twin served as a control. At 131 d gestation (term 145 d), fetal hearts were removed, perfused and myocytes isolated. For microarray analysis (Agilent sheep gene expression array), a separate group of pregnant ewes were used with one fetus receiving a continuous infusion of ANG II (n=4, dose as above) or the AT1 receptor antagonist losartan (n=4, 20mg/kg/d); non-catheterized twin pairs served as controls. Comparisons were made by ANOVA or t-test; significance defined as $p < 0.05$. Microarray data were normalized using Bioconductor and ComBat software. **Results:** ANG II resulted in a significant increase in heart mass (5.44±0.28 g/kg fetal weight) compared to control (4.38±0.27g/kg) and losartan (3.96±0.07g/kg) animals. The percent of mononucleated cardiomyocytes was significantly greater in the right ventricle (RV) from ANG II animals (84±5%) compared to controls (75±5%). A similar though not significant pattern was seen in the left ventricle (LV). ANG II significantly increased cardiomyocyte area in LV and RV mononucleated and LV binucleated cells. Microarray analysis, performed on LV RNA from ANG II, losartan and control fetuses, yielded 1507 significant genes (15,208 probes on chip) after correction for false positive discovery rate. Of these, 168 were known genes, the remaining being ESTs. Expression profiling and pathway analysis is ongoing. **Conclusion:** ANG II produces an increase in fetal cardiac mass via cardiomyocyte hypertrophy and probably hyperplasia. Microarray analysis should yield novel information regarding the molecular mechanisms of ANG II mediated cardiac growth in the fetus.

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PROXIMAL SIGNALLING IN ENDOTOXIN STIMULATED NEUTROPHIL PRIMING

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Background: Neutrophil (PMN) function is required for microbial killing, but is regulated to allow the response of the cell to be appropriate to the stimulus. Priming is a regulatory mechanism to allow cellular responses to be enhanced by previous interaction with a priming agent. Although priming is a necessary component of the host response to some infectious stimuli, priming has no physiologic advantage in many inflammatory responses. Many diverse stimuli elicit PMN priming including endotoxin. Although endotoxin signaling via toll-like receptor-4 (TLR4)-myeloid differentiation protein-2 (MD2) pathway has been well-characterized in many cell types, the proximal components of the cell signaling pathway in PMN priming by endotoxin have not been fully defined. **Purpose:** We hypothesize that PMN priming by endotoxin is dependent on lipooligosaccharide binding protein (LBP), CD14 and occurs in a TLR-4-dependent manner. **Methods:** Human PMNs were isolated and stimulated with endotoxin (meningococcal lipooligosaccharide; LOS) presented in combination with LBP or as a conjugate with either CD14 or MD-2. NADPH oxidase activity was assessed in primed and unprimed cells by lucigenin-enhanced chemiluminescence. Upregulation of intracellular protein stores was assessed by flow cytometry. **Results:** PMNs stimulated with LOS:CD14 complex produced a 10-fold increase in the primed NADPH oxidase-dependent generation of reactive oxygen species (ROS) when compared to an equal concentration of LOS in the presence of LBP. Addition of excess sCD:14 to LOS + LBP condition did not improve ROS production. LOS:MD-2 stimulation elicited very low-level priming compared to LOS:CD14 or LOS + LBP. Anaerobic conditions blunted priming in response to LOS:CD14, LOS + LBP, and LOS:MD-2. LOS:CD14 priming elicited greatest levels of cell surface CD11b by flow cytometry. **Conclusions:** Priming of PMN by endotoxin presented as a complex with CD14 elicits the most potent enhancement of ROS production and mobilization of intracellular protein stores. Our data suggest that presentation of LOS in the presence of LBP diverts some of the endotoxin to a non-inflammatory pathway. Failure of LOS:MD-2 complex to prime suggests that all TLR4 on the cell surface exists in a dimerized state with MD-2. Priming by endotoxin in oxygen-dependent.

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DECREASED SHP-1 TYROSINE PHOSPHATASE ACTIVITY MAY ENHANCE FC-RECEPTOR-MEDIATED PHAGOCYTOSIS IN NEONATAL MONOCYTE-DERIVED MACROPHAGES

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Background: Resident macrophages represent a first line of defense against invading microbial pathogens. Present in specific tissues prior to the arrival of PMN, resident macrophages are capable of utilizing both the innate and adaptive arms of the immune system to rid the host of pathogens. Phagocytosis of IgG-opsonized targets is mediated by Fc receptors on the macrophage surface. Fc receptor phagocytosis is a complex series of events involving the coordinated efforts of tyrosine kinases and phosphatases and cytoplasmic adaptor proteins. **Objective & Methods:** The objective of this study was to investigate the signaling mechanisms the govern macrophage phagocytosis via FcγRIIa receptors. Monocytes were isolated from adult or cord blood and were adhesion-differentiated into macrophages. Phagocytosis functional studies were performed with fluorescent IgG-coated latex beads. Investigation of Fc receptor signaling was done following antibody crosslinking of surface FcγRIIa receptors. All blood samples were obtained in accordance with the guidelines of the Institutional Review Board at Saint Louis University. **Results:** In this study, we found that ingestion of IgG2-coated latex beads was enhanced in neonatal MDM although the surface expression of FcγRIIa was similar between the two cell types. Crosslinking of surface FcγRIIa receptors for 2 minutes enhanced tyrosine phosphorylation of several proteins in both adults and neonatal MDM followed by a reduction in tyrosine phosphorylation after 5 minutes of crosslinking in adult MDM only. Crosslinking FcγRIIa receptors for 2 minutes induced a significantly larger increase in relative SHP-1 activity in adults as compared to neonatal MDM. The protein expression of SHP-1 was similar in adults and neonates; however baseline neonatal SHP-1 relative enzyme activity was about half that of adults. The cytoplasmic adapter protein Cbl binds to SHP-1 and FcγRIIa and is purported to negatively affect FcγRIIa -meditated phagocytosis. Using co-immunoprecipitation assays we show that SHP-1 and Cbl in neonatal MDM do not associate to the same extent as observed in adult MDM. **Conclusion:** Our data suggest for the first time that the observed enhanced phagocytic capacity of neonatal MDM may be related to decreased SHP-1 activity and inhibition of SHP-1/Cbl protein complex formation downstream of FcγRIIa crosslinking.

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NORMAL LYMPHOCYTE DEVELOPMENT REQUIRES THE ACTIN-BUNDLING PROTEIN L-PLASTIN

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Purpose: Actin cytoskeletal elements regulate T and B lymphocyte motility and activation. L-plastin (LPL) is an actin-bundling protein expressed only in hematopoietic cells. LPL stabilizes actin structures, such as lamellipodia. Following stimulation with chemoattractants, LPL is recruited to the leading edge of polarized T cells. Intact lymphocyte motility is required for normal development. We therefore tested the hypothesis that lymphocyte motility and consquent development would depend upon LPL. **Methods and Summary of Results:** We analyzed T and B cell motility, development and function in mice genetically deficient for LPL. Initial studies focused on T cells. Flow cytometric analysis of T cell subpopulations revealed increased numbers of phenotypically mature single-positive thymocytes and reduced numbers of peripheral mature T cells in LPL-deficient mice, consistent with defective thymic emigration. Defective thymic emigration was confirmed by intrathymic FITC injection. Reduced thymic emigration resulted from diminished chemotaxis of both immature and mature T cells towards the chemoattractants CCL19 and sphingosine-1-phosphate, both of which regulate thymic egress. CCL19 signals through the G-protein coupled receptor CCR7. Proximal signaling through CCR7 was intact in cells deficient for LPL, as assessed by Rac activation and F-actin polymerization. However, CCR7-mediated polarization of LPL-deficient T cells was reduced. Ongoing experiments extend these findings to B cell development. B cells from LPL-deficient mice also exhibited defective chemotaxis towards CXCL12 and CXCL13, chemokines critical for B cell development. LPL-deficient mice demonstrated a 40% reduction in the number of total splenic B cells and an 80% reduction in splenic marginal zone B cells. A preliminary study revealed reduced antibody responses to heat-killed *Streptococcus pneumoniae*, indicating a critical role for LPL in adaptive immune function. **Conclusions:** We describe a novel requirement for LPL in the polarization of lymphocytes in response to chemoattractants, and thus a critical role for LPL in lymphocyte motility, development and function.

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RNase 7 Expression in the Human Kidney and Urinary Tract

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Background: Urinary tract infections (UTI) are a common bacterial infection. While many consider the urinary tract sterile, little is known how the body maintains sterility. Recent studies stress the importance of antimicrobial peptides (AMP) in preventing infection. Ribonuclease 7 (RNase7) is an important AMP that has been studied in the skin. RNase7's role in the epithelium of the urinary tract is unknown. **Objective**: To characterize gene and protein expression of RNase 7 in the human kidney and urinary tract. **Design/Methods:** *Gene expression*: We isolated RNA from human kidney, ureter, and bladder tissue harvested from non-infected surgical pediatric patients. Isolated mRNA was reverse transcribed and quantified using real-time PCR. *Protein expression*: RNAse 7 expression was localized using immunohistostaining. To examine RNase7 protein expression in the urine, we developed a sandwich ELISA using two distinct antibodies to RNase7 and normalized urine concentrations to mg of creatinine. **Results**: *Gene expression*: Constitutive *RNase7* mRNA expression was detected in human kidney, ureter, and bladder tissue. Absolute quantification using real-time PCR and a standard curve revealed that *RNase7* is expressed (ng RNase7/ 10 ng total RNA) in the renal cortex at 270, outer medulla 190, and renal pelvis 170. *Protein expression*: Immunohistochemistry localized RNase 7 to the urothelium of the bladder, ureter, and a subset of cells in the collecting duct. Specifically, immunofluorescence localized RNase7 to intercalated cells of the collecting duct. RNase 7 was detected in non-infected human urine samples. Control urine normalized to urine creatinine demonstrated RNase7 protein expression ranging from 1.5 to 6.6 (mcg/mg creatinine) with a mean SD of 4.1 +/- 1.9. **Conclusion:** Our results characterize the expression a novel AMP, RNase 7, in the human kidney and urinary tract. *RNAse7* is expressed in the kidney, ureter, and bladder. Within the kidney, mRNA expression is greatest in the renal cortex. This potent AMP is specifically expressed in intercalated cells. In addition to maintaining acid-base homeostasis, intercalated cells have a role in the innate defense of the urinary tract.

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IMMUNOGENICITY OF LONGUS STRUCTURAL SUBUNIT OF ENTEROTOXIGENIC ESCHERICHIA COLI DELIVERED BY AN *E. COLI* PROBIOTIC VACCINE STRAIN

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Background: Longus from enterotoxigenic *Escherichia coli* (ETEC), also identified as a colonization surface antigen (CS) 21, is a typical type IV pilus and one of the most prevalent ETEC CSs. Longus major subunit, LngA, adheres to intestinal cells, protects ETEC against stress factors, and may actively participate in ETEC intestinal colonization. The objective of this work is to evaluate the immunogenicity of LngA when delivered by a mucosal delivery bacterial vector. Nissle E. coli is a non-pathogenic, well-tolerated, and safe probiotic strain clinically tested in adult and children volunteers. This strain colonizes the human intestine well and it has a potential to deliver heterologous antigens to induce specific immune responses. We propose to use Nissle strain as a mucosal delivery system and a safe live oral vaccine vector to induce protection against ETEC induced diarrhea. **Materials and Methods:** The *lngA* gene was cloned and expressed in two different host E. coli strains, DH5alpha and Nissle. LngA expression was detected by immuno blot. Immunogenicity was evaluated after oral or intranasal immunization of C57BL/6 mice by detection of anti-LngA antibodies in an ELISA assay. Serum obtained at different interval as well as intestinal fluid at the end of the experiment were tested for anti-LngA antibodies. **Results:** The *lngA* gene was expressed under the AraC promoter control in both, DH5 alpha and Nissle, E. coli strains. Both constructs as well as controls were delivered to C57BL/6 mice by three oral or intranasal immunizations. DH5alpha and Nissle E.coli expressing LngA induced specific anti-LngA antibodies. The anti-LngA response was significantly higher when Nissle strain was used as orally as a delivery system. **Conclusions:** The LngA vaccine candidate may be expressed and delivered by E. coli probiotic delivery systems. Oral immunization of mice with E. coli Nissle expressing LngA induces very strong anti-LngA antibody responses, including secretory IgA as well as systemic IgG subclasses. Our preclinical study may provide the basis for further testing of this ETEC vaccine in phase I clinical trials.

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A GENE TRANSFER APPROACH TOWARDS HEMOPHILIA A CORRECTION USING THE *PIGGYBAC* TRANSPOSON VECTOR

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Human Factor VIII (hFVIII) deficiency offers advantages as a disease target for gene therapy as small increases in FVIII levels will alter the bleeding phenotype. The insect derived *piggyBac (PB),* a nonviral DNA transposon, can be engineered to carry a therapeutic transgene between the inverted terminal repeats. We hypothesize that *PB* transposon vector carrying a reporter gene cassette along with a transposase will confer persistent gene expression and correction of the hemophilia A bleeding phenotype with the FVIII cDNA. We evaluated co- and hyp-transposase-mediated transposition in a human hepatoma cell line to verify function in hepatocytes. Using the PB hygromycin resistance vector, we demonstrated that the hyp-transposase generated higher transposition efficiency than an inactive mutant or co-transposase. We showed *in vivo* persistence following hydrodynamic tail-vein injection using firefly luciferase expression driven by a liver specific promoter. Luciferase expression measured via *in vivo* bioluminescence imaging persisted up to eight months in Balb/C liver (duration of experiment). Following partial hepatectomies at 5 months post injection, expression was observed only in animals receiving PB luciferase transposon and an active transposase while expression in those treated with the inactive mutant dropped to background levels. We furthered these experiments by introducing PB human alpha$_1$ antitrypsin (hAAT) via hydrodynamic tail-vein injection as before at either a low or high dose. Serum hAAT levels were measured and were significantly higher than in control mice. These data represent one of the first studies to show persistent transgene expression *in vivo* from *piggyBac* transposon gene transfer.

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MGLUR-INDUCED TRANSLATION OF AMYLOID PRECURSOR PROTEIN IN DENDRITES INVOLVES THE C-JUN N-TERMINAL KINASE (JNK) PATHWAY

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Background: Synaptic plasticity is required for normal learning and memory and is altered in a number of developmental disorders. Local protein translation in dendritic spines induced by glutamate receptor stimulation is required for induction of plasticity. Amyloid precursor protein (APP) is translated in response to metabotropic glutamate receptor (mGluR) stimulation and may directly contribute to impaired plasticity. The c-Jun N-terminal kinase (JNK) pathway is activated in response to mGluR activation, and has been implicated in regulating ***nuclear*** transcription of several genes. **Hypothesis:** We hypothesized that JNK activation ***in dendritic spines*** by mGluRs regulates local APP translation. **Methods:** We used synaptoneurosomes (SNs) prepared from 16-day old mice and primary E18 cortical neuron cultures to examine the role of the c-Jun N-terminal kinase pathway in regulating dendritic translation of APP. Incorporation of ^{35}S-methionine followed by immunoprecipitation of APP was used to measure APP translation in SNs. Immunofluorescence of cortical neurons was used to examine levels of activated (phosphorylated) JNK (p-JNK) and APP in dendritic spines. Western-immunblots were used to determine total APP and p-JNK levels in SNs. The JNK pathway was inhibited with SP600125 (20 mM). DHPG (100 μM) was used to activate group 1 mGluRs. **Results:** JNK is activated in WT SNs and cortical neurons in as little as two minutes after DHPG treatment. JNK inhibition reduces basal translation of APP and, to a greater degree, the increase in APP translation in SNs following DHPG stimulation as assessed by 35S-methinonine incorporation. The DHPG-induced increase in dendritic levels of APP is also reduced by JNK inhibitor as assessed by IF. **Conclusion:** Our data indicate that local, mGluR-induced dendritic translation of APP requires JNK signaling. This may provide a therapeutic target for diseases involving pathologic increases of APP including Fragile X Syndrome, Alzheimer's disease and Down's Syndrome.

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GENOTYPE AND PHENOTYPE CORRELATIONS WITH 17-HYDROXYPROGESTERONE CONCENTRATION IN PREMATURE NEWBORNS

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Background: 17-hydroxyprogesterone (17-OHP) is the analyte used to identify newborns with congenital adrenal hyperplasia (CAH) through newborn screening programs. Unfortunately, the positive predictive value (PPV) of 17-OHP in testing for CAH is low. Clinical and genetic factors that may contribute to variation in 17-OHP could potentially be useful for improving the PPV; however, few studies have examined factors other than gestational age and birth weight. **Objective:** To identify clinical and genetic contributors to variation of 17-OHP in newborns. **Design:** Clinical data from a population of preterm Iowa neonates (n=756) and 17-OHP measurements from the Iowa Neonatal Metabolic Screening Program (INMSP) were analyzed using linear regression to identify clinical and genetic associations with 17-OHP concentrations. A subset of Caucasian infants without congenital anomalies (n=447), their parents and any siblings were genotyped at 48 single nucleotide polymorphisms (SNPs) encompassing 6 candidate genes within the steroidogenic pathway and 12 related genes, including the glucocorticoid receptor (*NR3C1)* gene. Genetic analysis was performed using linear regression and quantitative transmission disequilibrium tests. **Results:** Placental abruption (p=0.01) and respiratory distress syndrome (p=0.002) were associated with higher concentrations of 17-OHP while congenital anomalies (p=0.002) were associated with lower 17-OHP concentrations independent of gestational age within the preterm population. The AA genotype of rs4674338 in *CYP27A1* was associated with higher 17-OHP concentrations in Caucasian newborns compared to those with the GG genotype (p=0.01). Additionally, the TT genotype of rs927650 in *CYP24A1* was associated with higher 17-OHP compared to those with the CC genotype (p=0.03). **Conclusions:** Clinical and genetic associations were observed to have an effect on 17-OHP concentrations and could potentially improve the PPV of CAH testing.

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PAIRED-BOX 2 REGULATION OF DISABLED-2 GENE EXPRESSION

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Background: During renal development, the Pax2 (Paired-box 2) transcription factor is necessary for the mesenchymal-to-epithelial transition essential to producing a viable metanephros. Absence of Pax2 results in failure of metanephric development, and over-expression of Pax2 has been documented in several genitourinary tumors. Dab2 (Disabled-2) is a pleiotropic phosphoprotein involved in endocytic trafficking and cell signaling, notably in its attenuation of the Wnt signaling pathway. Its presence is associated with well-differentiated epithelialized cell states, and its temporal expression in renal development contrasts that of Pax2, being most abundant in mature proximal tubule cells. Dab2 is down-regulated in several genitourinary tumors, and may function as a tumor suppressor. The transcriptional regulation of Dab2 is not fully understood; as such, we hypothesize that Pax2 directly regulates the expression of Dab2 in the developing kidney and that this mechanism is dysfunctional within certain tumors. **Objective:** To demonstrate a temporal, physical, and functional inverse relationship between Pax2 and Dab2, further clarifying their roles in genitourinary oncogenesis and renal development. **Methods:** Comparative analysis between immortalized E11 murine metanephric mesenchymal cells stably-transfected with an adenoviral-Pax2b vector and the parental cell line using a suppressive-subtraction PCR/differential display strategy revealed a down regulation of Dab2 in Pax2 expressing cells. This was confirmed with real time RT- PCR. 3T3 cells were co-transfected with a luciferase vector containing the human Dab2 promoter region (Switchgear Genomics), and a CMV Pax2bHA vector. Transfection efficiency was normalized with a CMV β-galactosidase vector. **Results:** Dab2 was isolated as potential target for Pax2 during screening. Real-time PCR revealed a nine-fold decrease in Dab2 mRNA expression in Pax2b transfected cells when compared to parental cell line. Increasing Pax2b gene dosage in 3T3 cells resulted in progressive diminution of luciferase activity (repeated in triplicate, $p < 0.05$), with a 39.8% decrease in luminescence noted between maximally transfected and sham transfected cells ($p < 0.05$). **Conclusion:** Dab 2 expression is down-regulated in the presence of Pax2. Functional analysis suggests that Pax2 directly targets the Dab2 promoter. *In-vivo* and physical association studies are under way. Establishment of this regulatory mechanism has implications in both renal development and genitourinary oncogenesis.

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CLONAL ANALYSES REVEAL SPATIAL RESTRICTION AND THE ABSENCE OF TRANSDIFFERENTIATION DURING APPENDAGE REGENERATION IN ZEBRAFISH

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Purpose: Regeneration is a process by which damaged or lost structures are perfectly or near-perfectly replaced. The zebrafish caudal fin has become a valuable structure for studying regeneration. Mammals, including humans, have the potential to regenerate only a limited number of structures such as liver and digit tips. In contrast to mammals, urodele amphibians and teleost fishes have the ability to regenerate multiple structures. During fin regeneration, it is thought that differentiated mesenchymal cells enter the regeneration blastema, proliferate and give rise to all mesenchymal structures in the regenerate. However, it remains unknown whether cells populating the regeneration blastema are pluripotent. **Methods and Summary of Results:** We used genetic markers to follow the lineage and position of individual cell types during zebrafish fin regeneration. In particular, two different ubiquitous promoters, β-actin and ef1α, were used to drive expression of a GFP reporter. Furthermore, two techniques were employed to label cells: blastula transplantation and plasmid injection. Using this combination of techniques, we were able to label arteries, veins, axons, epidermis, as well as several mesenchymal structures including intra-ray mesenchyme, inter-ray mesenchyme, osteoblasts and fin ray joints. We found no evidence for transdifferentiation during fin regeneration. Furthermore, dorsal/ventral migration of labeled cell clones was very limited. These data support a model of fin regeneration wherein cells are restricted in both lineage and space as they populate the regenerating appendage. **Conclusions:** Through dual methodologies of blastula transplantation and plasmid injection, we examined the contribution of multiple, unique GFP-labeled cell types to the regenerating zebrafish caudal fin. We conclude that this combined methodology labeled most subpopulations of cells in the fin (epidermis, artery, vein, nerve, joint scleroblast, inter-ray mesenchyme, and intra-ray mesenchyme). Second, we found that these GFP-labeled cells contributed singularly to their original cell type in the regenerate with no evidence for transdifferentiation observed. Further work on the mechanisms of regeneration will be needed to understand why differentiated cells can regenerate an amphibian limb or a teleost fin, but not evolutionarily related structures in mammals.

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NEONATAL SERTRALINE EXPOSURE INCREASES ADULT SYMPATHETIC TONE

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Background: Maternal depression occurs in 7-15% of pregnancies and the treatment of maternal depression is uniformly recommended. SSRIs (selective serotonin reuptake inhibitors) are first-line therapy for maternal depression. Over the past two decades, the prevalence of SSRI therapy during pregnancy has drastically increased despite no long term outcome data. Animal models targeting an analogous neurodevelopmental window have shown neonatal SSRI exposure decreases adult serotonergic signaling. We have previously shown neonatal exposure to the most commonly prescribed SSRI (sertraline) increases adult heart rates, exaggerates responses to sympathetic blockade, and increases very low frequency pulse interval variability. **Purpose:** We sought converging evidence to support our hypothesis that neonatal sertraline exposure increases central sympathetic activation in adult mice. **Methods:** Pregnant mice were allowed natural delivery. Pups were randomized to injections of saline, low-dose (5 mg/kg/d) or high-dose (15 mg/kg/d) sertraline from day of life 1-14. During adulthood, male mice were implanted with telemeters to record locomotor activity. Basal metabolic rates were obtained through indirect calorimetry and caloric intake was determined by recording food intake. Urinary noradrenaline (NA) excretion was quantified by ELISA. **Results:** Male mice exposed to neonatal sertraline had increased locomotor activity ($P<0.001$), metabolic rates ($P<0.05$), and caloric intake ($P<0.05$) compared to control mice but maintained comparable body weights. Urinary NA excretion was increased in sertraline-exposed mice. **Conclusions:** Neonatal sertraline exposure causes increased basal metabolic rates in adult mice. As a result of the increased basal metabolic rate, sertraline exposed mice have increased caloric intake and activity levels but maintained comparable weights to control mice thus suggesting increased sympathetic tone as the cause. Combined with earlier findings and the increased urinary NA excretion, there is converging evidence that neonatal sertraline exposure increases sympathetic activation. We hypothesize that SSRI exposure during a critical developmental window elicits a compensatory down-regulation in serotonin signaling, resulting in enhanced sympathetic tone once SSRI exposure ceases.

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GESTATIONAL DIABETES INDUCES ALTERATIONS IN THE FUNCTION OF NEONATAL ENDOTHELIAL COLONY FORMING CELLS

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Background: Diabetes (type 1, type 2, and gestational diabetes) is a common disease and affects ~7% of all pregnancies. Epidemiologic data suggest that a diabetic intrauterine environment predisposes offspring to develop hypertension. However, the underlying mechanisms are unknown. Endothelial colony forming cells (ECFCs) are endothelial progenitors involved in vascular repair and vasculogenesis. Previously, we demonstrated that ECFCs from the cord blood of infants born to women with type-1 and type-2 diabetes have reduced colony formation, decreased proliferation, decreased vessel forming ability, and premature senescence compared to healthy controls. The goal of the current studies was to examine whether intrauterine gestational diabetes (GDM) exposure has a deleterious effect on the function of neonatal ECFCs. We hypothesize that cord blood derived ECFCs from GDM pregnancies will exhibit impaired colony forming capacity, proliferation, and vessel-forming ability, while exhibiting increased cellular senescence both at baseline and after exposure to hyperglycemia compared to ECFCs derived from uncomplicated pregnancies. **Methods:** ECFCs were isolated from the cord blood of uncomplicated and GDM pregnancies as previously described. For hyperglycemia experiments, ECFCs were grown in media containing increasing glucose concentrations. Colony forming assays were plated. Thymidine incorporation assays were conducted to evaluate proliferation, and acidic β-galactosidase assays were performed to examine for senescence. Finally, matrigel assays were done to quantitate vessel forming ability. **Results:** Total colony formation of GDM ECFCs was unchanged from the controls ($n = 4$), however, GDM ECFCs demonstrated decreased vessel forming ability ($n=4$, $p < 0.01$). Proliferation was significantly increased in GDM ECFCs compared to controls ($n=7$, $p < 0.03$). Interestingly, hyperglycemia induced senescence was attenuated in GDM ECFCs ($n=4$, $p < 0.03$). **Conclusions:** GDM ECFCs exhibit resistance to hyperglycemia, increased proliferation, and decreased vessel forming ability. We speculate that GDM ECFCs may undergo proliferative exhaustion and premature senescence. Therefore, as an infant's vascular tree grows, the endothelium may have a decreased capacity for linear growth and repair leading to endothelial dysfunction and subsequent hypertension.

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LOCALIZED FETOMATERNAL HYPERGLYCEMIA INDUCES DIABETIC EMBRYOPATHY

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Purpose of Study: Diabetic embryopathy encompasses fetal loss, caudal regression syndrome, neural tube defects and other malformations in offspring of diabetic mothers (ODMs). It is postulated that these malformations are induced through exposure of the developing fetus to a variety of excess circulating fuels including glucose, insulin, ketones and lipids which are elevated in diabetic mothers. The objective of this study was to determine if localized hyperglycemia alone is sufficient to induce teratogenesis. **Methods:** Our approach took advantage of the bicornate uterus of the rat, each with its own blood supply. The left uterine artery was catheterized, allowing selective exposure of the developing pups in the left uterine horn to hyperglycemia while maintaining unexposed right sided pups as an ideal control. Positron emission tomography (PET) imaging using the glucose tracer, [^{18}F] fluorodeoxyglucose (FDG), was used to determine selectivity of glucose exposure. To determine the effects of hyperglycemia on embryopathy, glucose infusion (4 mg/min) during key phases of organogenesis was performed. A subteratogenic dose of systemic retinoic acid was used to potentiate embryopathy. Embryos were harvested and examined for embryopathy or resorption on gestational day 12. **Results:** As determined by PET imaging, each left-sided fetus accumulated 2.3±0.1% of the total administered FDG dose, whereas right-sided fetuses accumulated only 1.5±0.1% ($p \pm 2$ mg/dL, n=226). Furthermore, catheter placement did not impair pup size (3.52±0.06g on the left versus 3.54±0.06g on the right. p=0.87, n=76-79.) In the presence of a subteratogenic dose of systemic retinoic acid, glucose infusion on gestational day 7-9 led to increased embryo resorption and malformation compared to retinoic acid exposure alone (62.5% vs. 10.5%. p=0.005, n=27). To assure this systemic retinoic acid was subteratogenic, doses were compared, finding 8.3% malformations with 10mg/kg (n=12), 18.2% with 25mg/kg (n=27), and 100% with 40mg/kg (n=11) compared to a baseline resorption rate of 8 to 20% without retinoic acid. **Conclusion:** Selective and localized hyperglycemia is sufficient to induce embryopathy in the absence of other components of maternal diabetes. This rodent model allows study of the mechanisms of localized fetomaternal hyperglycemia, with the ultimate goal of a better understanding pathogenesis and prevention of diabetic embryopathy.

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BACTERIAL DIVERSITY IN THE INTESTINAL MICROBIOME OF PREMATURE NEWBORNS

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Background: Human milk is associated with a decreased risk of necrotizing enterocolitis (NEC), a devastating disorder that affects approximately 10% of premature infants. Bacteriologic culture methods have suggested that the type of feeding (human milk versus formula) for healthy term infants significantly affects intestinal colonization patterns. The effect of feeding on the premature enteric microbial composition is unknown. **Objective:** To use non-culture based methods to determine the effect of cow's milk-based formula and human milk on the development of enteric bacterial diversity in premature newborns. **Methods:** Microbial DNA was extracted from sequential stool samples of 10 infants with birth weight ≤1500 grams. The V1-V3 and V3-V5 regions of the genes encoding bacterial 16S rRNA were sequenced. These sequences were compared with known bacterial 16S sequences using the RDP classifier to determine the relative representation of specific microbial populations in the each specimen. Shannon and Simpson diversity indices were calculated for each week post-birth and regressed by chronological age (in weeks), total amount of feeding (human milk or formula), and medications. A mixed model with the patient as random effect was used. Bar graphs were further utilized to pictorially demonstrate the day-to-day relationship of feeding on bacterial composition. **Results:** Each subject had 10-26 stool specimens; collection was between weeks 1-10 of life. Intersubject variability in the microbial representation was evident, even within feeding types. Longitudinal patterns within subjects demonstrated a stable intra-subject environment throughout sampling. Diversity of this pattern was not significantly affected by feeding type, whether grouped at a genus level or by gram +/- status. The Shannon diversity index ranged from 0.04 to 2.34 and the Simpson diversity index ranged from 0.01 to 0.88. Diversity indices indicate that type of feeding has no effect on bacterial diversity. **Conclusions**: Although there was significant inter-subject variability in the microbial composition, the type of enteral nutrition did not affect the degree of diversity for this NICU preterm population. The stability over time of the microbiota within a subject suggests that factors other than type of feeding contribute to the establishment and maintenance of the premature newborn's enteric microbiome.

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BREAST MILK INHIBITS TOLL-LIKE RECEPTOR 4 SIGNALING IN ENTEROCYTES AND ATTENUATES THE SEVERITY OF EXPERIMENTAL NECROTIZING ENTEROCOLITIS

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Introduction: Necrotizing enterocolitis (NEC) is the leading cause of gastrointestinal death in preterm infants. NEC occurs via exaggerated Toll-like receptor 4 (TLR4) signaling and decreased enterocyte (IEC6) proliferation. Given that breast milk is known to be protective in human NEC and known to be rich in epithelial growth factor (EGF), we sought to test the hypothesis that breast milk could attenuate TLR4 signaling in enterocytes and determine the potential mechanisms involved. **Methods:** Breast milk was extracted from CFW wild type mice using a novel device developed in our lab using an electric, piston driven breast pump. NEC was induced using gavage feeds, intermittent hypoxia, with or without the EGF receptor inhibitor, gefitinib at a dose of 200 ug/g given with a gavage feed daily. TLR4 signaling in vivo and in vitro was determined by qRT-PCR of intestinal NOS and NFkB translocation by confocal microscopy. An EGFR deficient enterocyte cell line was created using a lentivirus expressing shEGFR. **Results:** In IEC6 cells, breast milk reduced TLR4-mediated NFkB translocation in a dose dependent manner and significantly reduced TLR4-mediated IL6 and iNOS expression. Breast milk did not prevent TLR4 signaling in EGFR -/- cells. Strikingly, breast milk markedly reduced the severity of NEC and iNOS expression in experimental NEC, which was reversed in the presence of gavaged EGFR inhibitor, gefitinib. **Conclusion:** Breast milk attenuates experimental NEC in a manner dependent on a signal via epithelial growth factor receptor suggesting a role for EGF in the pathogenesis and treatment of this devastating disease.

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EFFECT OF SURFACTANT PROTEIN A ON MILK IMMUNOGLOBULIN A PRODUCTION

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Background: Surfactant Protein A (SP-A) is a part of the lung's innate immune system. It communicates with the adaptive immune system and modulates cytokines and immunoglobulin production. Maternal SP-A is associated with improved neonatal survival among SP-A null mice in a bacteria laden environment, likely due to passive protection (placental or breastfeeding). Secretory(s) IgA is a major component of breast milk. The immediate neonatal period is characterized by a deficiency of IgA. Antigen specific milk sIgA formed in response to maternal respiratory and gastrointestinal pathogens provides focused immunoprotection to the neonate, especially preterm babies. Preterm birth is on the rise with prematurity the leading cause of neonatal mortality. **Objective:** To assess the role of respiratory SP-A for optimal sIgA production and function in breast milk. **Methods:** A murine model for respiratory exposure to 2,4-Dintirophenyl keyhole limpet hemocyanin (DNP-KLH) was used. Adult mice (wild type and SPA null on C3HeB/FeJ background) were paired and primed with 30 ul intranasal DNP-KLH (30 μg/μl) or deionized water and boosted two weeks later. Female mice were milked at 48 h and 7days (d) postpartum. Total and DNP-KLH-specific sIgA were measured by enzyme-linked immunosorbent assay (ELISA). Bronchoalveolar lavage (BAL) was performed to assess cellularity and IgA levels. **Results:** Exposure to DNP-KLH did not induce any differences in the cellularity, total IgA, or DNP-KLH specific IgA in lung lavage fluid in either strain of mice. No differences were noted in the concentration of total sIgA in milk between wild type and SP-A null at baseline or between DNP-KLH exposed mice. DNP-KLH specific sIgA in milk at 48h was, however, significantly higher ($p<0.01$) in the DNP-KLH exposed wild type mice compared to SP-A null mice. **Conclusions:** Lung lavage fluid concentrations of IgA were not altered in SP-A null mice. Total milk sIgA concentration was also not altered in the absence of maternal SP-A and not affected by DNP-KLH respiratory exposure in our murine model. SP-A is important for the production of DNP-KLH specific sIgA in milk. Therefore, maternal SP-A may be critical for antigen specific immunoprotection in expressed milk.

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INTEGRATION FREE REPROGRAMMING OF SURFACTANT PROTEIN B (SP-B) DEFICIENT HUMAN FIBROBLASTS INTO INDUCED PLURIPOTENT STEM (iPS) CELLS

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Background: Disruption of pulmonary alveolar type 2 cell surfactant metabolism by rare mutations in genes that encode surfactant protein-B (*SFTPB*), surfactant protein-C (*SFTPC*), and ATP binding cassette protein member A3 (*ABCA3*) cause lethal neonatal respiratory distress (*SFTPB*, *SFTPC*, *ABCA3*) and interstitial lung disease (*SFTPC*, *ABCA3*). Methodological challenges in maintenance of primary alveolar type 2 cultures from affected and normal individuals have limited investigation of specific pathogenic mechanisms encoded by these mutations and of genetic background and environmental stress. Availability of integration free (episomal) vectors to reprogram human fibroblast cell lines into iPS cells and methods to derive alveolar type 2 cells from human embryonic stem cells permits molecular and cellular characterization of patient-specific disruption of surfactant metabolism without complicating viral vector integration. **Objective:** To use integration free, episomal vectors to reprogram skin fibroblasts from SP-B deficient and normal infants and characterize the resulting iPS cells **Design/Methods:** After establishing skin fibroblast cell lines from a SP-B deficient (homozygous c.362 C>GAA) and a normal infant, we used nucleofection to introduce virus-free, non-integrating episomal plasmids that expressed the transcription factors OCT4, SOX2, NANOG, LIN28, c-Myc and KLF4. We picked colonies on day 28 and mechanically passaged them every 7 days on a fresh feeder layer of irradiated murine fibroblasts. On day 48, we characterized selected iPS cell colonies using live cell staining with the pluripotency markers TRA-1-81, TRA-1-60 and SSEA4. On day 50, we isolated genomic DNA from 1 colony using the GenElute kit and performed *Sfu1* restriction fragment analysis on a 310 bp *SFTPB* amplicon that included the homozygous c.362C>GAA mutation. We also amplified 2.5 kb of *SFTPB* in 1 amplicon and used Sanger methods to sequence the amplicon. **Results:** We picked 16 colonies per 1 million fibroblasts transfected. All selected colonies were morphologically similar to human embryonic stem cells (hESC). Only 5 colonies out of 48 were successfully expanded and used for further characterization. All 5 clones displayed TRA-1-81, TRA-1-60, and SSEA4 antigens. *Sfu1* digestion of DNA from the SP-B deficient iPS cells confirmed the presence of the mutation by yielding 2 fragments with mobilities of 187 bp and 125 bp. Sequence analysis also confirmed the presence of the homozygous c.362 C>GAA substitution. DNA from iPS cells reprogrammed from the normal fibroblast cell line was resistant to *Sfu1* digestion and had *SFTPB* sequence similar to the parent fibroblast cell line in exon 4. **Conclusion:** Integration free reprogramming of SP-B deficient and normal skin fibroblasts did not alter the *SFTPB* mutation or the *SFTPB* wild type sequence. We speculate that patient-specific alveolar type 2 cells can be derived from iPS cells by transfecting a construct that expresses *SFTPC* and carries selectable markers as previously described for hESC. Use of integration free vectors to reprogram skin fibroblasts and derivation of alveolar type 2 cells will permit molecular and cellular characterization of genetic disruption of surfactant metabolism without integrated viral vectors.

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CHRONIC HYPEROXIA RESULTS IN NEONATAL LUNG DISEASE ASSOCIATED WITH UP-REGULATION OF TRANSFORMING GROWTH FACTOR BETA-2 LIGAND MRNA EXPRESSION

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Background: Bronchopulmonary Dysplasia (BPD) is a chronic lung disease that affects preterm infants, characterized by fewer, larger, simpler alveoli due to an absence of secondary alveolar septation resulting in a reduced surface area for air exchange. Up-regulation of the Transforming Growth Factor Beta (TGFβ) superfamily signaling pathway has been associated with the development of BPD in multiple animal models as well as premature infants. Although it is known that TGFβ signaling is mediated via three ligands, TGFβ-1, 2, and 3, that serve overlapping yet specific functions in normal lung development, there is a lack of data describing how these ligands are affected by neonatal exposure to hyperoxia. We hypothesized that exposure to chronic hyperoxia will have differential effects on the expression of these isoforms in the neonatal mouse lung. **Methods:** Within 12 hr of birth, C57/B6 wild-type mice were subjected to 21 consecutive days of continuous exposure to 85% oxygen (O2) or room-air (RA) as controls. At the end of the study period, lungs were pressure-fixed with 4% Paraformaldehyde (for examination of distal airspace size and TGFβ-isoform mRNA in-situ hybridization) or frozen and homogenized for protein electrophoresis (to quantify TGFβ signaling via relative phospho-Smad 2/3 expression). **Results:** Exposure to chronic hyperoxia resulted in an arrest of alveolar development with enlarged, simplified distal airspaces. TGFβ-2 mRNA expression was increased in 85% oxygen-exposed mice compared to RA controls, with a punctuate pattern noted within the alveolar epithelium. TGFβ-1 mRNA appeared to be equivalent between the two groups, and TGFβ-3 mRNA was not present in either group. There was potentiation of TGFβ signaling levels as evidenced by increased phosphorylation of Smad2/3 protein (O2:RA increased 41%, $p<0.001$). Significantly, Periostin protein, a known down-stream effector of phospho-Smad 2/3 signaling, was increased in 85% oxygen-exposed mice compared to RA control lungs. **Conclusion:** Hyperoxia-induced arrested alveolar development is associated with long-lasting enhancement of TGFβ signaling. Further, we show that the potentiation of TGFβ signaling is mediated via specifically increased TGFβ-2 ligand expression. Future studies to delineate the spatio-temporal expression of TGFβ-2 mRNA as well as the cell-specific targets of TGFβ-signaling are needed, as this will be paramount to understanding the possible role of TGFβ in the development of neonatal lung diseases.

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THE EFFECT OF ERYTHROPOIETIN AND IRON SUPPLEMENTATION ON BRAIN GROWTH

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Background: Nutrition deficits, such as iron deficiency (ID), during fetal development can cause permanent alterations in brain growth and function, especially in the hippocampus. With limited iron stores, rapid growth and erythropoietic need, anemic premature infants may deplete their iron stores. The hormone Erythropoietin (Epo) stimulates erythropoiesis and is used to clinically treat the anemia of prematurity, but Epo increases iron utilization. Epo receptors are present on immature brain oligodendrocytes, but with ID, Epo may preferentially withhold iron from these cells. Epo promotes oligodendrocyte differentiation to myelin-producing cells. The goal of this study is to determine whether erythropoietic doses of Epo alter brain iron or structure under conditions of differing iron status. **Methods:** Sprague-Dawley newborn rats were used as models of premature newborns and iron ID. 4 groups of Sprague-Dawley rats were treated P4-P12: dam fed (Control, i.e. iron-sufficient, IS), IS+Epo (425U/kg/d SQ), ID (artificial milk via gastronomy) and ID+Epo (425U/kg/d SQ). All groups were given iron (ferrous sulfate 6 mg/kg/d). Brain tissues were collected and stained with Prussian Blue and Luxol Fast Blue. Photomicrographs were taken to measure oligodendrocyte iron, pyramidal cell density in the CA1 area of the hippocampus, and estimates of hippocampal and subcortical myelin. Tissue iron, as well as tests of erythropoiesis and iron status were measured. **Results:** In contrast to adult rodents, oligodendrocytes contained no storage iron. Qualitative myelin stain suggests better myelination in the IS groups, with possible improvement in +Epo groups. In hippocampal CA1 region, pyramidal densities in ID were slightly less than in IS ($P<0.05$), but Epo treatment had no effect. Brain weights (μg/g rat wt) and iron content (μg/g rat wt) in ID groups were less in ID, $p<0.05$, and with IS, slight improvement with +Epo, but no change in the ID+Epo group. Brain wt and brain iron content were neither correlated with measures of erythrocyte iron nor with storage iron, but correlated with plasma Epo level, $p<0.02$. **Conclusions:** ID decreased brain weight and iron content. However, Epo treatment did not worsen iron content, but brain wt and iron content may directly relate to steady-state plasma Epo levels. This finding is intriguing because Epo-treated human premature infants with higher plasma Epo levels may have better neurological function. Erythropoietic doses of Epo did not affect CA1 pyramidal proliferation, but Epo may increase oligodendrocyte differentiation. Further study should include quantitative markers of differentiation and myelination.

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IMMEDIATE AND LONG-TERM EFFECTS OF RECURRENT HYPOGLYCEMIA ON REGIONAL BRAIN INJURY, PLASTICITY AND BEHAVIOR IN YOUNG RATS

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Background: Recurrent hypoglycemia (RH) is common in infants and children. Despite its potential for neurologic sequelae, the effects of RH on the young brain are poorly understood. **Objective:** To investigate the immediate and long-term effects of RH on the young rat brain. **Design/Methods:** Young male rats were subjected to insulin-induced hypoglycemia (blood glc 30 mg/dl for 120 min), once daily from postnatal day (P) 24 to P28 (RH group). Rats in the control group were administered normal saline. On P29, injury in cerebral cortex and hippocampus was assessed using Fluoro-jade B (FJB) histochemistry (n=8). Brain derived neurotrophic factors (BDNF) III and IV and their receptor TrkB (markers of plasticity), and signaling molecules of dendritogenesis, profilin (pfn) I and II expressions were determined using qRT-PCR and Western blot methods on P29 (immediate effect) and P65 (long-term effect) (n=8). Behavioral effects were tested using prepulse inhibition (PPI, a measure of sensorimotor gating) on P29 and P42 (n=12), and hippocampus-dependent trace and hippocampus-independent delay conditioning of auditory fear (fear-potentiated startle; FPS) on P65 (n=16-20). **Results:** Compared with the control group, more degenerating neurons were present in the cerebral cortex in the RH group, predominantly in the prefrontal region (86 ± 21 vs. 0 ± 0 FJB positive cells/section, $p<0.01$). Neuronal injury was absent in the hippocampus. BDNF and pfn I and II expressions were decreased by 16-35% in the cerebral cortex on P29 ($p<0.01$), but not on P65. BDNF expression was not altered in the hippocampus on P29 or P65. Pfn I and II expressions were decreased by 22% in the hippocampus on P65 ($p<0.01$). PPI was decreased 28% on P29 in the RH group but recovered on P42 ($p<0.01$). Trace and delay FPS on P65 were similar in the groups. **Conclusions:** RH was associated with neuronal injury, decreased plasticity and altered dendritogenesis in the cerebral cortex of developing rats. Neuronal injury in the prefrontal cortex is likely responsible for the transient decrease in PPI. Lack of injury and BDNF expression, and intact trace fear conditioning suggest that the hippocampus is relatively resistant to injury in this model. However, decreased profilin expression on P65 suggests potential for long-term disruption in hippocampal dendritogenesis. These structural and molecular changes may explain the cognitive deficits common in infants and children with recurrent hypoglycemia (Funded by NICHD and Viking Children's Fund).

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LOW SERUM 25-HYDROXY VITAMIN D (25(OH)-D) LEVEL IS ASSOCIATED WITH ACUTE PHASE REACTION (APR) FOLLOWING INTRAVENOUS BISPHOSPHONATE (IVBP)

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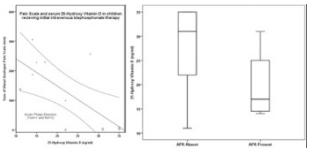
APR is common following the first course of IVBP and can be severe enough for the child to refuse therapy. Bertoldo et al [J Bone Miner Res. 2010;447] recently reported an inverse association between 25(OH)-D level and APR post-IVBP. We conducted a double-blind randomized cross-over placebo controlled study to evaluate Atorvastatin in alleviating APR in 12 children with various metabolic bone diseases and osteoporosis treated with IVBP. We analyzed our data for association between 25(OH)-D level and APR following IVBP. IVBP was given on 2 consecutive days referred to as a "cycle" given 3-4 months apart. Children completed the visual analogue pain scale (0-100) at baseline and at 4 different time points post-IVBP over a 48 hr period. We also recorded use of medications for pain or fever, as either 'Yes' or 'No'. APR was categorized as presence of fever needing acetaminophen and/ or musculoskeletal pain requiring oxycodone. Non-parametric Spearman correlation between serum 25(OH)-D level and sum of post-IVBP pain scales and multivariate regression modeling with backward elimination were performed using SPSS 18.0. The mean age of 12 children (10 girls) was 12.1 ± 4.2 yrs. In the first cycle 7 (58.3%) developed APR. There was no significant correlation between 25(OH)-D level and pain scale prior to IVBP ($p=0.3$). In contrast, post-IVBP a strong inverse correlation was found between pain scale and 25(OH)-D ($p=0.012$) [Fig 1]; 25(OH)-D was lower in children with APR. These findings remained true for 2nd cycle and for the two cycles combined. The two cycles were then combined for multivariate analysis. The correlation between 25(OH)-D and pain scales post-IVBP remained significant even after adjusting for confounding variables. 25(OH)-D was the only protective variable that reduced post-IVBP pain scale ($p=0.03$) when all other variables were held constant (R-square=0.74). To decrease APR, we recommend that prior to giving IVBP children be replete with vitamin D.



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LEAD AND IRON STATUS IN CORD BLOOD OF NEWBORNS AT-RISK FOR IRON DEFICIENCY

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Background: Proper development of the central nervous system (CNS) in the fetus depends on placental transport of iron, such that any interference with transport is highly detrimental. Working as a competitive inhibitor to tissue iron transport, lead has the potential to dramatically obstruct iron delivery. Lead is also a neurotoxin, with studies showing a greater impairment of CNS development in male vs. female toddlers, despite comparable blood lead levels (BLLs). Our goal was to examine the relationship of iron and BLLs in pregnancies with risk factors for impaired fetal iron status. **Methods:** In a prospective trial of 198 term newborns at-risk for iron deficiency at the Meriter Hospital Birthing Center, we compared measures of iron and BLLs in umbilical cord blood. Risk factors included maternal anemia, maternal diabetes, maternal psychosocial stress, and fetal growth retardation. Maternal psychosocial stress was determined by a 35-item question, retrospective questionnaire that assessed the maternal stress experienced during their pregnancy. Cord blood erythrocyte iron level was measured by zinc protoporphyrin/heme (ZnPP/H) and body iron stores were estimated by plasma ferritin. BLLs were measured by inductively coupled mass spectrometry. Unpaired t tests and simple linear regression were used to analyze the data. **Results:** We found that mean cord BLLs were below previously reported toxic levels. BLLs were similar in newborns affected and unaffected by maternal psychosocial stress and diabetes. However, in contrast to that anticipated, mean cord BLLs in newborns affected by maternal anemia were 20% lower than those without anemia ($p<0.05$). In all enrollees, cord BLLs were directly correlated to cord ZnPP ($p<0.0002$), but this association was predominantly defined by male offspring (males, $R^2=.138$ $p<0.0001$ vs. females, $R^2=.001$ $p=0.75$). Cord BLLs were not correlated with plasma ferritin. **Conclusion:** Our findings support that BLLs in male newborns had a stronger relationship to erythrocyte iron measures than in females. Despite relatively low lead exposure, we speculate that males may be more susceptible to toxicity, due to the combination of lead and lower iron levels. Additionally, iron/lead transport may function differently in maternal anemia than other maternal conditions. Our findings suggest that the interplay between iron and lead status in fetal development is complex and gender-specific. Further study of the cellular mechanisms is necessary.

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NEONATOLOGISTS' PRACTICE AND PERSPECTIVES REGARDING THE DIAGNOSIS OF VENTILATOR ASSOCIATED PNEUMONIA

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Background: The prevalence of neonatal ventilator-associated pneumonia (VAP) varies widely among reports. Whether this variability is related to variation in clinical care or variability in the clinical diagnosis of this condition is unknown. The National Health Safety Network (NHSN) has devised VAP diagnostic criteria for surveillance reporting. We hypothesized that many of the clinical factors used by the NHSN definition are not considered important factors in the clinician's decision making process. **Methods:** A questionnaire was developed to evaluate the importance of 24 potential clinical, radiologic and laboratory factors that may be used to diagnose VAP. SurveyMonkey™ was utilized to administer the survey and maintain respondent confidentiality. This questionnaire was distributed to ~3000 physician members of the AAP perinatal listserv. Physicians were asked to rank the importance of clinical factors using a 5 point Likert scale. The factors receiving the maximum importance rating as well as the percentage of respondents rating criteria commensurate with the NHSN definition of VAP were determined. **Results:** 274 survey responses were analyzed yielding a response rate of 9%. Of the respondents, 73.8% were from academic centers and the majority practiced in level IIIb units with >20 beds. Factors clinicians identified as maximally important for the diagnosis of VAP included: Chest x-ray with new, progressive or persistent infiltrate (62.4%), increased ventilator support (45.7%), increased FiO2 requirement (35.6%), 4+ growth on tracheal aspirate (TA) culture (35%), many white blood cells on TA microscopy (34.6%), and predominant growth of potential pathogen on TA culture (32.8%). Only 10.5% of neonatologists selected criteria consistent with the NHSN definition as maximally important. Excluding CXR and worsening gas exchange, 57% of neonatologists did not identify any of the additional NHSN diagnostic criteria as very important in the diagnosis of VAP. **Conclusions:** While chest x-ray findings and worsening gas exchange agree in part with NHSN criteria, a gap exists between neonatologists' diagnosis of VAP and the existing NHSN definition. Development and validation of a clinical predictive model aimed at assisting in a clinically relevant and consistent diagnosis of VAP in the neonatal population is needed.

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EOSINOPHILIA AT 6 MONTHS, AN INDICATOR OF RECURRENT WHEEZING IN INFANCY, AND IRON STATUS AT BIRTH

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Introduction: Recurrent wheezing in infancy is a risk factor for asthma, the most prevalent childhood chronic disease in the U.S. Some children with asthma also exhibit a higher incidence of infantile iron deficiency (ID). The epidemiological association between these two diseases may be biological, as lower plasma iron in cord blood was directly linked to persistent wheezing in infancy. Since eosinophilia (eosinophils $\geq$ 4% of total WBCs) at 6-12 months of age is a biomarker for recurrent wheezing in infancy, we hypothesized that poorer iron status at birth was directly correlated with eosinophilia at 6-12 months. **Methods:** Newborns of at least 35 weeks gestational age with one or more risk factors for ID were recruited. Risk factors included: maternal ID, maternal diabetes, fetal overgrowth or undergrowth, mothers from ethnic minority groups, or those with lower socioeconomic status. Cord blood indices of storage iron (plasma ferritin), steady state erythrocyte (RBC) iron (ZnPP/H), and recent RBC iron (reticulocyte-enriched ZnPP/H) were measured. At 6-12 months, eosinophil % and absolute eosinophil counts were measured. Linear regression and unpaired t tests examined the relationship between eosinophil % and iron status at birth. **Results:** No linear relationship was found between either 6-12 month eosinophil % or absolute eosinophil count and any cord blood iron status index. Similarly, no difference was found in 6-12 month eosinophil % or absolute eosinophil count between infants in the highest and lowest cord blood plasma ferritin terciles. We found a trend for higher cord blood recent RBC iron (reticulocyte-enriched ZnPP/H) in those with eosinophilia (168$\pm$45μmol/mol) versus those with a lower eosinophil % (121$\pm$6 μmol/mol), p=0.061. The difference between recent RBC iron and steady state RBC iron (ΔZnPP) was significantly higher in those with eosinophilia (60$\pm$34 μmol/mol) versus those with a lower eosinophil% (24$\pm$3 μmol/mol), p=0.036. **Conclusions:** Our findings suggest that late gestational ID may be correlated with eosinophilia at 6-12 months. We will continue to evaluate iron status at birth in the context of other risk factors for recurrent wheezing and asthma, such as family history and respiratory infections in infancy. There is need for further exploration of the biological mechanisms between infantile ID and childhood asthma.

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EPIDEMIOLOGY OF CHOLESTASIS IN EXTREMELY LOW BIRTH WEIGHT INFANTS

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Objectives: Cholestatic jaundice (CJ) is a frequent complication in extreemly low birth weight infants (ELBW) with small for gestational age infants (SGA) infants at particular risk. We sought to identify the risk factors for CJ in this population. **Design/Methods:** Data from preterm infants $\leq$28 weeks and $\leq$ 1000 grams were reviewed retrospectively over an eight year period. SGA infants with CJ (group 1) were compared to appropriate for GA (AGA) infants with CJ (group 2) and matched by GA in a 1:2 ratio with SGA infants without CJ (group 3). CJ was defined as conjugated bilirubin $>$1.5 mg/dl without elevated hepatic transaminases that persisted for $>$1 week. Data were analyzed using Wilcoxon tests for univariate analyses; significant covariates were subjected to multivariate analyses with logistic regression to detect significant risk factors. **Results:** 44/901 (4.9%) infants developed CJ of which 17 (38.6%) were in group 1 and 27 (61.4%) were in group 2; 33 infants were identified in group 3. On univariate analyses, GA, birth weight (BW), multiple gestation, maternal hypertension, antenatal steroid use, use of midazolam, hydrocortisone and ionotropes for hypotension, number of blood transfusions (BT), sepsis with hypotension, necrotizing enterocolitis, duration of total parenteral nutrition including Intralipid use, number of days of withholding feeds after first feed (days NPO) were found to be significant between the three groups. However, on multivariate analyses, only GA (**p=0.004**), BW (**p<0.001**), days NPO (**p=0.01**) and BT (**p=0.003**) were found to be significant between the groups. The mean ($\pm$SD) birth weight were higher in group 2 compared to group 1 (850.7 $\pm$ 195.6 vs. 598.9 $\pm$100.8 grams) and group 3 (850.7$\pm$195.6 vs.630.8$\pm$117.0 grams), **p<0.001** for both. Mean ($\pm$SD) GA was lower in group 2 compared to group 1 (25.4$\pm$1.4 vs. 26.2$\pm$1.2, **p=0.006**) and group 3 (25.4$\pm$1.4 vs. 26.3$\pm$1.4,**p<0.01**). The median (25th-75th centile) number of days NPO were higher in group 1 vs. group 2 (18.0 (9.235.0) vs. 11 (3.7- 27.7), **p=0.04**). The median (25-75th centile) number of BT were higher in group 1 vs. group 3 (6.0 (3.0-9.0) vs. 0.0 (0.0-0.7), **p<0.001**) and group 1 vs. group 2 (6.0 (3.0-9.0) vs.0 (0.0-4.0), **p=0.003**). **Conclusions:** SGA status, duration of NPO and BT are risk factors for CJ in ELBW infants.

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TREATEMENT OF INFANTILE HAMANGIOMAS WITH PROPRANOLOL

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Background: Propranolol has recently been described as a treatment for infantile hemangiomas in numerous case reports with 100% success. We have treated 23 infants and children with propranolol and the purpose of this study is to review our experience. **Methods:** Prior to institution of propranolol therapy, infants were evaluated by cardiology. Therapy was begun at 2 mg/kg/day. Infants $<$ 6 months were admitted for 24 hour heart rate monitoring for 48 hours. Photographs of the lesions were obtained prior to initiation of therapy and to document response. Patients were evaluated every 11-3 months for response to the drug and monitor dose. The dose of propranolol was adjusted to achieve a 10-15% decline in heart rate. **Results:** Twenty-three patients were begun on therapy. There were 20 females and 2 males. Mean age of patients was 6.6$\pm$10 months (range 1 $-$46 months). Eight patients had received prior therapy, including oral or intralesional steroids and/or laser therapy. Lesions were located on the head and neck (X), subglottic (2), leg (1). Indications for therapy included interference with vital structure or function (X). potential for tissue destruction (x) pain with ulceration (X) and cosmetic (x). Eleven patients had completed therapy and were treated for an average of 8.4$\pm$3.3 months. Of the 21 patients with cutaneous lesions, 19 had a response to therapy within 24 hours. **Conclusions:** Propranolol is an effective therapy for infantile hemangiomas with an acceptable side-effect profile. Treatment with propranolol is a reasonable first line medication, but some infants will require additional therapy. Within the first 24 hours we noted a change in color and/or softening of the lesion. Subsequest response was slower and more variable. 2 patients with sub-glottic lesions required multiple laser therapies and one require addition of steroid therapy. 4 patients required a reduction in dose and X patients required an increase up to 3-5 mg/kg/day to achieve a change in heart rate.

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RADIATION FREE (NON-FLUOROSCOPIC) ABLATIONS OF VENTRICULAR ARRHYTHMIAS IN CHILDREN AND YOUNG ADULTS

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Background: Electrophysiology has conventionally utilized fluoroscopy for catheter guidance, placing the patient and operator at risk for radiation exposure. The advent of 3-D mapping systems has led to minimal and even radiation free EP cases , which may be especially important in children, as they are at a greater risk than adults for radiation related adverse events, and may require multiple procedures over their lifetime. Ventricular arrhythmias often require longer fluoroscopic times and non-fluoroscopic procedures have yet to be described, The purpose of this study was to report the feasibility of minimal or no fluoroscopy in the ablation of ventricular arrhythmias in the pediatric population. **Methods:** A retrospective review was performed of all patients $<$21yrs who underwent ablation of ventricular tachycardia using 3-D mapping with no or minimal fluoroscopy at a single institution. Demographic, clinical, and procedural data was reviewed. **Results:** Four patients underwent EP study for VT or frequent PVCs. The mean age = 14$\pm$4.5 years and weight = 58$\pm$16kg. Two patients had the diagnosis of frequent right sided PVCs with dilated LV and evidence of depressed or low normal function. The third patient with right ventricular PVCs had an unknown etiology of syncope. The fourth patient presented with a left posterior fascicular VT which recurred on antiarrhythmic medication. No fluoroscopy was utilized in the patients with right sided arrhythmias. The patient with left posterior fascicular VT received 1.0 min of fluoroscopy to confirm appropriate femoral venous access and to correlate the site of successful ablation to a fluoroscopic image. Electroanatomic mapping with 3-D NavX was used in all patients. The sites of successful termination of the arrhythmia included $\sim$9 o'clock on the ventricular side of the tricuspid valve, two RVOT tachycardias, and the mid left ventricular septum. One patient developed a RBBB during the procedure which resolved in the recovery period with no other complications. The mean procedure time was 142.75 +/- 71.6 minutes. No complications occurred and there has been no recurrence at a mean follow-up 135$\pm$80 days. **Conclusions:** 3-D electroanatomic mapping can be utilized to perform fluoroscopic free ablations for the treatment of pediatric ventricular arrhythmias.

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ENGINEERED CARDIAC TISSUE AS A NON-HYPERTROPHIED MODEL SYSTEM OF HYPERTROPHIC CARDIOMYOPATHY.

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Mice in which cMyBP-C have been ablated (cMyBP-C KO) have been studied as a model for hypertrophic cardiomyopathy (HCM). cMyBP-C KO mice display severe cardiac hypertrophy as early as 10 days of age. Most physiologic studies of cMyBP-C ablation are performed in mice in which hyertrophic remodeling has already occurred, obscuring primary effects of ablation on contractile function. We have developed a novel method of culturing neonatal mouse cardiomyocytes in a 3-D matrix to form engineered cardiac tissue (ECT). We hypothesize that while hypertrophic remodeling occurs between postnatal day 1 and day 10 in the developing heart of cMyBP-C KO mice, the same remodeling does not occur in the mechanically unloaded ECT. In order to test this hypothesis, expression levels of several hypertrophic response genes were assessed. RNA was extracted from ECT, neonatal, 10 day, and 5 week old wild type and cMyBP-C KO cardiomyocytes. cDNA was reverse transcribed and quantitative PCR performed to measure expression levels of several hypertrophic marker genes. Expression levels of the early hypertrophic response marker genes, ANP and BNP, were unchanged in cMyBP-C KO vs. WT ECT. Expression levels of ANP and BNP were significantly higher in cMyBP-C KO hearts compared to WT not only at the 10 day timepoint, but also in neonatal cMyBP-C KO hearts ($P<0.001$, $N=5$ per group), indicating that upregulation of these genes precedes overt hypertrophy. An isoform switch from α to β MyHC is generally associated with the development of ventricular hypertrophy in mice. This switch was observed in hearts of the cMyBP-C KO at the 10 day timepoint as an upregulation in expression of β MyHC ($P<0.05$) and a downregulation in α MyHC. This switch was not observed in either ECT or hearts from neonatal animals. In conclusion, while hypertrophic remodeling takes place in hearts by day 10 and early response genes are already upregulated in the neonatal cMyBP-C KO heart, ECT do not undergo the same hypertrophic remodeling. This study provides evidence that ECT is a suitable model system for studying the functional effects of HCM causing mutations in the absence of confounding hypertrophy.

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AUTOPHAGY AND INNATE IMMUNITY DURING VARICELLA INFECTION IN CHILDREN

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Autophagy is a normal cellular process that degrades whole organelle components and has been observed to play a role in innate immunity by enhancing the presentation of viral antigens on the surface of an infected cell. Yet its role in innate immunity to viruses has been discovered only recently. Therefore, we postulated that autophagy may be a previously unrecognized immune mechanism following infection with varicella and also immunization with the live attenuated varicella vaccine. To investigate, we first observed that varicella infection induces the production of two sentinel autophagy proteins: the microtubule associated Light Chain 3 isoform B (LC3B) protein as well as the autophagy marker p62/SQTM1. Further, we observed organelles with double membranes characteristic of autophagosomes in images of the same Varicella Zoster Virus (VZV) infected cells. Since many of our studies of VZV induced autophagy were performed entirely in cell culture, we have included new experiments with cells from varicella and zoster vesicles to show that autophagy is a component of VZV infection during both primary infection and reactivation. Our studies documented that the cells extracted from the vesicle consisted of 77% keratinocytes and 22% T-cells (consisting of approximately equal numbers of CD4+ and CD8+ cells). All of the keratinocytes were infected and exhibited punctate LC3B staining indicative of autophagosomes. We found evidence that VZV induced ER stress may precede and induce autophagy, namely, we observed that the ER morphology in infected cells became disorganized and swollen and the alternative splicing of the X Box Protein 1. These virus-induced alterations closely resembled cellular morphology upon treatment with tunicamycin, one of the best characterized inducers of ER stress. In earlier studies in infected cells, we found evidence for the Unfolded Protein Response (UPR), a sequel of ER stress. When taken together, these results suggest that autophagy in VZV infected cells is likely induced by ER stress and the resulting UPR. In turn, these same mechanisms could extend the life of an infected cell. Finally, these results emphasize marked differences between autophagy after infection with VZV (which lacks an inhibitor) and HSV-1 (which has the ICP 34.5 inhibitor of autophagy).

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CORRELATES OF IMPULSIVITY AND INATTENTION IN CHILDREN BORN PREMATURELY

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Background: Previous research has demonstrated an increased risk for inattention/impulsivity issues in children born prematurely. Risk factors for this population include male gender, younger gestational age, lower SES and birth weight, higher neonatal risk, and increased abnormal neurological status. Method of measurement has often been based on parental/teacher reports and ratings by independent observers. The purpose of the current study was to evaluate levels of inattention and impulsivity on a computerized assessment in school-aged children born prematurely compared to those born at term. **Methods:** School-aged children born prematurely who participated in a red blood cell transfusion study at birth (n = 46; 59% male) and a control sample from the same geographic location (n = 55; 45% male) were tested at ages 7 – 16 years of age (Mean (SD) = 12.6 (2.17) Preterm & 11.8 (2.6) Full Term). Subjects completed a computerized test of inattention/impulsivity (CPT) and intelligence testing. Parents completed a demographic questionnaire and chart reviews were completed on hospital records from those born prematurely. **Analyses:** Levels of impulsivity, inattention, and processing speed were compared between children born prematurely and those born at term, evaluating potential gender interactions and controlling for age and SES. Follow-up ANOVAs and Pearson Correlations evaluated interaction effects and relationship to specific NICU variables within the preterm sample. **Results:** There was a group x gender interaction effect for level of inattention and processing speed; no significant differences were found for levels of impulsivity. Preterm girls had significantly higher omission errors (inattention) and slower processing speed compared to Full term girls and Preterm boys. Post-hoc Pearson correlations found that Preterm girls demonstrated a lack of improvement with age, compared to other sample groups. Worse performance for Preterm girls was correlated to incidence and treatment for sepsis (a marker for infection). **Conclusion:** Contrary to previous research, increased incidence of inattention was specific to girls born prematurely, not boys. This was not correlated to most illness severity factors (e.g., GA, BW or neurological markers), but to increases in incidence of sepsis. Findings are discussed in relation to gender-specific risks in premature birth and connection to learning disability research.

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COMPARISON OF NONINVASIVE AND CENTRAL ARTERIAL BLOOD PRESSURE MEASUREMENTS IN THE NEONATAL INTENSIVE CARE UNIT

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Objective: To determine if non-invasive and central arterial blood pressure measurements correlate reliably across neonatal weight groups. **Design:** We conducted a retrospective cohort study of infants admitted to a single center from October 2009 to March 2010 who required central umbilical arterial access. Data was collected on 21 infants ranging from 24-39 weeks gestational age, and 720-4250 grams. Paired central and non-invasive blood pressure (NBP) readings were compared. Prior to data analysis infants were grouped according to very low birth weight (VLBW > 2500 grams. The data was analyzed for intraclass correlation across the groups for systolic, diastolic, and mean arterial pressure. **Results:** We obtained a total of 684 total paired readings of which 294 readings were in the VLBW group, 182 in the LBW group, and 208 in the > 2500 gram group. For all measured variables, the reliability of the NBP decreased as infant weight group decreased. In the VLBW and LBW groups, the values for systolic, diastolic, and mean arterial pressure were below accepted cutoff values for reliability (0.7).

Group	Intraclass Correlation Systolic	Intraclass Correlation Diastolic	Intraclass Correlation Mean Arterial Pressure
VLBW <1500 grams	.493	.620	.626
LBW 1501–2500 grams	.652	.634	.676
>2500 grams	.803	.815	.790

Conclusion: In VLBW and LBW infants cuffed blood pressure measurements do not accurately reflect central arterial blood pressures, and should be used with caution in clinical decision making.

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PREMATURE THELARCHE IN INFANTS AND TODDLERS: PREVALENCE AND ENVIRONMENTAL DETERMINANTS

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Background: An increasing prevalence of early breast development in girls may be an effect of estrogen-like compounds in the environment. **Purpose:** The aim of this study was to measure the prevalence of premature thelarche in infant and toddler girls and to determine if environmental estrogens are associated with early breast development.**Methods:** This cross-sectional study recruited girls aged 12 to 48 months from a general pediatric clinic in an urban teaching hospital. We conducted an interview survey with the mother as well as a chart review of both the mother and the child. The presence of premature thelarche was assessed by visualization and palpation. Statistical analysis of the data included Wilcoxon Rank Sum test and odds-ratio calculations. **Results:** Among the 318 subjects, the prevalence of premature thelarche was measured at 4.7%. The prevalence by race/ethnicity was 4.2% among White Non-Hispanics, 4.6% among African Americans and 6.5% among White Hispanics. The peak prevalence occurred between 12-17 months of age. No statistically significant relationship was found between premature thelarche and environmental exposures. Upon follow up, 44% of the cases of premature thelarche had persistent breast development. **Conclusion:** Our study demonstrated a higher prevalence of premature thelarche than was previously reported. This study lacked power because of the small number of premature thelarche cases and the relatively small influence each environmental component has on this condition. Future studies need to employ a very large sample in order to accurately analyze the relationship between environmental toxicants and premature thelarche.

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LOCALIZED HYPERGLYCEMIA IS SUFFICIENT TO IMPAIR FETAL GROWTH

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Purpose: Intrauterine growth retardation complicates 7% of diabetic pregnancies. Postulated mechanisms include chronic uterine artery vasculopathy, placental insufficiency, and hyperglycemia. We sought to determine the effect of localized hyperglycemia on fetal growth in the absence of maternal pathology. **Methods:** Fetuses in the left uterine horn of pregnant Sprague-Dawley rats were selectively exposed to hyperglycemia via catheterization of the left uterine artery. Dextrose in 0.9% saline was infused on gestational days 18-20 at a rate of 0-4 mg/min and 20 μl/min. Blood flow to the two uterine arteries was traced by 15 μM fluorescent microspheres infused via the aorta. Upon completion of the infusion, fetuses were collected by Cesarean section. Measurements from the left versus right uterine horns were compared using Student's *t* test. **Results:** Infusion of 4 mg/min dextrose produced severe localized hyperglycemia (2397 $\pm$ 1245 mg/dL) in the left uterine artery, despite normal (89 $\pm$ 5 mg/dL) glycemia in the maternal systemic circulation. Fetal weight in the left versus right uterine horn was progressively reduced by the infusion of 1-4 mg/min dextrose. At 4 mg/min the mean fetal weights from the left versus right uterine horn were 2.89 $\pm$ 0.12 and 3.88 $\pm$ 0.07 g, respectively, $p < 0.00005$. By contrast, infusion of 0 mg/min dextrose produced no difference in fetal weights from the left versus right uterine horn (3.61 $\pm$ 0.09 and 3.79 $\pm$ 0.12 g, respectively). Likewise, dextrose infusion reduced blood flow to the placentae of the left uterine artery in a dose-dependent manner, with 4 mg/min dextrose reducing relative blood flow by 3.9 $\pm$ 1.0 fold ($p < 0.05$). **Conclusions:** Localized hyperglycemia in late gestation is sufficient to impair fetal growth associated with reduce blood flow to the placental. Our novel approach in the rat allows exposure of the fetus to hyperglycemia, without affecting maternal systemic glycemia thus avoiding a myriad of confounding secondary physiologic maternal responses.

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PREDICTING MORTALITY IN FETAL NEWBORNS

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Background: Resuscitation of fetal newborns (FN) [23 0/7–24 0/7 weeks gestational age (GA)] can result in medical, ethical, legal and financial dilemmas. Despite rare intact survival, these FN continue to be resuscitated. A paucity of data exists in this unique group. The objective was to determine if antenatal and postnatal variables were associated with lethal outcomes which might guide the Neonatal practitioner. **Study Design:** Over a 20 year period (1987–2007), we retrospectively evaluated our experience with 74 FN who were resuscitated and survived to be admitted to the NICU. Antenatal factors examined included chorioamnionitis, preterm premature rupture of membranes (PPROM), and mode and location of birth. Postnatal factors that were evaluated included five minute Apgar score, severe intraventricular hemorrhage (IVH) (grade 3 or 4) and gender. IRB approval was obtained and this project was supported by a grant. **Results:** Of the 74 FN evaluated, the mean GA and birth weight were 23 5/7 weeks and 581 grams, respectively, with 60/74 (81%) inborn. The mortality rate was 33/74 (45%) with the average length of stay until death of 82 days. Antenatal factors associated with a lethal outcome (Table 1) were the absence of chorioamnionitis (54% vs 16%), PPROM (57% vs 42%), and Cesarean section (C-section) (50% vs 39%). Birth in a non-tertiary center was not associated with a significant increased mortality (45% vs 43%). Postnatal factors associated with a lethal outcome were Apgar score $\leq$ 6 at 5 minutes (52% vs 29%), presence of severe IVH (44% vs 33%), and male gender (54% vs 30%). **Conclusions:** Absence of chorioamnionitis, presence of PPROM, delivery via C-section, 5 minute Apgar score of $\leq$ 6, severe IVH and male gender are factors that were associated with increased mortality in FN. This data could assist in the formation of management and end of life care decisions for these newborns.

Table 1. Clinical Variables Associated with Morality in FN (n=74)

Clinical Variable A	Clinical Variable B	Mortality A	Mortality B	Confidence
Chorioamnionitis (n=19)	No Chorioamnionitis (n=52)	3/19 (16%)	28/52 (54%)	99.5%
PPROM (n=7)	No PPROM (n=64)	4/7 (57%)	27/64 (42%)	63.9%
C-Section (n=30)	Vaginal Delivery (n=44)	15/30 (50%)	17/44 (39%)	78.2%
5 min Apgar $\leq$ 6 (n=50)	5 min Apgar > 6 (n=24)	26/50 (52%)	7/24 (29%)	94.5%
Mild IVH (n=24)	Severe IVH (n=45)	8/24 (33%)	20/45 (44%)	73.8%

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NATURAL HISTORY OF FETAL PELVIECTASIS

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Background: Pelviectasis (PVS) is a common anomaly detected on antenatal fetal ultrasound (AUS) and is seen in up to 1% of all pregnancies. Majority of PVS resolve spontaneously. The aim of this study is to follow the natural progression of PVS in an unselected obstetric population. **Methods:** This was a single-centered, retrospective study that included all Level II ultrasounds done at Mount Sinai Hospital in Chicago from Jan 2008 to Dec 2009. The AUS were classified for PVS as mild (5-7mm), moderate (7.1-9mm) or severe (>9.1mm) based on the measurements of renal pelvic diameter. Postnatal data included neonatal Renal Ultrasound (RUS), Voiding Cystourethrogram (VCUG) and Nuclear Scan (NS). Post-natal outcomes were classified as "Resolved", "Improving", "Stable" and "Worsened" based on the RUS, VCUG and NS. **Results:** During the study period 98 cases of fetal PVS were detected. Of these 16 patients delivered at an outside hospital and 34 infants did not have a complete postnatal follow-up at our institution and were excluded. Of the remaining 48 cases of fetal PVS, 19 (39.5%) were classified as mild, 12 (25%) as moderate and 17 (35.4%) as severe. In 40 of the 48 (83.3%) infants the PVS either resolved or remained stable on the postnatal follow-up, while in 8 of the 48 (16.6%) infants the PVS worsened. 5 patients (5/19 - 26.3%) in the mild group had worsening of disease, of which 2 had reflux on VCUG and underwent surgery. 1 patient (1/12 – 8.3%) in the moderate group had worsening PVS, however no reflux was present. 2 patients (2/17 - 11.7%) in the severe group had worsening PVS with no reflux and did not require surgical intervention. However, 3 (3/17 – 17.6%) patients in the severe group that remained stable in the postnatal period, later required surgical intervention for ureteropelvic junction obstruction/hydroureter. **Conclusion:** Although, majority of the cases (83.3%) either resolved spontaneously or remained stable, the degree of fetal PVS did not correlate with postnatal outcome. 26.3% of infants in the mild group had worsening disease. Hence all cases of fetal PVS should be followed postnatally till resolution or until a diagnosis is obtained.

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INTERFERON REGULATORY FACTOR 6 IS A REGULATOR OF CUTANEOUS WOUND HEALING

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Mutations in *Interferon Regulatory Factor (IRF6)* cause two orofacial clefting syndromes, Van der Woude (VWS) and Popliteal Pterygium (PPS). In addition to an oral defect, we recently show that VWS patients are more likely to have wound complications after corrective cleft surgery than patients with isolated cleft. Thus we hypothesize that *Irf6* is critical to wound repair. We used in vitro and in vivo approaches to test our hypothesis. In vitro, live imaging reveal that keratinocytes from *Irf6* null mice were delayed in closing an in vitro scratch wound, potentially because of a lack of directional migration. Furthermore, examination of cellular structure of *Irf6* null keratinocytes shows more stress fibers after phalloidin staining. In vivo, Irf6 is expressed in the suprabasal layer of the epidermis and in the migrating leading edge keratinocytes re-epithelializing the wound, and appears to require transforming growth factor beta 3 (Tgfb3) for proper expression. Indeed, injection of neutralizing antibody against Tgfb3 decreased Irf6 expression and promoted keratinocyte proliferation. We are currently testing directly the role of *Irf6* in an in vivo cutaneous wound healing by using a conditional knockout approach. Broadly, these data suggest that *Irf6* is necessary for events critical to proper cutaneous wound healing. Identification of genetic variants in Irf6 could have clinically relevant impact on wound healing, by better anticipation of complications or altering approaches or timing for surgical repair.

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ALCOHOL ON UNDERGRADUATE MALES' FACEBOOK PROFILES

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Purpose: Perceived peer alcohol use is a predictor of consumption in college males; frequent references to alcohol on Facebook may encourage alcohol consumption. The purpose of this study was to perform a content analysis of male undergraduates' Facebook profiles for references to alcohol use. **Methods:** Facebook profiles of male undergraduate students at a large Midwestern university were identified through a search of public profiles (n=225). Profiles were included if they reported age of 18-22 years old and were currently enrolled undergraduates. Content analysis of Facebook profiles included references to alcohol. Prevalence was identified and compared by grade and age. ANOVA was used to compare means between groups. **Results:** Profiles of 225 undergraduate males were evaluated; average age was 19.9 years. Alcohol references were present on 85.3% of profiles; the prevalence of alcohol was similar across each undergraduate grade. The average number of alcohol references per profile was 8.5, but increased with undergraduate year (p=.003; CI:1.5-7.5). Students who were of legal drinking age referenced alcohol an average of 4.5 times more than underage students. **Conclusion:** Our data showed similar prevalence of alcohol use to existing survey data; alcohol references on Facebook may reflect offline alcohol experiences by college students. The average number of references to alcohol per profile increased with age. This may be due to the upper grades having more students of legal drinking age, or an accumulation of references over time. Future research is needed to evaluate the validity of and motivation for displaying alcohol references on Facebook.

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USING HYDROCORTISONE TO FACILITATE EXTUBATION IN INFANTS WITH SEVERE BPD

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Hydrocortisone (Hc) use in early postnatal age (PNA) has been demonstrated to shorten mechanical ventilation days with no significant adverse effects on neurologic outcome. However, early use of Hc carries a greater risk of gastrointestinal (GI) perforation. The efficacy of late use of Hc (> 1 month PNA) has not been reported. **Objective:** We examined the efficacy and adverse effects of late Hc in ventilator weaning in infants with severe BPD over 1 month of age. **Methods:** This is a retrospective, IRB-approved chart review of all preterm infants treated with Hc (5 mg/kg/day taper every 3 days over 9 days) from January 2006 to January 2010. Data collection included demographics, respiratory support, and extubation rates. Extubation success was defined as extubation during therapy. Data on short-term and developmental evaluation performed at 6 to 12 months of corrected age were collected. Data were analyzed using chi-square and pooled variance t-test. **Results:** Fifteen infants treated with Hc during the time period were included. Mean gestational age(GA) and birth weight (BW) were 25.9 ± 1.2 weeks and 750 ± 204 grams. Extubation success (responder) occurred in 60% of infants; 2 infants were reintubated Between the responder and non-responder, there were no differences in gender, African American race, GA, BW, baseline FiO_2 and mean airway pressure (MAP). Cumulative Hc dosing regimen was similar between both groups (23.7 mg/kg vs 25.6 mg/kg). Infants that responded to extubation received treatment at a lower PNA and postmenstrual age (PMA) compared to non-responder (59.6 vs 97.9 days and 34.1 vs 39.7 weeks, respectively, $p < 0.05$). Days on ventilator prior to treatment were also shorter in the responder group (55.2 vs 73.1 days, $p < 0.05$). Death and transfer-out-of hospital were significantly higher in non responders. Elevated mean arterial blood pressure was the most common adverse effect. No infants developed GI perforation. Other adverse events were similar between the groups. Number of infants with Bayley Scales of Infant Development scores <85 at 6 to 12 months of corrected age was significantly less in the responder group. **Conclusions:** Late use of Hc appears to facilitate extubation in some ventilator-dependent BPD infants. However, the efficacy significantly decreases as PNA and PMA increase. Based on our findings, earlier initiation of Hc treatment for ventilator weaning in infants with severe BPD may be considered if Hc treatment is determined to be beneficial.

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UNRELENTING SUBCLINICAL ATHEROSCLEROSIS IN OBESE CHILDREN WITH MULTIPLE RISK FACTORS

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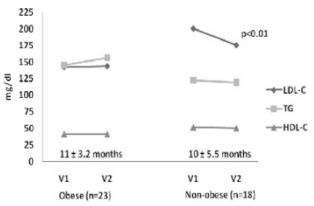
Obese children may have multiple, modifiable atherosclerosis promoting risk factors which can impact the vasculature prematurely. **Objectives:** Do clinic based, life style interventions change Carotid Artery Intima Media Thickness (CIMT) in high-risk, obese children compared to non-obese, dyslipidemic children? **Methods:** Risk factor profile {BMI Z score, systolic blood pressure (SBP), LDL C, triglyceride (TG), High density lipoprotein cholesterol (HDL C), Insulin and tobacco smoke exposure history} and CIMT of 23 obese and 18 non-obese children who were seen at least twice (Visit 1-V1 and Visit 2-V2) were analyzed. **Results:** Age at V1- Obese 11.5 ± 3.0 years, Non-obese 14.1 ± 3.1 years (p<0.01). Sex, race, and tobacco smoke exposure history were comparable. Children who were obese at V1 had a decreasing risk for obesity (OR=0.16, p=0.03) and those non-obese at V1 had an increasing risk for obesity at follow up (OR=6.5, p=0.03). SBP did not differ between groups and overtime. LDL C, HDL C and TG trends are illustrated in the graphic. Hyperinsulinemia, noted in a third of the obese group only, did not change over time. Modifiable risk factors # - (V1, V2) - Obese 3.6 ± 1.1, 3.8 ± 1.2; Non-obese 1.6 ± 1.1, 1.7 ± 1.1 (p<0.01). CIMT (mm) - (V1, V2) - Obese 0.57 ± 0.05, 0.57 ± 0.05; Non- obese 0.57 ± 0.06, 0.57 ± 0.06. A "statin therapy eligible" obese child had a 33% chance of being commenced on therapy compared to a 75% chance if non-obese. **Conclusions:** Obese children were younger but had a higher number of risk factors and comparable CIMT with little improvements noted with clinic based, life style interventions. The emergence of obesity in non-obese, dyslipidemic children may have impacted their vascular response to statins. Compliance with recommendations to begin statin therapy was unmet in many obese children, perhaps due to clinician anticipation of desirable change with life style counseling. High risk, obese children should be treated with lipid modifying therapy in addition to life style counseling *at the outset,* as they do not demonstrate desirable improvements with life style counseling alone.



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TRANSCRANIAL MAGNETIC STIMULATION IS WELL TOLERATED IN THE PRESURGICAL ASSESSMENT OF MOTOR TRACT FUNCTION

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Purpose: Intraoperative monitoring of motor tract function with transcranial electric stimulation (TES) is currently done routinely in patients during surgery in which there is potential risk of motor tract impairment so that the risk of paraplegia or paraparasis can be reduced. A preoperative assessment of motor tract function prior to these types of surgeries can be useful clinically. TES is generally not tolerated in patients who are awake. Transcranial magnetic stimulation (TMS) is a non-painful technique that produces a motor evoked potential by noninvasive cortical stimulation using a high intensity magnetic field. This is an ongoing prospective review of its use at our institution as a preoperative assessment in patients with scoliosis. **Methods:** TMS was performed with stimulation over the motor cortex and recording over the brachioradialis and tibilalis anterior muscles bilaterally. Using the Magstim Model 200 Stimulator (MagStim Company Ltd, Whitland, Wales, and UK), TMS was delivered through a double cone coil. An intensity of 70% stimulator output (~1.4T) was used. A hand held coil was used and positioned tangentially to the scalp to find the location of maximum reliability for MEPs approximately 2 cm anterior to the C3 or C4 positions on the 10-20 EEG electrode system. **Summary:** One hundred scoliosis patients have been tested at our institution using transcranial magnetic stimulation. No complications have occurred during testing and none reported after discharge from the testing. Potential complications can include tongue or lip lacerations, headaches or excessive or intolerable pain. **Conclusions:** This review suggests that Transcranial magnetic stimulation as a preoperative assessment is both safe and well tolerated in the pediatric scoliosis population. This testing may be very useful when done prior to surgery as a preoperative assessment of current motor tract function and can allow discovery of motor tract asymmetries which may be of clinical concern and require additional evaluation.

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DRINKING TO MAKE FRIENDS: EXPLORING THE LINKS BETWEEN ALCOHOL USE AND PEER GROUP SIZE IN FIRST-YEAR UNDERGRADUATE STUDENTS

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Purpose: Freshman year in college is a high-risk time in which binge drinking is common. Freshmen often report social motives for drinking. This study aimed to explore the links between types of alcohol consumption and peer group size among college freshmen. **Method:** Data were collected from college freshmen via an hour-long interview assessing 28-day alcohol use using the Time Line Follow Back (TLFB) method. Participants were categorized into abstainers, "social" drinkers (no binge drinking) and binge drinkers (at least one binge drinking episode). Peer group size was assessed via self-reported number of total new college friends and new close friends. ANOVA was used to compare number of friends across the three categories of drinkers. **Results:** Overall, 60 freshmen participated (68.9% response rate), and 60% were female. At least one binge drinking episode was reported by 63% of participants, with an average of 2.9 episodes in the past 28 days. Participants reported a mean of 27.5 (SD=16.6) new friends, of which 6.7 (SD=3.5) were considered close friends. Social drinkers reported highest number of new friends (mean= 33.3, 95% CI = 25- 41.5), followed by binge drinkers (mean= 27.9, 95% CI = 22.6 33.2), and then abstainers (mean= 12.9, 95% CI = .84- 25) (F=2.91, p=0.03). No significant differences were found in mean number of close friendships. **Conclusion:** Findings support the assertion that alcohol may aid in establishing a large social network in the first year of college. However, non-binge drinking appeared to be a more fruitful method for achieving this goal. Future binge-drinking prevention efforts may benefit from advocating social (rather than binge) drinking.

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METABOLOMIC MARKERS OF NEPHROTOXICITY IN NEWBORN RATS

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Background: Acute Kidney Injury (AKI) in the intensive care setting may be multifactorial and is associated with significant morbidity and mortality. Many of the pharmaceutical agents (i.e. gentamicin) that are widely used in NICU have nephrotoxic side effects which may result in neonatal AKI. Routinely used measures of renal function, such as blood urea nitrogen (BUN) and serum creatinine, have severe diagnostic limitations. **Objective:** The purpose of this study is to identify novel metabolomic urinary markers that provide real time evidence of evolving neonatal renal injury thereby allowing earlier intervention. **Methods:** Newborn rats were divided into 2 groups. The first group (n=6) was injected with gentamicin intraperitoneal daily on days 3-10 of life. Control rats (n=6) were injected with equivalent volumes of vehicle (0.9% saline). Urine was collected 24 hours after the last dose. Metabolites present in the urine were evaluated using LC-MS (liquid chromatography mass spectrometry); detected peaks were aligned into 247 groups according to their mass (m/z) and retention time (rt). Blood samples were collected at the end of the study for biochemical analysis. Comparisons were made by two-tailed T-test. Data are presented as mean ± SE. **Results:** BUN and creatinine values were significantly higher in the gentamicin group compared to controls, BUN: (51.1 ± 5.89 vs 31.5 ± 1.64 mg/dl p< .005) creatinine: (1.56 ± 0.38 vs 0.37 ± 0.08 mg/dl p< .005). At autopsy kidneys from gentamicin exposed rats weighed significantly less than the control group, kidney weight / body weight: (4.04 ± 0.44 vs 4.93 ± 0.14 p< .005). LC-MS demonstrated seven urinary metabolites with a significant (p<.005) fold change (FC) in the gentamicin group compared to controls: (m/z): 322 (rt: 99, FC: 8.9), 132 (rt: 144, FC: 3.23), 160 (rt: 100, FC: 6.64), 240(rt: 102, FC: 7.68), 166 (rt: 521, FC: 1.74), 254(rt: 134, FC: 3.3), 205 (rt: 555, FC: 1.81). **Conclusion:** Urine small molecules detected using LC-MS may provide candidate biomarkers of nephrotoxicity. Identification of detected metabolites is ongoing.

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IN-VIVO VASCULAR DYNAMICS IN AN ANIMAL MODEL OF PULMONARY HYPERTENSION: FEASIBILITY OF USING AN OVER-THE-WIRE ULTRASOUND PROBE

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Purpose: Intravascular ultrasound (IVUS) measures prognostically important pulsatile flow related indices compared to traditional continuous flow based indices in patients with pulmonary hypertension (PH). Guiding catheter size limits use in infants. Feasibility of using a commercially available IVUS probe and pressure wire without a guiding catheter to study real time vascular dynamics was evaluated in a neonatal swine model of hypoxic PH. **Methods:** Seven domestic swine (weight 1.4-2.2 kg, age 8-48 hours) were raised in a thermo-neutral isolette and exposed to isobaric normoxia (n=4) or hypoxia (n=3, FiO2 10-12%) for 72 hours. External jugular veins and internal carotid arteries were isolated and cannulated (5 Fr and 4 Fr introducers respectively) by surgical cut down. Heparin was administered for anticoagulation and a hemodynamic study was performed. The distal pulmonary artery was cannulated with a 5 Fr balloon tipped catheter and exchanged over a 0.014" guide wire for a 3.5 Fr. 20 MHz Eagle Eye Gold catheter (Volcano Corp., CA) without a guide. IVUS was performed at baseline and during 100% oxygen, and hypoxia and hypercarbia. **Results:** The IVUS probe was manipulated over a 0.014" wire without a guide catheter in all animals. Vessels ranging from 2.2-5.9 mm were adequately imaged. Hypoxic animals demonstrated thickening of the intima-media compared to normoxic animals (mean 0.23 vs. 0.35 mm). Clinically usable images to calculate pulsatile flow indices (pulsatility and distensibility) were feasible under all experimental conditions. The pressure wire did not accurately measure pulmonary artery pressure in neonatal animals. Simultaneous pressure measurement with a second catheter was feasible and performed in 4 animals. **Conclusions:** A major limitation for use in infants is overcome with adequate imaging without a guiding catheter. With proper wire calibration, simultaneous pressure measurement and placement of the IVUS catheter over the same wire would preclude repeated catheter exchanges and need for additional venous access. Simultaneous pulsatile and continuous flow indices can be easily measured while also obtaining anatomic information about the lumen and vessel wall. This would be a valuable addition in the armamentarium for assessment of PH in both the research and clinical setting. Over-the-wire feasibility enhances use in small animals and infants.

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RISK OF MORTALITY SCORES IN THE PEDIATRIC INTENSIVE CARE UNIT (PRISM III) IS A POOR PREDICTOR OF HOSPITAL CHARGES AND COSTS

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Introduction: US healthcare expenditure is rapidly expanding. It is documented that end of life costs represent 10-12% of the total US healthcare budget and up to 27% of the Medicare budget. However, this spending relationship to mortality has not been shown in the pediatric population. **Hypothesis:** Pediatric Risk of Mortality (PRISM III) score has a positive predictive relationship to hospital costs and charges. **Methods:** The study analyzed all patients hospitalized in an academic pediatric critical care unit between January 1 and December 31, 2009. PRISM III scores, clinical and demographic data from an internal clinical database were cross-referenced with cost and charge data from the hospital's decision support database. Statistical analysis was conducted with Stata IC/11.1. The Institutional Review Board approved this study. **Results:** Of the 850 patients identified through the clinical database, 45 patients (5.3%) were excluded from the analysis due to lack of appropriate financial data, as their hospitalizations were 0-1 days and no pediatric intensive care bills generated. 1 patient (0.1%) was excluded from the analysis due to a negative cost balance from the hospital decision support data. Of the remaining 804 patients, the male to female ratio was 53:47 and the medical to surgical patient ratio was 45:55. Mean values were as follows: age at admission – 8.0 years, length of stay – 6.1 days, PRISM III scores – 3.18, total charges – $72,574, total costs – $32,443, average daily charges – $12,020, and average daily costs – $5,428. Simple linear regressions of total charges, total costs, average daily charge, and average daily cost against PRISM III scores were conducted. For total charges, slope = 8078.6, 95% CI = 6274.9 to 9882.3, $t_{802} = 8.79$, $p < 0.001$, y = 46901.3 + 8078.6x, $r^2 = 0.088$. For total costs, slope = 3364.3, 95% CI = 2538.5 to 4190.2, $t_{802} = 8.00$, $p < 0.001$, y = 21752.0 + 3364.3x, $r^2 = 0.074$. For average daily charge, slope = 203.7, 95% CI = 76.8 to 330.6, $t_{802} = 3.15$, $p = 0.002$, y = 11372.4 + 203.7x, $r^2 = 0.012$. For average daily cost, slope = 32.3, 95% CI = −27.0 to 91.6, $t_{802} = 1.07$, $p = 0.285$, y = 5325.5 + 32.3x, $r^2 = 0.001$. **Conclusions:** PRISM III score has a positive relationship to total charges and costs. Despite p values being significant for both analyses, the low r^2 values indicated a poor fit of model. In comparison, the p value for average daily charge was statistically significant but that of average daily cost was not statistically significant. Nevertheless, both r^2 values continued to show a poor fit of model. Thus, although having an overall positive effect, given the consistently low r^2 values, PRISM III score was a poor predictor for total and average daily hospital charges and costs.

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REFERENCES TO DEPRESSION ON FACEBOOK

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Purpose: Depression is prevalent among college students and can have adverse effects on a college student's health. Despite the prevalence of depression on college campuses, many students do not seek evaluation or treatment. This study used the social networking site Facebook to determine whether status updates referencing depression were associated with self-reported depression symptoms. **Methods:** The public Facebook profiles of 18-20 year olds at two public universities were evaluated by trained coders. DSM-IV criteria were used to identify text references ("status updates") suggesting depression. A 20% subsample of profiles were evaluated by two coders for interrater reliability, Cohen's kappa for depression coding was 0.6. All participants were invited to complete a clinical survey measuring depression symptoms. Negative binomial regression was used for analysis. **Results:** Of 229 respondents, 45.5% were male and the average age was 18.8 (SD 0.7). The response rate for survey completion was 77%. Depression references were present on 32.7% of participant's profiles. Participants who displayed depression on Facebook had approximately 31% higher scores on the depression scale compared to those who did not display depression on Facebook (exp(B)=1.3, p=0.02). There were no noted gender differences. **Conclusion:** Many college students use status updates on Facebook to display reference to depression. Because these students had higher scores on the self-reported depression scale, it is possible that Facebook could be used as a tool to identify depression in students that may not otherwise seek help.

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FETAL CARDIAC MALFORMATIONS: CLOMIPHENE CITRATE AND OTHER INFERTILITY TREATMENTS

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Background: Nearly 12% of women of reproductive age in the United States receive infertility treatments. Clomiphene is one of the treatments used by women of child bearing age. It has a variable half life, and can be present in the maternal circulation for several weeks. Fetal cardiac development could be affected if exposed to clomiphene or its metabolites during cardiogenesis (6 to 8 weeks of gestational age). Congenital cardiac defects have been reported after clomiphene use, although the precise effect has not been documented. **Aim:** Our cross sectional study compared the prevalence odds of cardiac malformations in fetuses of mothers referred for fetal echocardiography who received clomiphene versus other infertility treatments. **Materials and Methods:** Fetal echocardiography data from the University of Minnesota hospitals were reviewed. Presence of cardiac malformations and types, risk factors, maternal history and indications for fetal echocardiography were documented. Fischer's exact test was used for analysis. **Results:** Among 1057 fetal echocardiograms there were ten fetuses conceived with maternal clomiphene use. Two (20%) had severe congenital heart defects. One of these had critical aortic stenosis with hydrops and in-utero demise and the other had hypoplastic left heart syndrome. Nineteen fetuses were conceived with other infertility treatments, one (5.26%) of which had transposition of great vessels. The prevalence odds ratio is 4.5 (P=0.26) for developing fetal cardiac defect with maternal clomiphene use compared to other inferitility treatments. **Conclusion:** Two severe congenital heart defects (20%) were identified with clomiphene use and one (5.26%) with other infertility treatments. Our observations suggest a potential association between cardiac defects and clomiphene treatment. Larger studies are warranted to further document this relation.

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EXPOSURE TO HYPERGLYCEMIA IN UTERO RESULTS IN SEX SPECIFIC CARDIOVASCULAR CHANGES IN ADULTHOOD.

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Purpose: The intrauterine environment strongly influences adult disease susceptibility. Our objective was to use a rat with maternal diabetes to test the hypothesis that offspring exposed to hyperglycemia *in utero* display increased blood pressure and alterations in *in vivo* vascular responsiveness in adulthood. **Methods:** Pregnant rats were administered streptozotocin on d 13 of gestation (term 21 d) to induce diabetes. Chronically catheterized adult offspring (6-12 months) were studied beginning 48 hrs after surgery. Blood pressure (BP) and heart rate (HR) responses to various doses of phenylephrine and nitroprusside were studied to assess baroreflex function. L-NAME (nitric oxide synthase inhibitor) and chlorisondamine (ganglionic blocker) were used to assess the contribution of nitric oxide and sympathetic tone to resting BP, respectively. Dose response to angiotensin II before and after L-NAME administration was performed to assess the contribution of NO in buffering the pressor response. **Results:** Male but not female ODM had increased mean BP compared to controls (122 ± 6 vs. 99 ± 5; $P < 0.05$), while resting HR was similar. For both sexes, BP and HR responses to phenylephrine were similar between ODM and controls while female but not male ODM had increased hypotensive responses to nitroprusside. Hemodynamic responses to angiotensin II, L-NAME and chlorisondamine were similar in both sexes between groups although blood pressure increases to angiotensin II after L-NAME were significantly greater in female but not male ODM compared to controls. **Conclusion:** Exposure to hyperglycemia *in utero* results in sex specific cardiovascular changes in adult offspring. Baseline hypertension in adult male ODM is not due to overt differences in autonomic and NO pathways. Female ODM have an exaggerated hypertensive response to angiotensin II following NO blockade with L-NAME, suggesting increased sensitivity to NO pathway activation in this group that may buffer the development of hypertension.

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ARRHYTHMIAS AFTER THE FONTAN OPERATION: EXTRA-CARDIAC CONDUIT

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The Fontan operation, first described by Fontan and Baudet in 1973, revolutionized the management of children with single ventricles. Since its inception, the procedure has undergone numerous modifications. Chief among these was the development of the total cavo-pulmonary connection (TCPC) which was shown to produce a better outcome compared to the original atrio-pulmonary connection. Currently there are two types of TCPC, namely the intra-cardiac lateral tunnel (ILT) and the extra-cardiac conduit (ECC). As their names imply, the ILT is a tube made out of the patient's own right atrium which connects the inferior vena cava to the pulmonary artery. The ECC, on the other hand, utilizes a tube graft which connects the inferior vena cava to the pulmonary artery, thus bypassing the right atrium entirely. One of the main advantages cited for the ECC is that by avoiding suture lines in the right atrium and by not subjecting the right atrial wall to higher pressures, there is likely to be a much improved long term outcome in terms of a lower incidence of arrhythmias. Studies so far have given conflicting results. The purpose of this research is to assess the incidence of bradyarrhythmias and tachyarrhythmias with the ECC (extra-cardiac conduit) type of TCPC (total cavo-pulmonary connection). A retrospective study of bradyarrhythmias and tachyarrhythmias with the ECC type of TCPC was performed. All patients of any age who underwent an ECC before 2/26/08 at CMH were included. Measures included; documented bradyarrhythmias causing death or need for pacemaker implantation, and/or documented tachyarrhythmias causing death or clinical symptoms and needing management with any of the following treatment options: drug therapy, antitachycardia pacing, direct current cardioversion or defibrillation, catheter ablation, or arrhythmia surgery.

Results of 105 patients demonstrated that 64% of children did not have arrhythmia < 6 months after ECC (95 % CI 0.54 to 0.72). 91% of children were arrhythmia free at 5 year follow up (95% CI 0.83 to 0.95). There was no significant difference between early and late bradyarrhythmias after ECC ($p = 0.41$). There was a significant difference between early and late tachyarrhythmias after ECC ($p = 0.03$).

In conclusion, there was found to be a low incidence of bradyarrhythmias and tachyarrhythmias with the ECC type of TCPC. There is a need to conduct a large study to compare incidence of brady and tachyarrhythmias between the ILT and the ECC to help better establish standard of care.

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EVIDENCE OF GENETIC INTERACTION BETWEEN SNPS IN CANDIDATE GENES THAT MAY CONFER INCREASED RISK FOR CL/P

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Clefts of the lip and/or palate (CLP) are a common complex birth defect caused by genetic and environmental factors and/or their interactions. CLP affects 180,000 births worldwide per year and its occurrence varies by geographic origin and socioeconomic status. In the past couple of years a handful of genes have been highly associated or linked to the occurrence of CL/P, including *IRF6*, *FOXE1, MAFB, ABCA4* and the gene desert region, 8q24. In a case control cohort from Denmark we calculated population attributable risk (PAR) and odds ratio (OR) for the most significant SNPs in the above mentioned candidate genes. The two most significant PARs were found for rs13041247 in *MAFB,* PAR 26 % - OR 1.8 and for rs987525 in 8q24, PAR 16 % - OR 3.1, for individuals being homozygous for the risk allele. Moreover, the combined analysis for these two SNPs showed an increased risk for those individuals homozygous for both variants of PAR 32 % - OR 10.7. We are presenting evidence of genetic interaction between SNPs in *MAFB* and 8q24 and more important a 10 fold increased risk for individuals in the Danish population that carry both risk alleles for the two variants on these genes. These finding could take us step closer to understand gene by gene interactions and apply our knowledge to genetic counseling and prevention.

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ZINC PROTOPORPHRYIN/HEME RATIOS (ZNPP/H) FROM FILTER PAPER SPOTS AS A NEWBORN SCREEN FOR IRON DEFICIENCY (ID)

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Background: Iron deficiency (ID), a common nutrient deficiency, can impair brain development. Earlier recognition of at-risk children can help prevent long-term neurocognitive morbidity. ZnPP/H is an available, cost effective and sensitive biomarker for incomplete iron incorporation into erythrocytes. ZnPP/H, a potential candidate for newborn screening of iron deficiency, is measurable on rinsed umbilical cord blood and is potentially measurable on filter paper blood spots. Bilirubin interferes with readings of ZnPP/H, but preliminary findings suggest that this interference may be reduced with bilirubin oxidase. Our aims were to examine methods to eliminate interfering substances to obtain accurate measurements of ZnPP/H on filter paper specimens. **Methods:** In de-identified cord blood samples collected at birth in EDTA, we measured whole blood and rinsed ZnPP/H levels by hematofluorometry. Blood was spotted onto Whatman 903 filter paper, similar to the collection of blood used for the newborn screening. Specimens were dried and eluted from the paper using different elutions. ProtoFluor® reagent was used to shift absorbance wavelengths of deoxyhemoglobin to oxyhemoglobin; catalase and superoxide dismutase were studied to prevent oxidation of lysed RBC's; and bilirubin oxidase was studied to reduce heme breakdown to bilirubin. A PBS elution was used as a control. **Results:** Filter paper ZnPP/H ratios after elution were higher, compared to rinsed blood. Elution with PBS was correlated with rinsed blood $R^2 = .74$, $p < 0.01$. Elution with ProtoFluor® was correlated with rinsed blood, $R^2 = .73$, $p < 0.011$. Elution with catalase/SOD was correlated with rinsed blood, $R^2 = .81$, $p < 0.01$. Bilirubin oxidase showed the best correlation with rinsed blood, $R^2 = .85$, $p < 0.01$. **Conclusion:** Of the treatments studied, bilirubin oxidase treatment yielded the strongest relationship between filter and rinsed ZnPP/H. Bilirubin oxidase reduces interference of the breakdown products of heme formed during lysis of RBC's on filter paper. Although further studies are needed to validate the use of ZnPP/H from blood on filter paper, using filter ZnPP/H on dried blood spots may be feasible.

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RETROSPECTIVE CHART REVIEW OF CHILDREN WITH TYPE 2 DIABETES MELLITUS EVALUATING THE EFFICACY OF METFORMIN VS. INSULIN VS. COMBINATION INSULIN/METFORMIN THERAPY

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Objective: Type 2 Diabetes Mellitus is a growing problem in the pediatric population and there is currently no consensus on the best treatment method. **Method:** We conducted a retrospective chart review on patients with type 2 diabetes mellitus who presented for treatment at the Diabetes Center at Nationwide Children's Hospital between March 2003 and March 2008. Data was collected on therapy type, BMI, and A1C over a 6 month follow up time. Therapy type was divided into metformin alone, insulin alone, or combination insulin and metformin. **Results:** At baseline, the only significant difference in A1C was between the metformin group and the combination therapy group ($p = 0.012$). BMI was not significantly different among the groups at baseline. ated analysis of variance with hemoglobin A1C at baseline as a covariate revealed that the only predictor of change in hemoglobin A1C over time was the hemoglobin A1C at onset of therapy ($p < 0.001$). Therapy type did not predict a change in hemoglobin A1C over time ($p = 0.905$). Additionally, repeated analysis of variance of BMI showed that a greater BMI at onset predicted a greater decrease in BMI ($p = 0.006$). Therapy type did not predict a change in BMI over time ($p = 0.517$). **Conclusions:** Metformin alone is as effective in the treatment of type 2 diabetes mellitus as insulin or combination therapy of insulin and metformin.

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VIEWS OF ADOLESCENTS ON TECHNOLOGIES TO PROMOTE AND IMPROVE FITNESS

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Purpose: Obesity is a significant health problem affecting adolescents. Adolescents' widespread use of technology may provide new opportunities to promote fitness, for example by personalized feedback and information. The purpose of this study was to investigate current use of communication technology by adolescents and perspectives on how technology could be used to improve fitness. **Methods:** Participants between the ages of 11-19 years old were recruited from a general pediatric clinic and completed a 10-question paper survey. Questions examined current concerns about weight, and assessed both current use and perception of potential for fitness-promotion of six technologies: social networking sites, email, cell phone, text messaging, Ipod, and video games. **Results:** Surveys were completed by 101 participants (84% response rate). They were 67% female and had a mean age of 15.5 years old. The majority of participants (90%) had concerns about their weight. For all six technologies assessed, reported use was greater than 70%, regardless of gender. Three technology choices were equally favored for future use as a fitness-improving method (1) emailing a health care provider or nurse, (2) utilizing a GPS device, and (3) Ipod + Nike (accelerometer). **Conclusions:** The majority of adolescents in a clinic setting report concerns about their weight, and are already utilizing many different forms of technology for communication. This familiarity with communication technology offers new opportunities to promote and reinforce physical activity and improved fitness in adolescents.

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LOWER INTAKE OF PHOSPHORUS, PROTEIN AND VITAMIN D IS ASSOCIATED WITH RICKETS AT SIX WEEKS OF LIFE IN INFANT WITH BIRTH WEIGHT LESS THAN 1250 GRAMS

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Background: Preterm infants are at significant risk of rickets. While deficiency of Vitamin D (VD) and phosphorus (PO4) are known predisposing factors, the impact of other nutritional components essential for bone development (calcium (Ca) and protein) on the occurrence of rickets in preterm infants is not clear. **Objective:** To evaluate the effect of Ca, PO4, VD and protein intake in the first 4 weeks of life as well as VD blood level on the development of radiological rickets at 6 weeks of life. **Design/Methods:** This is a prospective study involving infants with a birth weight less than 1250 grams. The study was approved by the IRB at UHCMC. Patients with congenital malformations or bone disease were excluded. Daily intake of Ca, PO4, VD and protein from enteral and parenteral sources was calculated. Rickets was diagnosed by wrist X-ray at 6 weeks of life by one radiologist blinded to the patients. Blood levels of VD and parathyroid hormone (PTH) were obtained at 6 weeks of life. Two way ANOVA was used to compare the effect of cumulative intake of all nutrients and student t-test was used to compare VD and PTH levels, between patients with and without rickets. Data is presented as mean±SE. **Results:** Twenty one infants completed the study with a mean GA of 25.3±0.4 weeks (23-31) and birth weight of 725±35 grams (500-1100). Ten infant developed rickets. The cumulative intake of PO4, VD and protein at 4 weeks of life was significantly lower in infants with rickets. VD and PTH levels were similar in the two groups. Table below summarizes our findings.

Cumulative Intake at 4 weeks	Normal (n=11)	Rickets (n=10)	P value
Ca (mg/kg)	1662 ± 108	1333 ± 125	0.081
PO4 (mg/kg)	1166 ± 52	924 ± 60	0.01
VD (IU)	1855 ± 93	1283 ± 107	0.001
Protein (gm/kg)	82.4 ± 0.9	75.8 ± 1.0	0.001

Conclusions: This is the first study to report a correlation between rickets and protein intake in preterm infants. Early emphasis on adequate supplementation of protein, VD and PO4 is essential in the prevention of rickets in the most vulnerable infants.

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GENETIC VARIANTS IN: *TFAP2B* IN PRETERM INFANTS WITH PATENT DUCTUS ARTERIOSUS

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Background: Patent ductus arteriosus (PDA) is a common complication of prematurity and has a significant inherited component. Our previous candidate gene study found association of PDA with DNA variants in transcription factor AP-2 beta (*TFAP2B*), the gene mutated in Char syndrome, a syndromic form of PDA seen in term infants. We hypothesized that sequencing the regions in *TFAP2B* with significant SNP associations would uncover the etiologic common variant causing the association with PDA and might further identify rare high-risk variants. **Methods:** DNA samples and clinical information was obtained from the University of Iowa neonatal DNA repository. Cases were defined as preterm infants born at ≤ 30 weeks gestational age with PDA, diagnosed by standard echocardiography. Controls were preterm infants ≤ 30 weeks gestational age without PDA. Exons 3, 4, 5, and 6 of *TFAP2B*, as well as three highly-conserved intronic regions, underwent DNA sequencing in 95 cases and 95 controls. **Results:** We identified 17 total sequence variations in cases, involving 11 different individuals and 7 unique loci. None of these polymorphisms were found in control samples. We also identified 3 sequence variations in controls involving 3 individuals (one polymorphism per individual), all at unique loci and none of these polymorphisms appeared in cases. The frequencies of observed polymorphisms between cases and controls were significantly different ($p=0.0014$, Fisher's exact test). All of the polymorphisms found in PDA cases were in conserved non-coding regions, with several in close proximity to known transcription factor binding sites, including IK3, HXA5, CUX1 and PBX1. **Conclusions:** Rare variants in conserved regulatory regions of *TFAP2B* may predispose extremely preterm infants to PDA. Replication studies are currently underway to validate these findings and future studies will focus on the biological function of the identified variants. Understanding genetic risk factors for PDA may lead to improvements in diagnosis and management.

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RADIOLOGICAL CONTRAST STUDIES IN NEONATES ADMITTED TO THE NEONATAL INTENSIVE CARE UNIT

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Background: Studies have quantified plain radiographs performed in premature neonates admitted to the neonatal intensive care unit (NICU) and the resulting burden of radiation.No study to date describes the radiological contrast studies in the NICU. **Aim:** To describe the number of contrast radiological studies neonates and compare the radiation to plain radiographs. **Methods:** A retrospective chart review of all surviving neonates admitted to the NICU in 2008 was performed.Neonates with incomplete charts were excluded.Data collected included demographic details , length of stay (LOS-),number of days on the ventilators and number of plain radiographs and contrast studies performed-.Contrast studies include upper and lower gastrointestinal studies (UGI and LGI) ,swallow studies, voiding cystourethrograms (VCUG)and computed tomography (CT) scans.Congenital defects included abdominal wall defects , bowel obstruction ,congenital heart defects and neural tube defects . Dose of radiation incurred from contrast studies was extrapolated from published studies (Wilson –Costello et al 1996, Damilakis et al 2006 and Staton et al 2007). **Results**: Neonates ($n=163$) with and without ($n=439$) congenital defects were compared.The BW, GA and LOS were significantly different in the two groups ($p=0.001$).The number of days on ventilator was not significantly different. Contrast studies were performed in 79/163 (47%)of babies with congenital defects and 62/439 (14%) in those without defects. One UGI study was estimated to be equivalent to 117 plain radiographs of the chest.A VCUG was equivalent to 12 and a CT scan of the head equivalent to 7800 plain radiographs based on estimates in literature.No estimate for LGI was found. **Conclusion:** Contrast studies are performed in about one fifth of neonates in the NICU.Almost fifty percent of neonates with birth defects are subjected to contrast studies.These studies expose neonates to considerably higher radiation than plain radiographs.Newer techniques of radiographic examinations may have decreased the dose of radiation.Studies are needed to investigate the clinical utility of these tests and studies to measure accurate dose of radiation from these radiological studies are needed.Risk of malignancy from radiation in the NICU is not established.

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AORTIC ARCH RECONSTRUCTION DOES NOT AFFECT AUTONOMIC TONE IN NEONATES UNDERGOING CARDIAC SURGERY

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Background: Heart rate variability (HRV) is a predictor of sudden death in adults for cardiac and non-cardiac conditions. Neonates who have undergone cardiovascular surgery involving the aortic arch appear to be at relatively greater risk for alterations in their autonomic tone because of the proximity of the sympathetic innervations. In addition, hypoxia appears to alter HRV. The purpose of this study was to evaluate HRV from birth to 2 months of age in newborns who have undergone cardiac surgery in comparison to normal newborn controls. **Hypothesis:** Infants undergoing cardiac surgery involving arch reconstruction will have decreased sympathetic tone due to disruption of the cardiac sympathetic innervation. **Methods:** Demographic, clinical data and 24 hour electrocardiogram (ECG) recordings were obtained at birth and post-operatively on infants with abnormalities undergoing: aortic arch reconstruction; Norwood operation: and a Blalock-Taussig shunt. Controls consisted of normal newborns without cardiac disease from birth to 2 months of age. ECG recordings were analyzed in 5 minute segments using time domain and frequency domain (Lomb periodogram) measures. Statistical analysis included one way ANOVA; post-hoc comparisons were performed with the Bonferroni test. **Results:** Pre-operatively, normal newborns had increased low frequency (LF) power, decreased high frequency (HF) power, and increased LF:HF (a indicator of higher sympathetic tone) compared to infants undergoing an aortic arch reconstruction or the Norwood procedure. After surgery, infants undergoing arch reconstruction had a similar LF power and LF:HF to normal newborns, which was significantly higher than the Norwood or BT shunt group. Subjects who underwent aortic arch reconstruction had significantly higher oxygen saturations before and after surgery compared to the Norwood and BT shunt groups. **Conclusion:** The significantly higher LF power and LF:HF ratio preoperatively in normal newborns was consistent with previously published data correlating severity of illness to sympathetic tone. After surgery, infants who underwent aortic arch reconstruction and normal newborns exhibited a similar autonomic balance, weighed toward more sympathetic tone compared to the Norwood and BT shunt groups. We conclude that hypoxia plays a larger role in the alteration of autonomic tone than aortic arch reconstruction.

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EVIDENCE FOR SHORTENED ATRIOVENTRICULAR SEPTA IN FAMILIES OF CHILDREN WITH NON-SYNDROMIC ATRIOVENTRICULAR SEPTAL DEFECTS

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Background: AVSD include a range of anomalies characterized by the involvement of the atrial and/or ventricular septa and the abnormal development of the AV valves. The atrioventricular septum (AVS) is the portion of the septal tissue that separates the right atrium from the left ventricle. Deficiency of the AVS contributes to the AVSD phenotype. Shortening of the AVS in families of children with non-syndromic AVSD might be reflected in the threshold model of disease where the liability for individuals who inherit an increased number of AVS shortening alleles exceed a threshold and an AVSD occurs. **Methods:** The AVS length (AVSL) was measured in three apical four-chamber views in echocardiograms of unaffected parents from families that were identified through a child with non-syndromic AVSD ($n=463$) and in parents of families with no history of congenital heart disease ($n=49$). Similar measurements were made in unaffected siblings of cases ($n=365$) and siblings of controls ($n=41$). The mean AVSL measurement was adjusted for BSA. Univariable and multivariable analyses were performed to evaluate for association with age or gender. The distribution of the standardized measurements was evaluated for evidence of admixture using a likelihood ratio test. Ten percent of the entire sample was re-measured by a secondary investigator. A paired t-test was performed to evaluate interrater reliability. **Results:** No significant differences were seen between case and control families in terms of % male, age, weight, and height; the normalized AVSL was significantly shorter in case parents and case siblings ($p<0.0001$). Age and gender were associated with normalized AVSL in the case parent and sibling groups. There was good interrater reliability of AVSL measurements ($p=0.831$). There was significant evidence for admixture in the case parent and sibling groups ($p=0.0303$); no evidence of admixture was noted in the control parent or sibling groups. **Conclusions:** Evidence for two component distributions from the analysis of case parents and case siblings suggests the presence of a minor phenotype for non-syndromic AVSD. Broadening the definition of AVSD to include those with a shortened AVSL may increase the power of genetic association and mapping studies to identify susceptibility genes.

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DOWN SYNDROME AND PROSTHETIC VALVE SIZE TO BODY WEIGHT RATIO PREDICTIVE OF DEATH FOLLOWING LEFT ATRIOVENTRICULAR VALVE REPLACEMENT AFTER PRIMARY ATRIOVENTRICULAR SEPTAL DEFECT REPAIR

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Background: While the results of atrioventricular septal defect (AVSD) repair have improved dramatically since the first repair, development of significant left atrioventricular (AV) valve regurgitation continues to occur in some patients following surgery, necessitating additional surgical interventions, including valve replacement. The aim of this study was to identify prognostic factors following left AV valve replacement in patients following primary AVSD repair. **Methods:** Using the Pediatric Cardiac Care Consortium database, descriptive analyses of reoperation characteristics were performed in patients with previously-repaired AVSDs. A prosthetic valve size to body weight ratio was calculated for each patient who underwent valve replacement. Univariable and multivariable linear regression analyses were performed. Survival curves were constructed evaluating freedom from repair or replacement. In addition, Cox proportional hazards models were constructed to aid in the identification of significant covariates. **Results:** A total of 370 patients were included in the study – 127 underwent left AV valve replacement, 243 repair. Time to first repair was 2.25 ± 3.17 years in the repair group and 1.35 ± 2.20 years in the replacement group; time to valve replacement was 2.00 ± 3.53 years. Multivariable age-adjusted predictors of earlier time to valve replacement included presence of Down syndrome, presence of post-operative left AV valve stenosis, and moderate to severe left AV valve regurgitation. One-year survival of patients undergoing left AV valve replacement was significantly worse compared to those undergoing repair ($p<0.0001$). Multivariable predictors of death following valve replacement included the presence of Down syndrome (hazard ratio 2.24, 95% confidence interval 1.03-4.86) and prosthetic valve size to body weight ratio (hazard ratio 1.92 per mm/kg, 95% confidence interval 1.25-2.95). **Conclusions:** A prosthetic valve size to weight ratio of greater than 2 is a predictor of death following left AV valve replacement. Additionally, patients who have previously undergone primary AVSD repair and have Down syndrome are at greater risk of death following left AV valve replacement.

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SCREENING OF THE LOCUS 8q24 IN PATIENTS WITH CLEFT LIP AND PALATE

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Cleft lip and/or palate (CLP) is a common birth defect of complex etiology. Clefts are the major birth defect disruptors of facial structures and affect 1 in 700 births and require surgical, nutritional, dental, speech, and behavioral interventions. About 70% of individuals are born with an isolated cleft and no other structural or cognitive abnormalities.

Family and population studies have confirmed a genetic component underlying nonsyndromic CLP. However, the precise genes involved in human CLP remain largely unknown. The first genome wide association study (GWAS) published on CLP reported a highly significant association with markers in a gene desert located within chromosome region 8q24.21. The study characterized a region of 640Kb on 8q24.21 as containing one or more common variants associated with CL/P in European populations. This novel locus for CLP was confirmed by two different replication studies with European populations.

We report the ongoing analysis of the 8q24.21 region using different approaches such as GWAS, direct sequencing, DNA microarray in samples of patients with CLP derived from European and Asian populations as well studies with mouse models.

The goals of this study are to screen the 8q24 region for potential pathogenic mutations and microdeletions that can contribute to cleft etiology. We sequenced conserved regions in the 640kb interval and identified a number of new variants in cases with cleft that were not found in control samples. Two particular region of interest have been confirmed by studies with mouse models as potential transcriptional enhancers that play important role during the craniofacial development. The association of gene desert regions with human disease highlights the importance of examining genomic regions outside of transcribed DNA that may contain important regulatory elements; the presence of mutations and/or microdeletions in such regions can cause misregulation of target genes involved in human diseases such as CLP. The identification of these genomic variants will help to understand the role of this new candidate region in cleft etiology and lead to the identification of new regulatory regions that play a role in craniofacial development.

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OVERCOMING BARRIERS TO ENROLLMENT OF LATINA FAMILIES IN PERINATAL HEALTH RESEARCH

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Background: Maternal minority status is a risk factor for infantile iron deficiency (ID). Preliminary research suggested that measures of iron in umbilical cord blood are abnormal in babies born to minority mothers. Consequently, a subsequent prospective study was designed to examine the impact of maternal minority status on infant ID. Because English may be a second language for many minority mothers in the population, it was anticipated that language and cultural differences might limit the research participation of these at-risk children. As part of a larger prospective examination of ID in infancy, the purpose of the present study is to quantify consenting Latina enrollees and refusals, and identify participation barriers and their possible solutions. **Methods:** Mothers, with their full-term newborns, were eligible for the study if the women were anemic, diabetic during pregnancy, of minority status, of lower socioeconomic status, and/or delivered an infant large- or small-for-gestation. Self-reported ethnicity and reasons for participation refusal were documented. **Results:** 255 mother-newborn pairs were enrolled. Because demographics of women delivering at the hospital were known, expected enrollment based on ethnicity was predicted. Based on the percentage of minority women admitted to the birthing center in a year, 25% minority recruitment was predicted, and 27% was achieved. Although the expected values for other minority enrollees were observed, the number of Latina enrollees was half the expected level (16.3%), with only 8% recruitment observed ($p<0.01$). Several recruitment barriers were encountered that fall into three main categories: system barriers, researcher perception barriers, and participant perception barriers. Because the Latina participation refusal rate did not make up for the 50% lower Latina enrollment, both researcher and caregiver bias may have been significant enrollment barriers. Additionally, in comparison to the other groups, Latina women seemed to weigh more heavily upon the involvement of other family members in the decision to participate. **Conclusion:** Enrollment barriers may pose a significant challenge in the recruitment of Latina women, but inclusion of this at-risk and fast-growing population in research is vital. Better understanding of enrollment obstacles early in study implementation can help to offset them.

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INFANT CPRANTIME: SELF-INSTRUCTIONAL CPR TRAINING FOR PATIENTS OF HIGH-RISK INFANTS

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Background: Premature infants (PRE) or infants with congenital heart disease (CHD) have a high risk of respiratory or cardiac arrest within the first year. Our purpose was to assess a self-instructional DVD kit for families of high risk infants. We hypothesized that comfort level of providing CPR would increase; the kit would be shared with other caregivers and would be reviewed at regular intervals. **Methods:** Parents were given a self-instructional CPR kit, completed a questionnaire, reviewed the DVD, and practiced CPR before discharge. Parents were asked to share the kit with other care-providers, practice CPR every 3 months and respond to questionnaires at 4 and 12 months. The questionnaire surveyed prior CPR training, comfort level doing CPR, plans to share the kit with other care providers and to review the kit. Four and 12 month surveys assessed comfort level (5 point Likert scale), number of persons who reviewed the kit, and review frequency by the parent. **Results:** We enrolled 311 parents. Seventy-five percent of parents had prior CPR training. Comfort level increased from 2.8 at baseline to 3.3 at 4 months to 3.5 at 12 months ($p = .0281$). The kit was shared with 2.8 persons and was reviewed by the parent 1.8 times during the 12 months. There were 8 events: choking (3) and CPR (5). Six infants survived. **Conclusions:** There was a significant increase in caregiver comfort level at 4 and 12 months compared to baseline, despite a high level of prior CPR training. There was a marked multiplier effect for the number of persons trained. Easily available self instruction provides an excellent method of CPR training for parents of high risk infants and likely contributed to a high survival rate.

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ADOLESCENT'S DISPLAY OF DEPRESSION ON FACEBOOK: A KEY TO DIAGNOSIS?

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Purpose: Depression is a common illness among college students that is underdiagnosed and undertreated. This project examined depression references on Facebook status updates- a feature that is used to display one's current experiences and emotions. The purpose of this study was to determine whether people who update their Facebook profiles more frequently were more likely to disclose references to depression. **Methods:** Facebook profiles of undergraduate students at two large public universities were identified through a search of public profiles. Profiles were included if they reported age of 18-20 years old and were currently enrolled undergraduates. Content analysis of Facebook profiles included examining status updates for depression using DSM-IV criteria, a status update count, and demographic information. A 20% subsample of profiles was evaluated by two coders for interrater reliability; Cohen's kappa for depression was 0.6 Analysis compared the mean number of status updates between those who did and did not display references depression using tests. **Results:** Of the 252 profiles included in the study 59.9% were male. Overall 25% of profiles displayed one or more references to depression. The mean number of status updates for depression displayers was 19.1 (+/-17.9) and for non-displayers 9.2 (+/- 12.5) ($p=0.00$). **Conclusion:** Our data showed that the more status updates displayed on a Facebook profile, the more likely one is to display depression. College student Facebook users who display depression may be using Facebook as a way to express depression; alternatively, more frequent Facebook use may lead to higher levels of disclosure of emotions including depression.

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SURVEY OF PEDIATRIC HEALTH PRACTITIONERS' COMFORT WITH ADOLESCENT HEALTH TOPICS

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Purpose: Health care providers commonly identify adolescent health topics as challenging. It is unclear whether this discomfort stems from inadequate training or could be remedied by further education once in practice. The purpose of this study is to determine which adolescent health topics are inadequately addressed during adolescent medicine training, whether comfort level changed since training completion, and whether additional training is desired. **Methods:** A paper survey was administered at a regional general pediatrics conference attended by providers in pediatrics and family medicine. The survey used a 5-point Likert scale and asked for participant's current comfort level, satisfaction with training, perceived change in comfort level, and interest in further training in five common adolescent health topics (sports medicine, contraception, sexual orientation, substance use and eating disorders). Analyses included ANOVA to assess differences in mean scores across health topics. **Results:** Surveys were returned by 75 people (75% response rate). Participants were 73% female, 65% MD, 17% mid-level providers, 58% pediatrics, in practice for a mean of 7.5 years. Participants reported an overall comfort level of 4.5 regarding working with adolescent patients. Participants reported being least comfortable addressing sexual orientation (mean 2.6, $p=0.01$), they reported the lowest satisfaction with training about sexual orientation (mean=2.6, $p=0.005$) and that they were no more comfortable addressing sexual orientation than when they finished residency (mean=2.6, $p=0.07$). However, sexual orientation was the least desired area for additional training once providers were in practice (mean 3.4, $p=0.005$). **Conclusion:** This study identifies a lack of interest to fill an acknowledged educational gap regarding addressing sexual orientation with adolescent patients. This may reflect discomfort with the topic as a result of limited training.

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GLIDESCOPE VIDEO LARYNGOSCOPE: AN EVALUATION OF LEARNING CURVE AND EASE OF USE

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Background: The GlideScope Video Laryngoscope (GVL) has been used for years in the adult population, but a neonatal model has only recently been introduced. GVL is a laryngoscope with a camera in the blade that projects to a video monitor. This allows for intubation without alignment of the oral, pharyngeal, and tracheal axes. Neonates have different airway anatomy than adult and pediatric patients with larger heads and tongues and shorter tracheas making the previous adult and pediatric studies difficult to apply to the neonatal population. Additionally, some adult studies have shown it takes up to 57 attempts with the conventional direct laryngoscope (CDL) before being competent; there are no such learning curve studies with GVL. Finally, GVL has been shown to be a more efficient intubation device with more successful intubations on first attempt than with CDL. GVL may therefore make intubations easier and less traumatic for neonates. **Objectives:** The purpose of our study was to determine the learning curve of experienced neonatal practitioners for intubation of neonatal manikins using GVL versus CDL. We also determined the efficiency of intubation with GVL as compared with CDL. Finally, we examined the relationship of type and length of clinical experience to success with GVL. **Methods:** 46 neonatal practitioners were recruited and randomized to start with GVL or CDL to intubate a Gaumard Scientific neonatal manikin. In part 1, the learning curve was determined by tracking the number of intubation attempts completed until a plateau in time was reached (3 attempts under 30 seconds and within 5 seconds of the previous attempt or 3 attempts under 20 seconds). Once competent, in part 2, 3 time trials were completed tracking time to airway visualization (T1), time to correct placement of the endotracheal tube (T2), and total time of intubation (TTI). Times were compared between GVL and CDL to determine equivalence. **Results:** The learning curve to plateau in time for GVL was 5 attempts (95% CI 4.7-6.2) versus 3 attempts for CDL (95% CI 3.1-3.7). In the time trials, participants were successful in all 3 attempts 77% of the time with GVL versus 98% with CDL. For those who were successful, T1, T2, and TTI were equivalent between the two methods (P-value <0.001, 0.005, and 0.002). There was no correlation between type of practitioner or years of experience with number of attempts to plateau or time intervals in the time trials. **Conclusion:** Approximately 5 attempts were required to achieve competence in endotracheal intubation of a neonatal manikin using the GVL. With adequate training, GVL may be as efficient an intubation method as CDL in neonates.

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COMPARISION OF ECG CHARACTERISTIS FOR AUTOMATED EXTERNAL DEFIBRILLATOR ALGORITHMS IN CHILDREN AND ADULTS

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Background: An automated external defibrillator (AED) is recommended for rhythm detection during cardiac arrest (CA) for children < 8 years if the algorithm has been validated in children. We hypothesized that ECG characteristics of potential cardiac arrest rhythms are different between pediatric and adult ECGs. **Methods:** Digitized recordings of NSR, supraventricular tachycardia (SVT), and ventricular tachycardia (VT) were obtained from PhysioNet's public database, and the ZOLL pediatric database. Rhythm strips of ≥3 sec were evaluated from 199 children and 170 adults (> 36,000 QRS complexes). Heart rate (HR) and amplitude (AMP) were determined by R-R interval detection and peak to peak QRS measurements. Conduction velocity (CV) was defined as maximum QRS slope from the first major deflection; SVT was defined as a narrow complex QRS without P waves and rate > 150 bpm. VT was any wide-complex tachycardia. Differences were defined by t-testing. **Results:** HR and AMP differed significantly during NSR and SVT (77 ± 15 vs 109 ± 25 bpm, 2.14 ± 0.8 vs.1.2 ± 0.06 mV $p< 0.01$). CV was different during NSR (88 ± 40vs 73 ± 40 mV/s, $p < 0.05$). VT characteristics were not different. **Conclusions:** Significant differences exist between adult and pediatric ECG characteristics of normal and potential CA rhythms. The most significant differences existed among adult and pediatric HR and AMP for NSR and SVT. If the detection algorithm does not include pediatric rhythms children may receive inappropriate shocks. This study confirms the recommendation that an AED rhythm algorithm be validated with ECG strips obtained from children.

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POSTNATAL GROWTH FAILURE IN VERY LOW BIRTH WEIGHT (VLBW) INFANTS.

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Background: Postnatal growth failure of very-low-birth weight (VLBW) infants may result from a complex interaction of genetic and environmental factors, including inadequate nutrition, morbidities affecting nutrient requirements, endocrine abnormalities and treatments. As growth failure is associated with impaired neurocognitive development later in life, its prevention is important for long-term outcome in VLBW infants. Despite advances in neonatal nutrition, post natal growth failure remains one of the leading morbidities among VLBW infants. **Aim**: The aim of this study is to document the incidence of postnatal growth failure, defined as weight th percentile for the corrected gestational age at discharge in VLBW infants in an inner city urban neonatal intensive care unit (NICU). **Methods:** Retrospective review of medical records of all infants admitted to Sinai Children's hospital NICU from 2007 to 2009. Exclusion criteria included infants, who were small for gestational age at birth, had congenital malformations, who were transferred out or expired prior to discharge and those who had incomplete records. During the study period standardized nutritional protocol was in place, which included starting hyperalimentation with amino acid on the first day of life. **Results:** During the study period 181 infants were admitted to the NICU. 68 infants were excluded and the data for the 113 infants is presented in the table below.

Birth Weight (grams)	N	Growth failure at discharge	%
500–750	11	5	45.4
750–1000	31	10	32.3
1000–1500	71	25	35.2
Total	113	40	35.4

Conclusion: Despite aggressive nutritional practices 1/3 of VLBW show growth failure at discharge from the NICU.

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THERAPY FOR PDA IN PRETERM INFANTS: WHICH DRUG AND WHEN?

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Purpose: Medical therapeutic choices for closure of a PDA in preterm neonates currently include indomethacin & ibuprofen, largely based on physician preferences. There is paucity of data on the comparative performance of the 2 drugs in the post-FDA approval period, beyond the rigid 'controlled trial' environment. Further, efficacy & safety comparative data of early (< 1 week) & late (≥ 8 days of life) therapy are scarce. The objectives of our study were to compare the effectiveness & safety of A) ibuprofen & indomethacin therapy for a hemodynamically significant PDA in preterm infants & B) early & late medical therapy. **Methods:** Retrospective chart review of all preterm infants (< 32 weeks gestation) at our center, with a PDA, & treated with at least 1 dose of either ibuprofen or indomethacin was performed. Demographic, laboratory & clinical data were collected. Statistical analyses (SPSS version 17) included chi square & t test & significance was set at $p < 0.05$. **Results:** Our cohort (n=91) included 44 infants (group1) treated with indomethacin & 47 (group 2) with ibuprofen. The mean gestational age (26.2 (2.3) vs. 26.2 (2) weeks), birth weight (913 (362) vs. 851 (283) grams), proportion of males (54.5% vs. 48.9%) & those who received antenatal steroids (73.8% vs. 86.7%) were comparable between groups. A 2nd course of therapy was required in 11 (25%) & 13 (27.6%) infants & ligation in 14 (31.8%) & 17 (36.2%) infants in the 2 groups. Rates of BPD (34 vs. 45%), grade 3-4 intracranial hemorrhage (18 vs. 15%), NEC (18 vs. 32%), mortality (25 vs. 23%), ROP needing laser (7 vs. 6%) & sepsis (43 vs. 57%) showed no statistically significant differences between groups. BUN, serum creatinine & platelets before, at the end of & 1 week after therapy were also comparable. The mean age at treatment for our cohort was 6.8 (5.3) days, with a similar proportion in both groups receiving late (> 1 week age) therapy (29 vs. 32%). Rates of BPD (35 vs. 50%), IVH (17.5 vs. 14.3%), NEC (21 vs. 36%) & death (22 vs 29%) were comparable between early & late-treated infants. The number of babies needing a 2nd course of therapy and needing PDA ligation was 11.3% & 28.6% in the early vs 25% and 46.4% in the late treatment group respectively. **Conclusions:** The therapeutic equivalence of indomethacin & ibuprofen, in our real-world comparative efficacy study is reassuring. However, the relatively high failure rate of medical therapy beyond 1 week is disturbing. Further investigations into pharmacokinetics in infants treated late & neuro-developmental outcomes related to ibuprofen are warranted to enhance efficacy & aid in a rational drug choice for PDA.

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PLACENTAL TRANSFERRIN RECEPTOR EXPRESSION AND FETAL IRON STATUS WITH UTERINE SPACE RESTRICTION IN OVINE PREGNANCY

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Background: Fetal iron transport is impaired in growth-restricted fetuses, resulting in lower iron tissue. In humans, cell surface transferrin receptor (TfR) is involved in placental iron transport, but it is not known if TfR is similarly involved in sheep pregnancy. Using a sheep uterine space restriction (USR) model that produced progressive fetal growth restriction between 120-130d gestation (term=145d), we hypothesized that placental TfR expression increases in response to lower tissue iron concentrations. **Methods:** To promote USR, nonpregnant ewes underwent a surgical severing of the intercorneal vascular connections followed by ligation of a single uterine horn 2-3 months prior to conception. Singletons, twins and triplets were studied at 120 or 130d. Non-space restricted fetuses (NSR) were defined as ligated and nonligated singletons and nonligated twins (53.6 ± 2.0 placentomes/ fetus). USR sheep were defined as ligated twins, nonligated triplets, and ligated triplets (26.9 ± 1.6 placentomes/ fetus). Fixed placentomes were sectioned and stained with Gomori Trichrome (collagen I) and Prussian Blue. Immunohistochemistry (IHC) slides were stained and Western Blots were probed with anti-CD71 TfR. Tissue iron (μg total and μg/g tissue) from kidneys, was quantified with a non-heme iron assay. **Results:** In NSR, trichrome staining was predominantly found in vascular stroma, but extravascular staining, indicating significant scarring, was observed in the USR group. Prussian Blue stain was minimal, except for small areas in the hemophagic zone. With IHC, TfR was found on both maternal and fetal sides of placentomes, but staining was distributed more evenly within both compartments in USR. Total fetal kidney iron (μg) in USR was similar to NSR at either 120 or 130d, however kidney iron increased between 120 and 130d in both groups, $p<0.001$. Because kidneys were relatively smaller in USR at 130d, the kidney iron concentration was higher in USR @ 130d, $p<0.03$. On the Western blot, we found a trend for decreased TfR expression in USR, compared to NSR at 130d, $p<0.06$. **Conclusions:** In fetal growth retardation, in contrast to the hypothesis, we found similar peripheral tissue iron content, higher tissue iron concentration, and a trend for decreased TfR expression. However, consistent with previous rat work, fetal tissue iron concentration in sheep appears to regulate placental TfR expression. This model is well suited for further investigation of the molecular mechanisms regulating TfR in the placenta and fetus. *NIH HL49210, HD38843, HL87144.*

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THE EFFECT OF LATE PRETERM BIRTH ON THE SURVIVAL OF INFANTS WITH MAJOR CONGENITAL HEART DEFECTS

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Introduction: Infants with congenital heart defects (CHD) are more likely to be born prematurely and have increased risk for death and serious morbidities. In infants without CHD, late preterm infants (34-36 weeks) have a risk of death comparable to those born at term. **Objectives:** We evaluated the effect of late preterm delivery hospital mortality of infants with CHD. **Methods:** Retrospective review of data of infants born at or later to 34 weeks, cared for in a single subspecialty perinatal center between 1999 and 2009. Factors associated with death prior to discharge from the hospital were ascertained using univariate and multivariate analysis. We did not include infants with isolated atrial septal defects, patent ductus arteriosus, ventral septal defects, coarctation of the aorta, rhythm abnormalities or cardiac myopathies in the analysis. Severe intraventricular hemorrhage, intubation in the delivery room, chest compressions in the delivery room, mode of delivery, place of birth, presence other congenital anomalies, late preterm birth, being small for gestational age, multiple birth, race and gender were the variables retained in the forward multivariate logistic regression analysis model. **Results:** Of the 753 infants with CHD, 115 were born at 34-36 weeks. The heart defects included hypoplastic left heart syndrome (34.8%), transposition of the great arteries (24%), pulmonary atresia (12%), tetralogy of Fallot (9.3%), double outlet right ventricle (7.2%), single right ventricle (6.4%), interrupted aortic arch (5.8%), tricuspid atresia (5%), total anomalous pulmonary venous return (4.6%), truncus arteriosus (3.7%), and common atrioventricular canal (3.3%). Using logistic regression analysis, white race (OR; 95% CI) (0.67; 0.41–1.07), late preterm delivery (2.54; 1.55–4.16), intubation in the delivery room (OR, 3.15; 1.92 −5.17) and severe intraventricular hemorrhage (3.61; 2.29–5.68) were independently associated with death. There were no interaction terms between these variables. **Conclusions:** In a population of infants with major CHD, late preterm birth was independently associated with increased risk of hospital death compared to delivery at more mature gestational ages. Analysis of larger data bases is needed to define the effect of this risk factor in various types of major CHD.

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CARDIAC SPECIFIC ATP- SENSITIVE K+ CHANNEL (KATP) OVEREXPRESSION RESULTS IN EMBRYONIC LETHALITY

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The KATP channel is formed of regulatory Sulfonylurea receptor subunits (SURx) and pore forming Kir6.x subunits. Transgenic mice overexpressing the KATP subunits SUR1 and gain of function Kir6.2 (ΔN30, K185Q) in the heart, demonstrate arrhythmia susceptibility and premature death. Pregnant mice, crossed to carry double transgenic (DTG) progeny, harboring high levels of both overexpressed subunits do not deliver any DTG pups. We hypothesized that KATP channel overexpression interferes with normal cardiac development accounting for in utero embryonic lethality. Pregnant females were imaged by ultrasound and then sectioned at different stages of pregnancy. The embryos were extracted, yolk sacs were genotyped and hearts were dissected. Embryonic cardiomyocytes were isolated and cultured. Cellular electrophysiological properties were studied using whole cell patch clamp and excised patch techniques.

Breeding of heterozygous single transgenic mice, which overexpress either SUR1 or Kir6.2(ΔN30, K185Q) under cardiac α myosin heavy chain - specific control, yielded 14 pregnancies with a total of 111 embryos. 23/29 (20.7% of total) of the DTG embryos, displayed embryonic lethality (either non beating or absorbed embryo). 7/48 (6.3% of total) of the single transgenic embryos displayed embryonic lethality. None of the wild type embryos displayed embryonic lethality (0/22, 0% of total). Genotyping of 12 (10.8% of total) of the yolk sacs was inconclusive. Ultrasound imaging of pregnant females revealed that all DTG embryos develop normally at 8.5 days post conception (dpc), but none of the DTG embryos are alive after 11.5 dpc. At 9.5-10.5 dpc some of the DTG embryos develop and beat normally, while others exhibit no heart beat, pericardial effusion and growth retardation. Whole cell and excised patch recordings from cardiomyocytes isolated from 9.5-18 dpc embryos detected KATP channel activity in both wild type and over expressed cells.

In conclusion, KATP channel activity is readily detected in embryonic cardiomyocytes during early heart development. Overexpression of KATP leads to embryonic lethality which is dose dependant, with the extreme phenotype in the DTG which die between 8.5-11.5 dpc, probably as a result of arrested excitability.

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PRENATAL CONSULTATION WITH A NEONATOLOGIST FOR CONGENITAL ANOMALIES: AN URBAN INNER-CITY EXPERIENCE

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Background: The widespread use of ultrasound has increased the number of fetal anomalies detected in antenatal period. The neonatologist can be a valuable source of information for the pregnant woman and her partner faced with making difficult decisions. In specific clinical situations, a focused, thorough consultation by a neonatologist provides benefits for the parents, their child, the physicians, and the health care delivery system as a whole. **Aim:** The aim of this study is to identify reasons for consults and the impact on patient management over last six year period at an inner city level III neonatal intensive care unit (NICU). **Methods:** Patients with fetal anomaly on ultrasound, abnormal QUAD screen result or maternal conditions that impact neonatal outcome were referred for prenatal consultation to a neonatologist. All consults were done by a single neonatologist who is board certified in both Neonatal-Perinatal medicine and Developmental-Behavioral-Pediatrics. Data from Jan 2005–May 2010 was analyzed. The main outcomes measured were reasons for consultation, impact on plan of care, neonatal outcome and follow-up within the Sinai Health System. **Results:** During the study period a total of 92 consults were done. One patient terminated the pregnancy, and there was one fetal death. The data on the remaining 90 patients is presented. 9/90 (10%) mothers (3 cases of diaphragmatic hernia and 6 complex cardiac disease) were referred to a tertiary center for delivery. 7/90 (7.8%) of neonates (3 cases of meningomyelocoele, 2 cases of complex cardiac anomaly, one patient each with neuroblastoma and diaphragmatic hernia) were transferred after delivery to a tertiary care center. 8/90 (8.9%) infants (one each with trsomy13, Trisomy18, holoprosencephaly, hydroencephaly and multiple congenital malformations and 3 infants with Potter's syndrome) died in the neonatal period. 3/90 (3.3%) mothers have not yet delivered. 7/90 (7.8%) infants were lost to follow-up after discharge from the NICU. The remaining 56/90(62.2%) of infants continue to follow-up within the Sinai Health System.**Conclusion**: Prenatal consults with a neonatologist helped prepare the parents for the perinatal course of their infant with congenital malformation. Majority of the parents elected to continue the pregnancy and want to be allowed to hope for the best possible outcome.

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ABSENCE OF CLC-3 PARTIALLY ABROGATES RENAL TUBULAR INJURY IN THE FOLATE INDUCED ACUTE KIDNEY INJURY MOUSE MODEL

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Background: Acute kidney injury (AKI) is associated with high morbidity and mortality. Inflammation plays a significant role in the patho-physiology underlying AKI. The mediators of these processes are not completely understood. One of the most common forms of renal injury noted in the Pediatric ICU is toxin induced tubular damage. ClC-3 is a chloride-proton antiporter required for intracellular production of superoxide anion by NADPH oxidases (Nox1, Nox2). It is highly represented in renal tubular cells. Vascular smooth muscle cells from ClC-3 null mice have reduced Nox1-dependent intracellular superoxide production and impaired ROS signaling. This includes failure to activate critical inflammatory mediators such as NF-kB in response to cytokines (TNF-a, IL-1b). Neutrophils lacking ClC-3 have reduced intracellular Nox2 activity and impaired ROS-dependent intracellular signaling in response to endotoxin. Given the importance of inflammatory pathways in the initiation, establishment and progression of AKI, we hypothesized that ClC-3 may play a significant role in the AKI injury cascade. **Objective:** To evaluate the potential protective effect the absence of ClC-3 in a classic folate-mediated AKI model in ClC-3 null and wild type (WT) mice. **Methods:** A pilot study was undertaken whereby 4 ClC-3 null mice and 4 littermate control WT mice received folate (300mg/kg in .3 M NaHCO3) injected IP. Urine and Serum BUN/Creatinine were drawn 24 hr prior to and 18 hr after folate injection. Mice were euthanized at 18 hr post folate injection and kidneys were preserved in paraffin and then sectioned for histological inspection. They were stained with both PAS and TUNEL techniques. Histology was performed in a blinded fashion by a veterinarian pathologist. **Results:** All mice injected with folate experienced renal failure as evidenced by the blood and histological samples. There was a 50% reduction in tubular damage using TUNEL and H&E staining as a read-out in the ClC-3 nulls compared to WT littermates. WT mice expressed higher BUN and Creatinine levels at 18 hr compared to the null littermates. **Conclusion:** ClC-3 null mice demonstrate a reduction in degree of folate-induced AKI compared to WT littermates. These results suggest that blockade of the ClC-3 antiporter may provide a novel AKI therapy. An expanded analysis is under way, along with metabolomic, proteomic and pharmacologic analysis.

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ASSOCIATION BETWEEN DISPLAYED DEPRESSION AND ALCOHOL USE ON COLLEGE STUDENTS' FACEBOOK PROFILES

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Purpose: College students with mental health concerns are at increased risk for problem alcohol use, but do not always seek help for either problem. Facebook, a commonly used Social Networking Site, is regularly used by this population and often contains references to depression and alcohol use. The relationship between Facebook displays of depression and alcohol use, and potential gender differences in these displays, is not well understood. **Methods:** Publicly available Facebook profiles from college students at two universities were examined. Profiles that displayed text references that fit Diagnostic and Statistical Manual IV for depression were examined for concurrent display of alcohol use. A 20% subsample of profiles was evaluated by two coders for interrater reliability; Cohen's kappa for depression was 0.6 and for alcohol was 0.69. Logistic regression was used to examine the relationship between display of depressive symptoms and display of alcohol use for each gender. **Results:** Of 229 profiles examined, 45% were male, 32.7% displayed depression references, and 35.4% displayed alcohol. Of the profiles that displayed depression, males were 2.2 times as likely to display alcohol (CI 1.3-3.8, p=0.004). There was no significant association among females. **Conclusion:** Males who display references to depression on Facebook are more likely to display references to alcohol, a pattern that is not seen among females. These findings suggest that males and females either display alcohol on Facebook or possibly use alcohol in different ways, concomitant with Facebook references to depression.

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IMPLEMENTING FAMILY CENTERED ROUNDS (FCR) IN A NEONATAL INTENSIVE CARE UNIT (NICU): A CASE STUDY FROM AN URBAN ACADEMIC MEDICAL CENTER

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Introduction: Effective physician-parent communication is associated with improved patient health status, recall, treatment adherence, and satisfaction. A critical need of families of intensive care patients is information and communication. Redesigning traditional medical rounding practice to FCR is a potential way to address these needs. **Objective:** Describe the implementation plan used to establish a sustainable practice of FCR in a large teaching hospital. **Implementation Plan:** The first priority was to create staff "buy in". We found FCR champions in the different groups of staff to promote FCR among their peers. Educational sessions were held to present background data on FCR, the benefits to the parents-patients and staff, and success of other programs. Focus groups were held for all groups of staff to discuss ideas and concerns about FCR. A committee was formed to develop a plan for implementation. This committee defined roles for each staff member on rounds, determined how to ensure confidentiality, and addressed ways to conserve efficiency. After the specific implementation plan was devised, education sessions were held to present the plan as well as information on communication with parents. A trial rounding week was conducted with a few select families. Feedback was collected from the staff and families. Handouts were given to parents inviting them to join rounds. The handout also described how medical information would be presented, teaching of residents and other team members would take place, and that FCR represents one opportunity to speak with the medical team but that the team would continue to communicate closely with them, if they were unable to attend the FCR. Once FCR was implemented, debriefing sessions were held with staff to assess FCR and make adjustments as needed. Within 6 months FCR were in place for our unit. **Results:** FCR was implemented in March 2009. FCR has continued in our unit for over a year with highly positive responses from staff and families. **Conclusions:** To implement a new practice in a NICU, a well constructed plan is essential. Enlisting members of all groups of staff from the inception is crucial to establishing buy-in, promoting education, developing a plan that works from all perspectives, receiving ongoing feedback and, ultimately, sustaining change.

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PHOCOMELIA WITH HUMERUS BURIED WITHIN SOFT TISSUE OF THE CHEST WALL

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In this report, we describe a novel variant of longitudinal deficiency in which the humerus was buried within the tissues of the chest wall. Surgical treatment was performed to provide better function and to allow for subsequent fitting and use of a prosthesis. This case is unique in that an empty soft-tissue envelope intended for the humerus radiated out from the shoulder with no bone present, while the humerus was located within the subcutaneous tissue of the upper chest wall.

An 8-month-old boy presented with hypoplasia of the left upper extremity. An empty soft tissue envelope extended from the left shoulder, creating a very shallow pseudo-axilla, and the humerus could be palpated within the soft tissue overlying the left chest wall. Since there was palpable movement of the buried humerus pre-operatively indicating the presence of functional muscle attachments, surgical treatment was planned to liberate the buried humerus and place it into the soft tissue envelope. This would allow the patient to actively flex, adduct and abduct the arm, thus improving function and leading to prosthesis use in the future. Through an incision along the medial aspect of the soft tissue envelope extending onto the chest wall, the humerus was dissected free, and a muscle slide was performed by dividing distal attachments of musculature along the length of the humerus. The humerus was elevated and placed into its intended soft tissue envelope and anchored distally with a button. A four-flap z-plasty was then performed to create an axilla of appropriate contour and depth.

We report a unique example of upper-extremity longitudinal dysplasia. A number of clinical features suggested that reconstruction would be beneficial in this case: 1) the child's ability to move the buried humerus; 2) a functional glenohumeral articulation, by clinical and radiographic examination; 3) the absence of osseous structures distal to the buried humerus (i.e. the skeletal element and its intended soft-tissue envelope were a good size match); and, 4) a supple soft tissue envelope for the humerus that would provide stable soft tissue coverage. Taking these factors into consideration, a surgical approach was devised to address the unique anatomical features in this patient to improve function and allow for future prosthesis use. Long-term follow up (45 months) has demonstrated that the patient has useful abduction, adduction and flexion of the left arm with stable soft-tissue coverage of the transferred humerus and a supple, anatomic-appearing axilla.

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REMOVAL FROM LIFE-SUSTAINING SUPPORT IS THE PRIMARY MODE OF DEATH IN THE NICU

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Background: In the United States, infant mortality rate is 6.86 per 1000 live births, with the majority being neonatal (4.92). Neonatal deaths generally occur within a NICU setting, and are preceded by an end-of-life decision. In 1973, the first open description of the withdrawal of care in neonates was published in the *New England Journal of Medicine*. Subsequent publications have suggested that the frequency of this practice has increased. However, data describing circumstances of the dying neonate are unavailable. **Purpose:** Determine the decisions related to mode of death for infants dying at a referral level IIIc NICU. **Methods:** This study is a retrospective descriptive study involving infants that died in the NICU at a children's hospital from January 1st, 1999 to December 31, 2008. Independent variables (GA, BW, diagnosis, age at death and sex) and dependent variables (care withheld, care withdrawn, versus CPR) were collected. Diagnosis was categorized as: congenital anomaly, prematurity, sepsis, HIE, NEC, respiratory insufficiency, intracranial bleeding, or other. Unstable was defined as having any two of the following criteria: persistent desaturation despite 100% oxygen on mechanical ventilation, hypotension despite volume infusion and inotropes, protracted bradycardia or anuria for >24 hours. Primary outcome was level of clinical service provided at end-of-life (care withheld, care withdrawn, or CPR). **Results**: Over 10 years, 414 infants died, 59% male. Median gestation age, birth weight, and age at death were **34.5** weeks, 2165 grams, and 10 days, respectively. Diagnoses resulting in death were: congenital anomaly (44.7%), prematurity (12.6%), NEC (10.1%), HIE (8.9%), respiratory insufficiency (7.5%), sepsis (6%), intracranial bleeding (2.9%), or other (7.2%). Over the ten year period, 61.6% had care withdrawn, 20.8% had care withheld, and 17.6% received CPR. Over time, there was not a significant difference in percent having care withdrawn, however there was a significant difference in care withheld, which increased from 15% to 25% (p= 0.01) and CPR which decreased from 22% to 14% (p = 0.01). **Conclusion:** Over the 10 year period**,:** the primary mode of death in this regional referral NICU was withdrawal of life-sustaining support. When death is eminent, or when medical care is considered futile, the approach is thought to provide a peaceful, "controlled" setting for the infant and their family. Decrease in frequency of end-of-life cardiopulmonary resuscitation and the concomitant increase in withholding of care may suggest improvement in recognition of medical futility and desire to provide a peaceful death for the families of the dying infant.

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RIGHT TO LEFT OR BIDIRECTIONAL SHUNT ACROSS THE PATENT DUCTUS ARTERIOSUS [PDA] IN PRETERM NEONATES: A PORTENDER OF DEATH?

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Purpose: There is paucity of data on the implications and outcomes of a bidirectional (BD) or right to left (RL) shunt across the PDA in preterm neonates. Our purpose was to delineate the natural history and evaluate the clinical characteristics of infants with moderate or large RL/BD shunt across the PDA in preterm infants. We further compared the clinical characteristics and outcomes of preterm infants with a moderate to large RL or BD shunt and those with a left to right (LR) shunt. **Methods:** All preterm infants with gestational ages <32 weeks, who, on an echocardiogram performed >24 hours of age for clinical suspicion of a PDA, had a moderate or large (>1.5 mm) BD or RL PDA on any ECHO (group 1) were included. The comparison group (group 2) comprised consecutive infants with a similar size LR PDA. Infants with a congenital heart defect were excluded. Data were obtained by review of medical records and serial echocardiograms. **Results:** Our cohort (n=161) comprised 74 infants (group 1) with a RL (n=22) or BD ductal shunt (n=52) and 87 (group 2) with a LR PDA. Of the 74 infants in group1, 58% had a transient RL/BD shunt, meaning had this type of shunting on only 1 ECHO. 15% changed direction to a LR shunt and 27% of the infants had a persistent RL/BD shunting across the ductus arteriosus, meaning had this type of shunting on 2 or more ECHOs. Twenty (27%) infants in group 1 underwent medical (n=14) or surgical therapy (n=6). Comparison of the clinical characteristics and outcomes of preterm infants with a moderate to large RL/ BD shunt and those with a left to right (LR) shunt showed comparable baseline characteristics and outcomes between groups except for significantly greater surfactant use [98.6% vs 94.2%; P value <0.001], higher rate of PDA therapy [27% vs 92%; P value <0.05] and higher mortality [48.6% vs 21.8%; P value <0.05] in group1. On binary regression analysis, significant associations with mortality were noted with lower gestation: OR 1.45 (95% C.I:1.15–1.83) and RL/ BD PDA: OR 4.74 (95% C.I: 2.18–10.3) p=0.0001. **Conclusions:** A BD or RL ductal shunt in preterm neonates was persistent in about a quarter of cases. Persistent RL/ BD shunting was seen in infants with lower gestational age and lower birth weight. Persistence was significantly associated with increased BPD and showed a trend towards increased incidence of severe IVH. A moderate to large RL/ BD ductal shunt when compared to a LR PDA in preterm neonates was associated with significantly higher mortality. Whether a BD or RL ductal shunt is merely a marker, a cause or a consequence of 'sickness' and subsequent death is unclear. Further investigation into the etiology and therapy of pulmonary hypertension in this population is warranted.