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1

PROGRAMMING OF CORONARY ARTERY DYSFUNCTION BY EARLY GESTATION DEXAMETHASONE EXPOSURE.

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BACKGROUND: There are strong epidemiological links between poor fetal growth and subsequent development of hypertension and atherosclerosis. Studies suggest that programming of these adult diseases may be a consequence of excessive exposure of the fetus to maternally derived corticosteroids.
HYPOTHESIS: Early gestation glucocorticoid exposure alters postnatal coronary artery vascular reactivity by augmenting vasoconstriction and/or impairing vasorelaxation.
METHODS: Dexamethasone (0.28 mg/kg/day iv for 48 hours) was administered to pregnant ewes at 27–28 days gestation (term being 145 days). The ewes were allowed delivery, and offspring were studied at a postnatal age of 127 ± 5 days (N = 7) as were non-dexamethasone exposed age-matched control lambs. Vascular catheters were placed 48 h prior to recording blood pressures. The contractile responses of circumflex coronary and second order mesenteric artery rings were then measured by wire myography. Protein expression was evaluated by immunoblotting.
RESULTS: Lambs exposed to maternal dexamethasone had higher mean arterial blood pressures than the saline-treated controls (93 ± 3 vs. 83 ± 5 mmHg, $P < 0.05$). Coronary vessels, but not mesenteric vessels, from dexamethasone-exposed sheep exhibited enhanced vasoconstriction to angiotensin II, acetylcholine, U46619 and potassium chloride (all $P < 0.05$). There was no difference in response of the coronary or mesenteric arteries to a wide range of vasodilators (sodium nitroprusside, isoproterenol, forskolin, and membrane permeable analogs of cGMP and cAMP). Angiotensin II receptor protein expression was not significantly increased in the steroid exposed group.
CONCLUSION: Early gestation glucocorticoid exposure programs postnatal elevations in blood pressure and selectively enhances coronary artery vasoconstriction to a variety of vasoactive compounds, including angiotensin II. The interactions of angiotensin II with oxidant stress and endothelial cell dysfunction may provide a mechanistic link between an adverse intrauterine environment and later coronary artery disease.

2

FAS LIGAND (FASL) AND FAS IN THE PATHOGENESIS OF NECROTIZING ENTEROCOLITIS IN AN EXPERIMENTAL RODENT MODEL

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Background: Necrotizing enterocolitis (NEC) is associated with abundant apoptosis of enterocytes. The FasL-Fas system of apoptosis is an important pathway of programmed cell death. FasL is a membrane bound protein that induces apoptosis of cells expressing its receptor Fas. This is one of the mechanisms by which cells that express FasL protect themselves from lymphocytes. There are no data to suggest that FasL and Fas play a role in the pathogenesis of NEC. **Purpose:** To test the hypothesis that enterocyte FasL is downregulated in the presence of lymphocyte FasL and that enterocyte Fas is upregulated in the rodent model of NEC. We also hypothesized that the upregulation of enterocyte Fas is mediated during inflammation by cytokines and Nitric oxide (NO). **Methods:** We performed in-vivo studies on the neonatal rodent model of NEC induced by hypoxia and formula feeding. Breastfed pups served as controls. The animals were sacrificed on days 1–4 and terminal ileum harvested. Expression of FasL and Fas on enterocytes was determined by immunohistochemistry and Western immunoblotting. Activation of Caspases 8 and 3, proteins downstream of Fas was determined in the mucosal lining using a colorimetric assay. We cultured IEC-6 cells (rat small intestinal cell line) with TNF-α, IL-1β, IFNγ or a NO donor (SNAP). **Results:** Enterocyte FasL expression was decreased in formula fed-hypoxic pups with NEC when compared to breast fed pups on day 4. Of note the FasL on intestinal lymphocytes did not decrease. In contrast, enterocyte Fas expression was increased in formula fed-hypoxic pups when compared to breast fed pups on day 4. In addition, animals that develop NEC demonstrated an increase in caspase 8 and 3 activity in the mucosal lining ($p < 0.05$). Our in vitro studies demonstrate that proinflammatory cytokines and SNAP increase enterocyte Fas expression. **Conclusions:** We conclude that enterocyte FasL is decreased and enterocyte Fas, Caspases 8 and 3 activities are increased in the presence of intestinal lymphocyte FasL in NEC. Complementing our in vivo studies proinflammatory cytokines and Nitric oxide increased Fas expression on IEC-6 cells. We speculate that the decreased enterocyte FasL is an early event in the pathogenesis of NEC and inflammatory mediators enhance lymphocyte-induced enterocyte Fas-mediated apoptosis. Therefore, strategies targeting FasL / Fas may be effective therapeutic intervention.

3

LONG-TERM FOLLOW-UP: NEURODEVELOPMENTAL OUTCOME AND GASTROINTESTINAL FUNCTION IN INFANTS <801 GRAMS DIAGNOSED WITH NECROTIZING ENTEROCOLITIS.

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Objective: Little is known about the long-term implications of neonatal NEC. The null hypotheses are: 1)ELBW (< 801g BW) infants who had neonatal NEC will have no differences in growth or neurodevelopment (ND) up to 8 yrs of age compared to those w/o NEC. 2) ELBW infants with NEC will have no long-term GI co-morbidity compared to those w/o NEC up to 18 yrs. **Methods:** This was a comparative follow up study of growth and ND to 8 yrs of age and GI function to 18 yrs of age for ELBW children with and w/o a history of neonatal NEC. Infants treated for NEC were identified and matched 1:2 with a non-NEC infant by wt and gender. Growth measurements and ND outcomes were determined at a 6 or 8 yr visits. Families completed a questionnaire based on Rome II criteria to determine current GI function. **Results:** 25 patients with NEC (21 medically managed) and 50 w/o NEC were evaluated w/o differences in GA (26.2 ± 1.5 and 25.8 ± 1.4 wks, with and w/o NEC, respectively) or BW (687 ± 71 and 701 ± 78g). There were no significant differences in neonatal co-morbidities (CLD, IVH, and PDA). At follow up, children who had NEC were lighter (17.2 ±2.3 and 20.1 ±4.7 kg for 6 yr olds; 21.5 ± 3.4 and 23.0 ± 3.6 kg for 8 yr olds, $p = .05$) and shorter (107 ± 4.4 and 114 ± 6.9 cm at 6 yrs; 121 ± 6.4 and 124 ± 9.7 cm at 8 yrs, $p = .01$. Children who had NEC were significantly more likely to have triceps fatfold measurements less than the 10th %tile. There were no apparent differences in ND outcome measures. 36 subjects completed the GI questionnaires. There were no significant differences in GI diagnoses between the two groups (9/18 with NEC and 9/18 w/o NEC). During the NICU stay, the infants with NEC required TPN longer (53 ± 22 vs. 23± 14 d, $p = .00$), had longer length of stay (113 ± 29 vs. 94 ± 17 d, $p = .001$) and were lighter at discharge (2.18± 0.43 vs. 2.23± 0.34 kg, $p = .018$). **Conclusion:** ELBW children who had NEC, compared to those w/o NEC had significantly lower weight and height measurements at 6 or 8 yrs, in spite of no differences in ND or GI outcome measures. There were no apparent differences in other neonatal complications to account for long-term growth discrepancies. Significant differences in growth parameters were evident by NICU discharge for ELBWs with NEC compared to those w/o NEC. Further investigation is needed to determine which factors associated with NEC may affect long-term growth.

4

***IN VIVO* CHARACTERIZATION OF NOVEL GENETICALLY ENGINEERED INACTIVATION RESISTANT COAGULATION FACTOR VIII.**

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Hemophilia A results from a qualitative or quantitative deficiency of coagulation factor VIII (FVIII). Thrombin (IIa)-activated FVIII (FVIIIa) is a heterotrimer of subunits A1/A2/light chain and rapidly inactivates due to spontaneous dissociation of the A2-domain or proteolytic cleavage. Inactivation resistant FVIII (IR8) is a novel genetically engineered factor VIII protein that has enhanced *in vitro* stability after IIa activation due to resistance to spontaneous A2-domain dissociation and proteolytic inactivation. IR8 protein was derived from stably-transfected CHO cells and has previously demonstrated effective hemostasis in the Chapel Hill Hemophilia A dog model. The specific activity of IR8 (78,235 mU/ml) was markedly higher than FVIII wild-type (WT) (1766 U/mg). We hypothesized that IR8 could provide effective hemostasis at much lower doses of protein compared to FVIII WT. We studied this in a murine model of hemophilia A (FVIII exon 16 knockout). Hemophilia A mice were injected via tail vein with either IR8 or FVIII WT protein to obtain 0–100% correction of FVIII activity. Blood loss was quantitated over 20 min after the tail was transected at a diameter of 2 mm. The mean blood loss (μl/kg/min) from 17 mice injected with FVIII WT was 528 at 0–5% correction, 682 at 5–10% correction and 183 at 50–100% correction. The mean blood loss from 12 mice injected with IR8 was 574 at 0–5% correction, 184 at 5–10% correction, 217 at 10–50% and 121 at 50–100%. Therefore, IR8's markedly increased specific activity enabled control of tail cut induced bleeding superior to FVIII WT at a 20-fold reduced amount of protein infused. We also tested the efficacy of IR8 expressed *in vivo*. Five hemophilia A mice were injected with 100ug of IR8 within a mammalian DNA expression plasmid via hydrodynamic tail vein injection. Expression was confirmed by FVIII activity analysis. In all five mice, FVIII activity was corrected into the normal range from 545-1038 mU/ml. Mice expressing IR8 had complete correction of tail cut induced bleeding. IR8's hemostatic efficacy, high specific activity and resistance to inactivation make it an interesting and potentially useful novel FVIII therapeutic for FVIII replacement or as part of gene therapy applications for patients with hemophilia A.

5

NKX 2–5 TRUNCATION IN THE DNA BINDING DOMAIN PROLONGS ATRIOVENTRICULAR CONDUCTION IN XENOPUS EMBRYOS.

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Congenital heart disease often includes anatomic and conduction abnormalities. Early reports of humans with mutations in the transcription factor, *Nkx 2–5*, showed an autosomal dominant pattern of heart block and secundum atrial septal defects. The conserved expression of *Nkx 2–5* across species has allowed description of the related anatomic abnormalities in animal models. However, developmental evaluation of the conduction defects is limited. We have examined the role of two *Nkx 2–5*mutations on cardiac development in the frog, *Xenopus laevis*. mRNA corresponding to the human point mutations Nkx2–5 Gln170ter or Nkx2–5 Gln198ter was injected at the single cell stage. Both mutations lead to a protein with a truncated C-terminus. The Nkx2–5 Gln170ter mutation eliminates much of the DNA binding domain while the Gln198ter mutant leads to termination just after the DNA binding domain. Either form of truncated mRNA resulted in anatomic abnormalities, including enlarged atria, abnormal atrial septae, and atrioventricular valve defects as visualized by confocal microscopy. To evaluate the effects on the embryonic conduction system, non-invasive imaging techniques were developed to assess the timing of cardiac contraction during embryogenesis. Magnified digital movies of the embryonic heart were acquired in pharmacologically paralyzed, stage 46 embryos. Grayscale measurements were made in the atria and ventricle through ten cardiac cycles to depict filling and contraction of the chambers. Grayscale waveforms allow measurement of the atrial and ventricular cycle lengths as well as the delay between atrial and ventricular contraction, the AV interval. Analogous to 1st degree heart block, embryos injected at the single cell stage with Nkx2–5 Gln170ter ($n = 24$) demonstrate a significantly prolonged AV interval of 237 ms $\pm$ 18 ms compared to the 187 ms $\pm$ 9 ms AV interval in water injected control embryos ($n = 37$, $p < 0.05$ by Tukey's F-test). The AV interval was normal in embryos injected with Nkx2–5 Gln198ter, averaging 173 ms $\pm$ 17 ms ($n = 23$, $p = 0.404$ vs control). These findings are consistent with the hypotheses that *Nkx 2–5* conduction abnormalities manifest during embryogenesis and that disruption of the DNA binding domain increases the effects on cardiac conduction.

6

GENETIC VARIATIONS IN THE CODING SEQUENCE OF THE IRF6 GENE OCCUR IN DIFFERENT ETHNIC POPULATIONS.

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BACKGROUND: The population prevalence of cleft lip and palate varies by ancestral origins. The etiologic factors leading to the differences in prevalence have yet to be determined. Mutations in interferon regulatory factor 6 (IRF6) cause Van der Woude syndrome (VWS). VWS is an autosomal dominant form of cleft lip and palate. Diagnosis of variants in IRF6 causing VWS, requires the ability to distinguish mutations from rare single nucleotide polymorphisms (SNPs). Currently, limited data on polymorphisms in IRF6 exists, especially within minority populations.
OBJECTIVE: The aim of the study is to determine and characterize genetic variants in IRF6 by direct sequencing DNA from a population of normal individuals with different ethnic backgrounds.
DESIGN/METHODS: DNA was obtained from 1,064 individuals from the CEPH diversity panel (a population of non-affected individuals from various geographic locations). DNA from the coding regions and all the exon-intron junctions of IRF6 were amplified by PCR, sequenced, and analyzed.
RESULTS: We sequenced 60% of the exons from 1,064 non-affected individuals. We identified 4 SNPS not previously reported within the coding region of IRF6. Three resulted in no change in the amino acid sequence, while one C to T substitution in exon 7, resulted in an amino acid change from methionine to isoleucine. This finding occurred in a non-affected Bedouin male, unexpectedly in a region where mutations are usually associated with clefting. Of the remaining 3 SNPS, a G to A substitution in exon 7, resulted in no change in threonine and occurred in 2 Palestinian females. A second SNP in exon 3, of G to A occurred in 9 subjects (of Mongola, Daur and Japanese descent) and resulted in no change in leucine. A third substitution of T to A, in one Tujian male, occurred in exon 3 and resulted in no change in arginine.
CONCLUSION: We found population specific genetic variation in IRF6. The identification of these new polymorphisms provides important information for the epidemiological study of risk factors for clefting. These new SNPs provide a resource for developing an expanded linkage disequilibrium map now under construction. The characterization of polymorphisms in IRF6 is important for genetic counseling to distinguish between mutations causing Van der Woude syndrome, and rare nonetiologic variants.

7

NEBULIZED DEXAMETHASONE IN ACUTE BRONCHIOLITIS- A RANDOMIZED CONTROLLED TRIAL.

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Objective: To evaluate the efficacy of nebulized Dexamethasone in children with bronchiolitis.
Methods: A randomized double-blinded placebo controlled study was carried out in an inner city emergency room. 73 children with bronchiolitis (age 2–24 month) were randomized to receive either nebulized Dexamethasone 1.5 mg/kg or Normal Saline, after receiving 1st dose (2.5mg) of nebulized albuterol. All patients were assessed for heart rate, respiratory rate, oxygen saturation and BAS (Bronchiolitis Assessment Score) at baseline, 30, 60, 120, and 240 minutes. The primary outcome measure was a change in BAS from baseline to 4 hours.
Results: Both groups were similar at baseline .BAS was not significantly different at 30, 60, 120, 240 minutes between the two groups.

Within dexamethasone group there was a statistically significant improvement from baseline to 240 minutes .Within Saline group there was a statistically significant improvement from Baseline to 240 minutes . Duration of stay (hours) in the ER for Dexamethasone group ($n=24$) was 4 ± 2.4 and for Saline group was 4 ± 1.3 with p-value $=0.87$. 24/34 (70.5%) got discharged in the Dexamethasone group as compared to 22/39 (56.4%) in the Saline group (p-value = 0.23). Duration of staying in the hospital in hours was ($n=11$) 36 hours ±18 for Dexamethasone group while for the Saline group was ($n=17$) 46 hours±34 with p-value = 0.34
Conclusion: Dexamethasone in nebulization form does not improve clinical scores in children with mild to moderate bronchiolitis, nor does it affect short term out come.

BAS	Dexamethasone	Saline	p-value
Baseline	4.68±1.52	5.25±1.48	0.21
30 minutes	3.89 ±1.41	4.14± 1.71	0.60
60 minutes	3.37 ±1.60	3.36± 1.70	0.98
120 minutes	2.36± 1.42	3.07 ±1.42	0.37
240 minutes	2.47± 1.61	3.00 ±1.78	0.38

8

FINE MOTOR ABNORMALITIES IN CHILDREN PRENATALLY EXPOSED TO COCAINE.

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Purpose of the study: Children with in utero cocaine exposure may be at risk for adverse neurodevelopmental outcomes. Studies have linked prenatal cocaine exposure with deficits in motor domains but data on its correlation with fine motor skills is scant. Our study aims to evaluate the relationship between prenatal cocaine exposure and the object assembly subtest of the Wechsler Preschool and Primary Scale of Intelligence- Revised (WPPSI-R) at age 6–7 years. ***Methods:*** Singleton children born to non-HIV positive African-American women between 1989–1991 at a Midwest university maternity center were enrolled in the study. Birth mothers were extensively screened during pregnancy for tobacco, alcohol, cocaine and other drug use. Prenatal cocaine exposure was defined at two levels: no exposure versus any exposure during pregnancy. Exposure was considered positive by self report, interview, medical records or laboratory analysis. Background data was collected prospectively and at age 6–7 years. Fine motor skills: perceptual organization and visual motor proficiency were assessed at age 6–7 years with the object assembly subtest of WPPSI-R. ***Results:*** The study sample consisted of 504 children aged 6–7 years, 211 (41.9%) of whom were prenatally exposed to cocaine. A multiple regression analysis was run to look at the relation between prenatal cocaine and object assembly. Maternal IQ and child's lead levels were the control variables significant in predicting object assembly. These confounders were entered into the regression , followed by forced entry of the three prenatal exposures (cocaine, cigarettes and alcohol). Overall all the variables accounted for 6.1% of variance in object assembly scores ($p<0.001$). Of the three prenatal exposures, only prenatal cocaine was significantly related to object assembly scores ($p= 0.015$) after controlling for lead and maternal IQ. ***Conclusion:*** Children with prenatal cocaine exposure score lower on object assembly scores than non exposed children after controlling for confounding variables like maternal IQ, child's lead levels and other prenatal exposures. Thus cocaine exposure may cause deficits in visual motor proficiency and/or fine motor skills

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SECRETION OF TNF A BY DONOR EFFECTOR CELLS IS CRITICAL IN THE DEVELOPMENT OF IDIOPATHIC PNEUMONIA SYNDROME AFTER ALLOGENEIC STEM CELL TRANSPLANTATION.

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Idiopathic pneumonia syndrome (IPS) is a frequently fatal complication of allogeneic (allo) stem cell transplantation (SCT). The pathophysiology of IPS includes the recruitment of donor-derived T cells, monocytes, macrophages and the secretion of inflammatory cytokines as TNFα. Using an established murine allo SCT model (B6-B6D2F1), we investigated the importance of donor- vs. host-derived TNFα in the development of IPS. Lethally irradiated B6D2F1 mice received SCT from syngeneic (syn) B6D2F1 or allo B6 donor mice. Flow cytometric analysis revealed that the turnover of donor lymphocytes and macrophages in the bronchoalveolar space was complete by day 14 and 28 after allo SCT respectively. By week 5, significant lung injury was present in allo SCT recipients compared to syn controls (4.7 ± 1.0 vs. 1.0 ± 0.7). Compared to syn controls analyzed at week 5, pulmonary macrophages from allo mice secreted significantly higher amounts of TNFα when restimulated with LPS in vitro (375 ± 127 vs. 136 ± 31 pg/ml), and the numbers of TNFα secreting, CD4 and CD8 T cells were significantly increased in the BAL fluid of allo SCT recipients (CD8: 154 ± 15 vs. 2 ± 0.2 (x1000); CD4: 76 ± 7 vs. 29 ± 2 (x1000)). These findings led us to hypothesize that TNFα production by donor effector cells is critical to the development of IPS after allo SCT. We next used B6 wild type (wt) or B6 TNFα deficient (TNFα-/-) mice as SCT donors to test this hypothesis. SCT with TNFα-/- donor cells resulted in significantly reduced lung injury (1.2 ± 0.3 vs. 4.1 ± 0.7) and BAL fluid TNFα levels (4.8 ± 3.7 vs. 67.4 ± 27.0 pg/ml) compared to wt controls. These findings were further confirmed in a second SCT system wherein donor and host differ at class I MHC only (B6-bm1). Next we completed mixing experiments in the B6-F1 system wherein wt or TNFα-/- bone marrow (BM) was supplemented with either wt or TNFα-/- T cells in order to determine the importance of the cellular source of TNFα (accessory (BM) cells vs. T cells), and found that TNFα from both cell sources significantly contributes to IPS. Finally, we found that lung injury did not differ between groups when B6TNFα-/- mice were used as recipients in a bm1 → B6 system, thus demonstrating that host-derived TNFα is not critical to the development of lung injury after allo SCT. In conclusion, these data reveal a critical role for both donor T cell and macrophage derived TNFα in the development of IPS and suggest, that neutralizing TNFα may be effective in preventing or treating this serious complication after allo SCT.

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DIFFERENTIAL REGULATION OF THE MITOGEN-ACTIVATED PROTEIN KINASES IN THE FETAL SHEEP HEART IN RESPONSE TO CHRONIC ANEMIA.

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The postnatal heart responds to biomechanical stress by activating mitogen-activated protein kinase (MAPK) pathways. The roles of these pathways in the loaded fetal heart are not well characterized. The purpose of this study was to test the hypothesis that myocardial p38, c-jun-n terminal kinase (c-JNK) and extracellular regulating kinase 1/2 (ERK 1/2) are activated (phosphorylated) by increased cardiac load in anemic fetal sheep. Anemia was created in chronically instrumented fetal sheep by daily isovolemic hemorrhage (60–100ml) for 7 days ($n = 7$) beginning at 134 d gestation (term 145 d). Cardiac output and stroke volume are increased 30–50% in this fetal anemia model. Catheterized, non-bled twins served as controls. Right and left ventricle MAPK kinase protein levels were determined by Western blot. Isovolemic hemorrhage resulted in decreased fetal hemoglobin (12.7+/-0.4 versus 6.0+/-0.3 g/dL) and arterial oxygen content (6.4+/-0.4 to 2.8+/-0.2 mL 02/dL). Total protein levels of p38, c-JNK, and ERK 1/2 were similar myocardium from anemic and control fetuses. Myocardial levels of activated p38 and c-JNK were also unchanged in anemic and control animals. In contrast, myocardial phosphorylated ERK 1/2 was markedly decreased in anemic fetuses relative to controls ($n = 4$, $p < 0.05$, see Figure). No differences in right or left ventricle protein expression were seen within either group. Volume loading of the fetal heart, as occurs with anemia, leads to differential regulation of MAPKs. Defining the myocardial signaling profile in the stressed fetus may suggest potential mechanistic and therapeutic bases for heart failure in the fetal and pediatric populations.

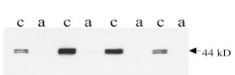

Figure: Immunoblot of phosphorylated ERK 1/2 in ovine RV. c=control; a=anemic

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P53 AND IKKα MEDIATE DOXORUBICIN- AND ETOPOSIDE-INDUCED APOPTOSIS OF N-TYPE NEUROBLASTOMA.

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Neuroblastoma (NB) is the most common malignant sympathetic nervous system tumor of childhood. NB tumors consist of two main cell populations—N-type (neuroblastic) and S-type (stromal). N-type neuroblastoma cell lines, represented by SH-SY5Y are N-*myc* amplified, express the anti-apoptotic protein Bcl-2, and do not express caspase-8. Doxorubicin (Dox) and etoposide (VP16) are two drugs that are part of all standard protocols to treat NB. Our previous studies suggest that activation of the NF-κB transcription factor is required for Dox to kill N-type NB (*J. Biol. Chem.* 2001, 276: 48921–48929). The present study was designed to characterize the mechanism leading to NF-κB activation in NB cells responding to Dox and VP16. In other systems, p53 activation is known to mediate NF-κB activation (*Nature* 2000, 404:892–7). To determine whether p53 is important in Dox and VP16-induced NF-κB activation, a SH-SY5Y cell line stably expressing dominant negative (DN) p53 was generated. As expected, Dox and VP16 failed to induce NF-κB activation in DNp53/SH-SY5Y cells. More importantly, the cells were significantly resistant to Dox or VP16. In complimentary experiments, p53 was inactivated by expressing human papillomavirus E6 in SH-SY5Y cells. E6 expressing SH-SY5Y cells (E6/SH-SY5Y) were resistant to Dox and VP16-induced NF-κB activation. To dissect the mechanism of p53 induced NF-κB activation, SH-SY5Y cells were transiently transfected to express either dominant negative IkappaB kinase complex alpha or beta (DN IKKα, DN IKKβ). Overexpression of DN IKKα, but not DN IKKβ, blocked the induction of NF-κB by these drugs. Our results suggest that p53 induced NF-κB activation requires IKKα. Through the activation of IKKα, NF-κB could be released from its complex with I-κB**,** an inhibitory NF-κB species, and then translocate into the nucleus. Therefore, it can be concluded that both IKKα and p53 are an important signal molecules to the activation NF-κB in N-type NB. By discovering the mechanism by which Dox and VP16 activate NF-κB, we can begin to investigate potential points along this pathway that could be exploited for targeted therapeutic activation (e.g. direct kinase activation).

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IN VIVO CHARACTERIZATION OF THE THROMBOGENIC POTENTIAL OF A NOVEL GENETICALLY ENGINEERED INACTIVATION RESISTANT FACTOR VIII.

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Hemophilia A is an X-linked bleeding disorder associated with decreased plasma factor VIII (FVIII). With IV replacement therapy, patients may have transiently supranormal FVIII plasma levels. Growing literature has established elevated FVIII levels as a significant risk factor for thromboembolic disease. Factor VIII circulates in plasma bound to von Willebrand factor and upon thrombin (IIa) activation is released as an unstable heterotrimer (subunits A1/A2/light chain), which is rapidly inactivated due to spontaneous dissociation of the A2-domain subunit. Inactivation resistant FVIII (IR8) is a novel genetically engineered FVIII protein that has enhanced in vitro stability after IIa activation due to resistance to spontaneous A2-domain dissociation and proteolytic inactivation. IR8 has a markedly increased specific activity and has demonstrated hemostatic efficacy in a murine model at up to 20-fold lower doses of protein compared to FVIII wild-type (WT). We investigated the thrombogenic potential of IR8 compared to FVIII WT *in vivo* within a murine model of photochemical-induced thrombosis. A Doppler probe is placed on the exposed right carotid artery to measure flow. Rose bengal dye is injected into the mouse via tail vein. The carotid artery is irradiated with a 1.5 mW green light (540nm)-emitting laser which activates the rose bengal creating a mild endothelial injury in the irradiated area. The doppler probe records blood flow through the carotid artery and the time to generation of an occluding thrombosis is recorded. FVIII knockout mice (FVIII -/-) were analyzed following tail vein infusion of varying degrees of FVIII plasma correction with FVIII WT or IR8 protein. The % plasma correction for the infused protein was determined by the activity of the infusate and the weight of mouse. In FVIII -/- mice corrected with FVIII WT, the time to occlusion shortened inversely to the level of correction (117 min at 10%, 37–44 min at 50–77%, 19 min at 300% correction). Suprisingly, FVIII -/- mice corrected with IR8 have prolonged occlusion times (≥90 min at 100% and 35 min at 260% correction) compared with FVIII -/- mice corrected with FVIII WT at similar levels of plasma correction. Thus IR8 is potentially less thrombogenic than FVIII WT in vivo when infused at similar activity levels. These results increase enthusiasm for the potential utility of IR8 for replacement therapy or gene therapy strategies for patients with hemophilia A.

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NESTIN-CRE-SPECIFIC DELETION OF *PITX2* IN THE MOUSE CENTRAL NERVOUS SYSTEM DOES NOT DISRUPT CORPUS CALLOSUM DEVELOPMENT OR SUBTHALAMIC NUCLEUS GENE EXPRESSION.

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Pitx2 is a homeobox transcription factor that is necessary for the development of multiple organs. Prior studies have shown that *Pitx2* is expressed in and required for proper neuronal differentiation in discrete regions of the developing mouse brain. In mice, *Pitx2* loss of function leads to midgestation embryonic lethality around e15, a time at which the neural tube has formed and intense neurogenesis is occurring. The early lethality of $Pitx2^{-/-}$ mice precludes analysis of the $Pitx2^{-/-}$ phenotype at later embryonic timepoints or in the adult mouse, making it impossible to predict the effects of *Pitx2* deficiency in mature neurons. To circumvent this problem, we have generated a conditional deletion of *Pitx2* using a neural specific Cre deleter strain, *Nestin-Cre*. Nestin is expressed in neural progenitors throughout the brain and in other areas of the body, such as the heart and craniofacial regions, coincident with and in a broader expression pattern than *Pitx2*. The Cre recombinase transgene in *Nestin-Cre* mice excises DNA between loxp sites, and is predicted to result in a *Pitx2* null allele in Nestin-expressing cells and their progeny. Analysis of $Pitx2^{-/flox}$; $Nestin\text{-}Cre^{+/tg}$ mice indicates $Pitx2^{-/flox}$; $Nestin\text{-}Cre^{+/tg}$ mice are viable to at least e18.5, but most do not survive beyond the immediate postnatal period. $Pitx2^{-/flox}$; $Nestin\text{-}Cre^{+/tg}$ embryos are smaller at e18.5 than their doubly heterozygous $Pitx2^{-/+}$; $Nestin\text{-}Cre^{+/tg}$ littermates, and they have elongated noses, small eyes, a fused mouth, and a kinked tail. Cresyl violet staining shows that the corpus callosum, a region disrupted in humans with loss of *PITX2* function, is present in e18.5 $Pitx2^{-/flox}$; $Nestin\text{-}Cre^{+/tg}$ embryos. $Pitx2^{-/flox}$; $Nestin\text{-}Cre^{+/tg}$ embryos also express *Pitx2* mRNA in the subthalamic nucleus, a region disrupted in mouse $Pitx2^{-/-}$ embryos at earlier time points. These experiments suggest that Nestin-specific deletion of *Pitx2* is not sufficient for recapitulating the $Pitx2^{-/-}$ CNS phenotype, and is permissive for normal development of the corpus callosum. Further experiments are underway to determine the phenotype of *Pitx2* deficiency using other neural-specific Cre deleter strains.

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PROTEOMIC ANALYSIS OF DIFFERENTIAL PROTEIN EXPRESSION IN NORMOXIC AND HYPEROXIC NEONATAL RAT LUNG.

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Purpose of Study: Oxygen therapy is frequently given to preterm infants with respiratory distress. After the treatment, many infants have lung injury because of oxygen toxicity and develop bronchopulmonary dysplasia (BPD). In this study, we utilized high resolution two-dimensional gel electrophoresis (2-DE) and mass spectrometry to study the protein expression patterns of the neonatal rat lungs in normoxic and hyperoxic conditions.

Methods: Neonatal rats at 4 days of age were randomly assigned to normoxic and hyperoxic groups. The rats in the normoxic and hyperoxic groups were treated with room air and 80% O2 for 3, 6, and 10 days, respectively. The lung tissue was collected and a total protein extract was prepared. 200 ug of proteins from each sample were applied to 2-DE analysis. The protein spots were visualized with Commassie blue staining. The candidate protein spots were excised, digested, and analyzed by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry for protein identification.

Results: More than 200 protein spots were displayed after 2-DE analysis. At least five protein spots from the samples collected at 10 days showed significant changes between the normoxic and hyperoxic groups. The molecular masses of these five proteins ranged from 33 to 60 kDa, the isoelectric points (pI) from 5.5 to 6.0. The protein showing the most dramatic change was about 57 kDa with pI of 6.0. It appeared as a discrete spot on the gels of the normoxic group and the same protein was not visible on the gels of hyperoxic group. This protein was identified as a protein disulfide isomerase isoform, ERp57, by MALDI-TOF mass spectrometry. ERp57 is mainly localized in the endoplasmic reticulum (ER). During protein folding in the ER, ERp57 catalyzes thiol/disulfide exchange, including both intrachain and interchain disulfide bond formations in proteins.

Conclusions: This study demonstrated significant changes in the patterns of protein expression in the lung tissue of neonatal rats between normoxic and hyperoxic conditions. The decreased expression of ERp57 in the lung tissue of the hyperoxic group may be implicated in the lung injury and the development of BPD.

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REDUCED STATURAL GROWTH AND LOW IGF-1 EXPLAIN APPARENT OSTEOPENIA IN PREPUBERTAL CHILDREN WITH CYSTIC FIBROSIS.

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It remains controversial whether children with cystic fibrosis (CF) have osteoporosis. The purpose of our pilot study was to evaluate bone metabolism in young children with CF for the presence of osteoporosis. Methods: 20 children (12 ♀) aged 6–14 years, Tanner stage I, with mild to moderate pulmonary disease (mean FEV_1, 96% predicted) were studied. Subjects completed the Block Food Frequency Questionnaire. Stadiometer height and body mass were recorded. Lumbar bone mineral density (L-BMD) was measured using Dual Energy X-Ray Absorptiometry (DXA), and expressed as a *Z*-score for both chronological (CA) and height age (HA). Serum insulin-like growth factor (IGF-1), parathyroid hormone, 25(OH)- and $1,25(OH)_2$-vitamin D, and markers of bone turnover (OC, BAP, NTX, OPG, and RANKL) were measured. Results: Dietary analysis revealed calcium intake of 98–228% and vitamin D intake of 74–540% of the RDA; none were vitamin D deficient. Height and weight *Z*-scores for CA ranged from –3.3 to +1.3 (median value, –0.4) and from –2.6 to +1.7 (median value, –0.4) respectively. L-BMD *Z*-scores for CA ranged from –3.3 to +1.7 (median value, 0.05), and increased to –2.0 to +1.7 (median value, 0.1), when recalculated using HA rather than CA ($p=0.06$), and only 1/20 had reduced bone mass (*Z*-score more negative than –1.0). All patients with low L-BMD-CA had significant growth stunting. Six of 16 subjects had serum IGF-1 levels low for age, while one was above. Four of six subjects with IGF-1 values below normal for age, and one subject with IGF-1 above normal for age, were below the 15th percentile for height or BMI, and/or had L-BMD-CA *Z*-scores more negative than –1.0. Only 4/20 subjects had OC levels low for age, and 2/20 subjects had RANKL/OPG ratio = 0. Other bone markers (BAP, NTX, OPG, RANKL) were within age-appropriate ranges. L-BMD and serum IGF-1 correlated well with height *Z*-score for CA ($r = 0.60$ and 0.46 respectively, $p<0.05$). Conclusions: Low bone mass in CF does not correlate with nutritional or bone biochemical values, but results from decreased somatic growth, often with deficient IGF-1 levels. Pharmacological intervention needed for augmentation of linear growth in children with CF should have bone mass evaluation as an outcome.

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DUCTUS ARTERIOSUS PATENCY & CEREBRAL BLOOD FLOW VELOCITY ASYMMETRY.

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OBJECTIVE: To test the hypothesis that left (L) Middle cerebral artery (MCA) blood flow velocity (BFV) is lower and more variable than right (R) MCA BFV when the ductus arteriosus (DA) is open, but not after it has closed. **DESIGN/METHODS**: Seventy-one newborns ≤ 33wk GA were prospectively enrolled. BFV was measured in the L followed by the R MCA on days 1 & 7 of life. Average systolic (S), mean (M), and end-diastolic (Ed) BFVs and corresponding coefficients of variation (CV) were calculated from 9 consecutive waveforms. DA patency was determined by echocardiography. Data from the 67 infants whose DA was open on d1 and closed on d7 (25–33wk, 517-2371g) were analyzed by paired Student's t-test with Bonferroni correction for multiple comparisons ($p=0.025$ significant). **RESULTS**: MCA BFV was lower in the L than the R MCA on day 1 of life but not on day 7 (Table 1, m±SD, L v. R, *$p = 0.01$). The CVs for M and Ed were higher (Table 2, m±SD, L v. R, *$p < 0.05$) in the L than R MCA on d1, but not d7. **CONCLUSIONS**: In infants ≤ 33 wk GA, L MCA BFV is lower than RMCA BFV on day1 but not on day 7. Left-sided M and EdBFVs are more variable on day1. We speculate that the physiologically open DA causes the left sided cerebrovascular disturbance.

Table 1

	Blood flow velocities					
	Systolic		Mean		End-diastolic	
	Left	Right	Left	Right	Left	Right
Day 1	27.45 ± 7.37*	29.34 ± 7.17	13.98 ± 4.03*	15.74 ± 4.73	5.35 ± 2.25*	6.76 ± 2.91
Day 7	44.56 ± 8.08	44.05 ± 7.67	23.34 ± 5.36	23.48 ± 5.39	10.09 ± 3.06	9.89 ± 2.95

Table 2

	Coefficients of Variation					
	Systolic		Mean		End-diastolic	
	Left	Right	Left	Right	Left	Right
Day 1	0.68 ± 0.06	0.66 ±0.06	0.14 ± 0.10*	0.09 ± 0.06	0.20 ± 0.15*	0.14 ± 0.08
Day 7	0.06 ± 0.07	0.08 ± 0.01	0.10± 0.12	0.12 ± 0.19	0.14 ± 0.09	0.15 ± 0.10

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ROLE OF REACTIVE NITROGEN AND OXYGEN SPECIES IN COMPROMISING THE RESPIRATORY EPITHELIAL BARRIER DURING HAEMOPHILUS INFLUENZAE INFECTION.

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Haemophilus influenzae (Hi) invasion into the respiratory mucosa is potentially a critical step in pathogenesis. In *in vitro* models, Hi traversal across the bronchial epithelium occurs via paracytosis (between cells). The generation of reactive nitrogen (RNS) and oxygen species (ROS) during infection has been implicated in promoting lung injury and facilitating the invasion of the respiratory mucosa by bacterial pathogens. In prior work, we had observed that Hi infection of bronchial epithelial cells (16HBE14o-) resulted in loss of intercellular zonula occludens-1 (ZO-1, a tight junction (TJ)-associated protein) staining, and an increase in expression of Ca^{2+}-independent nitric oxide synthase (iNOS). In the current study, we undertake a more comprehensive assessment of TJ proteins during infection and evaluated the importance of RNS and ROS in Hi-mediated breach of the epithelium in an air-interface transwell model. By microscopy, ZO-1 staining at the cellular margins became fragmented during infection, and was most marked at points where organisms had become associated with the TJ. In contrast, no redistribution of claudin-4, E-cadherin, occludin, or ZO-2 was noted. We also detected by immunoblotting a significant reduction in the association of ZO-1 with cytoskeletal (TX-100 insoluble) in comparison to cytoplasmic (TX-100 soluble) protein pools derived from infected cells. Functional barrier compromise was assessed by measuring transepithelial electrical resistance (TER) and apical to basolateral flux of FITC-BSA (primarily transported via the paracellular route). Hi infection resulted in a progressive decline in TER and increase in the permeability flux, accompanied by an upregulation of iNOS transcription and nitric oxide synthesis, as well as, depletion of GSH (reduced glutathione) and activation of an antioxidant stress response (induction of Mn-SOD and Heme oxygenase-1 transcription, increase in GSH production). Similar declines in barrier function were elicited by apical exposure of monolayers to either nitric oxide, peroxynitrite or superoxide generating compounds. Hi-induced disruptions in barrier integrity were most attenuated by prior loading of monolayers with either iNOS inhibitors, a cell-permeable superoxide dismutase mimetic (Mn(III)TBAP) or the superoxide scavenger Tiron; the antioxidants N-acetyl-cysteine and ascorbate were relatively less effective. Our studies indicate that both oxidative and nitrosative stress may play roles in unlocking the paracellular route for Hi invasion and are potentially important factors in the dynamic regulation of airway epithelial permeability.

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ENGINEERED LENTIVIRAL LYTIC PEPTIDE ACTIVITY AGAINST CF PATHOGENS.

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The lentiviral lytic peptides (LLPs) are cationic antimicrobial peptides derived from a portion of the HIV1 transmembrane protein. The 28-residue parent peptide is similar to human cathelicidin (LL37) both in its structure and ability to kill bacteria selectively. Engineered LLP derivatives (eLLPs) have been designed for increased activity against a variety of multiply resistant bacterial pathogens. This study evaluates their ability to selectively eliminate pathogens associated with cystic fibrosis (CF) airway infection, specifically *Staphylococcus aureus* (SA), *Pseudomonas aeruginosa* (PA), and *Burkholderia cepacia* complex (BCC). Broth dilution assays were used to compare the minimum bactericidal concentration (MBC) of the eLLPs and LL37 in phosphate buffer with and without 150mM NaCl. WLSA5, a tryptophan-rich eLLP, demonstrated greater bactericidal activity (MBC 0.1–3.0μM) versus LL37 against SA and PA in 150mM NaCl. WLSA5 showed equivalent activity with LL37 against SA and PA and greater activity against BCC in phosphate buffer alone. WLSA5 at 25μM demonstrated bactericidal effects against genomovars 1 through 5 of BCC, with at least 75% reduction in the number of bacterial colonies. LL37 activity against the five BCC genomovars was variable but consistently decreased compared to WLSA5. We also examined the activities of WLSA5 and LL37 in a CF airway epithelial cell culture model utilizing adherent PA. The MBC of WLSA5 in this model was increased tenfold when compared to standard broth dilution assays described above, while LL37 had no activity. Furthermore, 10μM WLSA5 or LL37 only transiently perturbed the transepithelial resistance of the cell monolayer. At 50μM, the reduction in resistance was higher and in some instances did not return to baseline after 24h. WLSA5 stimulated a less vigorous IL-8 response in the cell culture model when compared to LL37. Based on these results, we conclude that eLLPs can be generated that are more potent, yet no more toxic, than host-derived antimicrobial peptides such as the cathelicidins and merit serious consideration for their potential use in CF airway infection.

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EFFECTS OF HYPEROXIA ON PLACENTA GROWTH FACTOR IN NEONATAL RAT LUNG.

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Purpose of Study: Hyperoxia is one of the major factors in the pathogenesis of Bronchopulmonary Dysplasia (BPD). Decreased level of vascular endothelial factor (VEGF) has been demonstrated to play a critical role in hyperoxic lung injury and repair. The aim of this study is to examine the role of placenta growth factor (PLGF), a recently identified member of the VEGF family, in rat lung development and to study the effects of hyperoxia on PLGF expression in neonatal rat lungs.
Methods: Neonatal rats at 4 days of age were randomly assigned to normoxic and hyperoxic groups. The rats in the normoxic and hyperoxic groups were treated with room air and 80% O2 for 3, 6, and 10 days, respectively, and the lung tissues were collected. PLGF mRNA was measured by quantitative real-time polymerase chain reaction (Q-PCR). Its protein expression was studied by Western blot analysis. Immunohistochemistry was also performed to determine the cellular localization of PLGF in formalin fixed lung tissue.
Results: The levels of PLGF mRNA and protein expression were relatively high in the lung tissue of neonatal rats and the levels were significantly lower in the lung tissue of adult rats. PLGF mRNA and protein expression showed no significant changes in the hyperoxic group after a 3-day oxygen treatment, but decreased by 30% after a 10-day O2 treatment compared to the normoxic group. PLGF was found predominantly in the epithelial cells of the bronchial tree. Low immunoreactivity was also present in vascular endothelial cells.
Conclusions: The presence of high level of PLGF in the newborn lung tissue indicates that PLGF may play an important role in lung development. Prolonged O2 exposure decreases PLGF mRNA and protein levels in the neonatal lung tissue. Decreased PLGF expression may be implicated in hyperoxic lung injury and the development of BPD.

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EARLY AND LATE BEHAVIORAL EFFECTS OF DEVELOPMENTAL IRON DEFICIENCY ANEMIA IN THE RAT.

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Iron deficiency anemia (IDA), a common nutritional disorder during development, has neurobehavioral sequelae. Animal studies have demonstrated altered striatal and hippocampal function with IDA. However, functional outcomes associated with these systems have not been well-described. The objective of this study was to determine the effect of developmental IDA on behaviors sensitive to striatal and hippocampal function in the rat. Methods: Nine-week old dams were randomized to Control (C) or IDA groups and placed on iron sufficient or deficient diets during early gestation. Diets continued until postnatal day (P)21 then all pups received the iron sufficient diet. Pup hematocrit and weight were monitored. Sensorimotor function was assessed using a developmental battery every three days during lactation. Performance on the Morris watermaze was assessed at P35 and 9 months. Data were analyzed using one-way and repeated measures ANOVA. Results: IDA pup hematocrit was reduced by 40% vs C ($p<0.05$) during lactation and normalized by P35. IDA pup weight was reduced by 18% vs C at P25 ($p<0.05$) and normalized by P90. The following measures of sensorimotor development were delayed during postnatal development for IDA pups vs C ($p<0.05$): auditory startle, barhold, negative geotaxis, surface righting, and head-on and vibrissae placing (Fig. 1). Previously IDA pups had longer latencies to reach a stationary platform in the watermaze at P35 ($p<0.01$). At 9 months of age, IDA and C rats did not differ for latency to reach a stationary platform. However, previously IDA rats had longer latencies to reach the platform when the location alternated by trial (Fig. 2). Conclusions: The emergence of functional behaviors dependent on intact striatal function is delayed with IDA. Despite iron treatment, performance on a spatial learning task dependent on hippocampal function is poorer for previously IDA animals. Behavioral measures provide sensitive indicators of specific neurobiologic system deficits during anemia and after iron treatment.

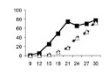

Fig 1. Vibrassae placing (% trials)*

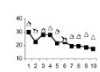

Fig 2. Watermaze-Latency (sec)* (C ■ ID △ *RPM $p<0.05$)

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INSPIRATORY NEURONS IN THE VENTROLATERAL MEDULLA ARE INHIBITED DURING SUPERIOR LARYNGEAL STIMULATION IN PIGLETS.

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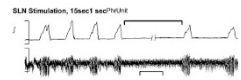
Stimulation of laryngeal mucosa in newborns results in apnea. Such reflex apnea is central in origin and is mediated through the superior laryngeal nerve (SLN). The inspiratory group of neurons inhibited during SLN stimulation has not been identified. We have identified an area in the ventrolateral medulla where microinjection of $GABA_A$ receptor blocker bicuculline was able to block reflex apnea induced by SLN stimulation. In this study we aimed to study the pattern of firing of neurons located in the specific area where bicuculline microinjection prevented reflex apnea. The studies were performed in 3–7 day old newborn piglets (n=3). The animals were intubated, decerebrated, vagotomized and paralyzed. The phrenic and SLN nerves were dissected, isolated and placed on measuring and stimulating electrodes, respectively. The dorsal surface of the medulla was exposed and the calamus scriptorius identified. A metal microelectrode (tungsten, Fred Haer Inc) with a 10 micron tip and impedances between 5–7 Megaohms was used for single unit recording. The microelectrode was advanced to an area 5 mm rostral to the calamus scriptorius, 5 mm lateral to the midline and 2 mm dorsal to the ventral surface of medulla using a hydraulic microdrive (Kopf Instruments). Single units encountered during electrode advancement were determined to be either respiratory or non-respiratory according to firing synchronization with phrenic nerve discharge. The phrenic nerve and single unites activity measurements were performed before and in response to SLN stimulation at a level that caused complete cessation of phrenic nerve activity. At the identified area we encountered 14 single units that fired in synchrony with the phrenic nerve and so identified as inspiratory, and seven units that were characterized as expiratory. All inspiratory units were silenced during SLN stimulation and only started firing with the recovery of phrenic activity. Three early expiratory units were activated while four other expiratory units were not affected by SLN stimulation. We conclude that the identified site consists mostly of inspiratory neurons that are preferentially inhibited during SLN stimulation. We speculate that such neurons are rhythm-generating neurons and belong to the pre-Botzinger complex.



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COMPLICATIONS AND OUTCOMES IN PREMATURE TWINS <35 WEEKS GESTATION: SPONTANEOUS CONCEPTION VERSUS ASSISTED REPRODUCTIVE TECHNOLOGY.

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Although a substantial proportion of multiple gestations arises from assisted reproductive technology (ART), it remains controversial whether outcomes are systematically different. To assess differences in neonatal complications and survival, we retrospectively compared premature twins ≤34 weeks gestation born at the University of Michigan from 1991–1999 who resulted from ART to those who were spontaneously conceived. All infants of this gestation were routinely admitted to the neonatal intensive care unit (NICU) during this period. Infants conceived with pharmacologic assistance alone (clomiphene, pergonal or gonadotropins) were excluded, as were infants with major anomalies and those whose gestational siblings were not liveborn. Outcomes of interest included RDS (radiography and requirement for positive airway pressure) , infection (proven by culture of blood, CSF or other sterile site), NEC (proven by pneumatosis, portal gas, or surgery), PDA (requiring ligation or indocin), IVH grade II or greater, PVL, length of hospitalization in survivors (LOS), and requirement for supplemental oxygen at 36 weeks post-conception (CLD). During this period, 406 liveborn preterm twins (90 ART, 316 non-ART) were admitted to the NICU. ART mothers were more likely to be preeclamptic (18 v 9%, $p=0.05$) and receive antenatal steroids (55 v 22%) and less likely to smoke or use illicit substances (0 v 16%; $p<0.0001$). No significant differences between ART and non-ART were found for delivery route, BW (median 1641 v 1630 gm), GA (31 v 31 wk), male gender (53 v 51%), infection (12 v 9%), RDS (60 v 55%), PDA (22 v 19%), IVH (18 v 11%), PVL (3 v 3%), or LOS (39 v 33 d). CLD (32 v 18%) and death (10 v 4%) were significantly more frequent ($p \leq 0.05$) in ART twins. Although outcome is good in most liveborn premature twins, these data suggest they may at increased risk for some major adverse outcomes. Absence of data regarding long-term outcomes, singletons, higher orders of multiples, severity of index outcomes, twins who may have been liveborn but not admitted to the NICU, as well as possibly incomplete data about maternal risk factors and which twins resulted from ART, necessitate caution in interpretation of these observations.

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INHALED PGE1: A COST-EFFECTIVE SELECTIVE PULMONARY VASODILATOR IN NEONATAL PPHN WHICH PREDICTS THE NEED FOR ECMO.

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Background: Extensive experience with inhaled nitric oxide (NO) in neonatal PPHN shows lack of sustained improvement in 30–40% of patients. Moreover, NO is a toxic molecule requiring specialized delivery systems and monitoring, making treatment expensive and limiting availability. Aerosolized prostaglandins I2 (iPGI2) and E1 (iPGE1) are inexpensive and pharmacologically safe alternatives. **Objective:** Comparative cost effectiveness analysis of the use of iPGE1 in a Phase I, dose escalation, safety and efficacy study in neonatal PPHN. **Design/Methods:** Twenty neonates with PPHN associated with RDS (5), meconium aspiration (9), pneumonia (1) and idiopathic (5) received $iPGE_1$ for OI≥20. $iPGE_1$ was delivered by a jet nebulizer in incremental doses (25, 50, 150, 300 ng/kg/min for ~30 min each) over ~2 hrs. Two groups were defined based on disease severity. Group I patients received $iPGE_1$ before receiving INO (n=13). Group II patients were refractory to INO (n=7). Adverse effects were monitored. Response was defined as an increase in PaO_2 of ≥25 torr (full) or 10–25 torr (partial). **Results:** The (mean [SD]) birthweight, gestation and postnatal age were 3.1 [0.6] kg, 37.7 [2.4] wks, and 46 (69.5) hrs respectively. There was significant improvement in OI ($p<0.005$) and PaO_2 ($p<0.01$) following $iPGE_1$. The mean (SD) OI at baseline and end of $iPGE_1$ administration was 25.9 (6.3) and 14.5 (8.2) in Group I and 37.0 (4.8) and 21.8 (15.2) in Group II. Two neonates in Group I exited without receiving iPGE1; three other failed to respond to $iPGE_1$, were refractory to INO and were placed on ECMO. The remaining 8 of 13 neonates in Group I showed an increase in PaO_2 with $iPGE_1$. Four of these deteriorated on withdrawal of $iPGE_1$; two responded to INO and 2 other to ECMO. Of the 7 infants in Group II, 3 had full response; 1 had partial response and was placed on ECMO; three others did not complete the study and were placed on ECMO. No adverse events were identified. In this cohort, the charge-saving for treatment for an infant with PPHN following iPGE1 for ~ 2 hrs was $2516 for Group I and $ 2087 for Group II. A full response to iPGE1 had a sensitivity of 100%, a specificity of 77.8%, a PPV of 81.8% and a NPV of 100% in predicting the need for ECMO in this small unblinded study. **Conclusions:** $iPGE_1$ improved oxygenation in neonates with PPHN even when given for a short duration (~ 2 hrs) with charge-saving. Prospective, large scale studies with long-term follow-up are needed to validate the findings of this study.

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MEASURE OF INSULIN RESISTANCE IN ACTIVE POSTPUBERTAL ADOLESCENT GIRLS ACROSS THE WEIGHT SPECTRUM.

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Objective-The incidence of type 2 diabetes in youth is rising and adolescent girls have declining rates of physical activity as they progress through puberty. Post-pubertal girls across the normal weight spectrum were recruited to determine predictors of insulin-resistance by homeostasis model assessment ($HOMA_{IR}$).

Research Design and Methods- Fifty-three adolescent girls of 16 to 20 years, were enrolled. Maximal oxygen consumption (VO_2 max) was determined by an exercise treadmill test and body composition was measured by total body dual X-ray absorptiometry. During an overnight admission at the University of Michigan General Clinical Center, testosterone, sex hormone-binding globulin, glucose, insulin and IGF1 were measured. Insulin resistance was estimated by the $HOMA_{IR}$ index derived from three fasting consecutive glucose and insulin and was used as the dependent variable.

Results-Cardiovascular fitness was normal for age (mean VO_2 max 40.5 ± 1.0 mg/ml.min) and average BMI was 23.3 ± 0.45. The mean value for $HOMA_{IR}$ was 2.9 ± 0.15. Percent body fat and VO_2 max were significantly related to $HOMA_{IR}$ ($p= 0.017$ and 0.001, respectively) while BMI, androgenicity index, IGF1 and diet had no predictive value. When both percent body fat and cardiovascular fitness were analyzed together, the strongest predictor of insulin resistance was VO_2 max ($p=0.01$), while percent body fat lost its significance ($p= 0.36$).

Conclusion- In post-pubertal adolescent girls, VO_2 max is a more critical predictor of insulin resistance than BMI and percent body fat. Relative inactivity may represent a greater risk than obesity alone and aerobic exercise might be the most effective strategy for the prevention of type 2 diabetes in youth.

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IDENTIFICATION OF CAPSULAR POLYSACCHARIDE AS A VIRULENCE DETERMINANT IN *BURKHOLDERIA CENOCEPACIA*.

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The *Burkholderia cepacia* complex consists of several phylogenetically closely related bacterial species that are important pathogens in persons with cystic fibrosis (CF). Unlike other respiratory pathogens in CF, these species cause necrotizing pneumonia and septicemia. Among the species in this group, *B. cenocepacia* is the species that most frequently infects CF patients. Unfortunately, little is known about specific *B. cenocepacia* virulence determinants. In previous work, we used subtractive hybridization to identify a novel insertion element, designated IS1363, in *B. cenocepacia* isolate PC8. This isolate is a member of the PHDC clonal lineage, which is known to infect CF patients in 24 US states. Analysis of DNA sequences flanking IS1363 revealed open reading frames (ORFs) with homology to *dedA*, *yggB* and *wcbA*, three genes included in capsular polysaccharide biosynthesis gene clusters previously described in the related species *B. mallei* and *B. pseudomallei*. By screening and DNA sequence analysis of a PC8 genomic library, we identified a 27 kb gene cluster downstream of *wcbA* containing 19 ORFs, 13 of which also had high homology to *B. mallei* and *B. pseudomallei* capsular polysaccharide biosynthesis genes. By using reverse transcriptase PCR analysis, we demonstrated expression of several genes in this cluster. We also used insertional mutagenesis to interrupt several of these. By using Alcian blue staining and Western blot analysis, we showed that mutagenesis of the *wcbC* homolog was associated with the greatest disruption of capsular polysaccharide expression. We assessed the wild type and *wcbC* mutant strains in a model of polarized 16HBE14o$^-$ epithelial cell monolayers grown in an air-liquid interface. In previous studies we found that *B. cenocepacia* strains were able to traverse this epithelial layer by disruption of tight junctions. They also decreased transepithelial electrical resistance and increased transepithelial flux of FITC-labeled bovine serum albumin. Although the wild type parent strain traversed the cell layer by 12 hours, the *wcbC* mutant did not. The *wcbC* mutant also induced significantly greater release of β-glucuronidase from cytochalasin B-treated human neutrophils than did the parent strain, indicating a role for *B. cenocepacia* capsular polysaccharide in evading neutrophil killing. In summary, we have shown that disruption of *B. cenocepacia* capsule expression by mutation of *wcbC* results in decreased capacities for human epithelial cell layer transmigration and neutrophil evasion. These data suggest that capsular polysaccharide contributes to the virulence of *B. cenocepacia* in human infection.

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SOLUBLE HEPARIN SULFATE INHIBITS MURINE GAMMAHERPESVIRUS 68 INFECTION.

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Murine gammaherpesvirus 68 (MHV-68) provides a useful tool in the study of the pathogenesis of the human gammaherpesviruses Epstein-Barr virus (EBV) and human herpesvirus 8 (HHV-8). Identifying the cellular receptors for MHV-68 is an important step in fully understanding the its pathogenesis. Herpesviruses employ multiple cell surface receptors for attachment to cells and for entry into cells. The majority of herpesviruses, along with many other types of viruses, use cell surface glycosaminoglycans (GAGs) such as heparan sulfate for the initial step of attachment to target cells. To test the hypothesis that MHV-68 uses heparan sulfate as a receptor, we examined the effects of soluble heparin sulfate sodium on MHV-68 infection of susceptible cell lines. Soluble heparin reduced in a dose-dependent manner, but did not totally eliminate, infection of owl monkey kidney (OMK) cells by recombinant MHV-68 encoding enhanced green fluorescent protein (eGFP). A related GAG, chondroitin sulfate, inhibited infection only at high concentrations. Soluble heparin almost completely eliminated plaque formation on NIH 3T3 cell monolayers. Again, chondroitin had a substantial effect on plaque formation only at the highest concentration used. Heparin-mediated blockade resulted in decreased MHV-68 gene expression in infected OMK cells as measured by ribonuclease protection assay. Incubation of OMK cells with heparin or chondroitin after MHV-68 infection did not alter patterns of viral gene expression, suggesting that inhibition of infection was not caused by direct effects of these substances on viral replication. Likewise, inhibition of infection was not caused by increased death of target cells, as incubation of OMK cells with either heparin or chondroitin resulted in only minimal decreases in cell viability. Lastly, treatment of NIH 3T3 cell monolayers with heparinase I to remove cell-surface heparan also resulted in substantially decreased, though not absent, plaque formation by MHV-68. Collectively, this data suggests that MHV-68 does utilize heparan sulfate as a cellular receptor. The fact that blockade with soluble heparin and enzymatic digestion of heparan sulfate does not totally abrogate infection indicates that heparan sulfate is likely not to be the exclusive receptor for MHV-68.

Poster Presentations Program #27–53

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GENOTYPIC AND PHENOTYPIC DIVERSITIES OF THE COMPLEMENT C4 AMONG ASIAN INDIANS IN THE US.

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The human complement component C4 genes, which are located in the major histocompatibility complex (MHC) class III region, have been extensively studied in the Caucasian population. The two isotypes C4A and C4B although sharing >99% sequence identities, have different hemolytic activities, serological activities, and covalent binding affinities to antigens and immune complexes. In Caucasians, the number of C4 genes present in an MHC varies mainly from 1 to 3. The long C4 genes have a frequency about 76% while the short gene has a frequency about 24%. C4 is a constituent of the 4-gene module, termed the RCCX, which also contains the genes TNXA, RP2 and CYP21A or CYP21B. In this study we focus on the polygenic and gene size variation of C4A and C4B in the Asian Indians that would provide essential information for epidemiologic studies of infectious and autoimmune diseases associated with this important ethnic group. The quantitative and qualitative variations in the C4 proteins in 56 healthy Asian Indians were determined by single radial immuno-diffusion and immunofixation experiments. The variations in the number and size of C4A and C4B genes were determined by PmeI pulsed field gel electrophoresis (PFGE) of genomic DNA prepared from fresh white blood cells, and by TaqI and PshAI-PvuII RFLP analyses. Significant differences were observed on comparison to normal Caucasian population previously studied. The C4 gene dosage of Asian Indians varied from 3 to 6.Fifteen percent of the subjects have low gene dosage (i.e. <4 genes) and 38% have high gene dosage (i.e., >4 genes). This was significantly different on comparison with those of Caucasians ($p=0.025$). PFGE experiments revealed several individuals with quadrimodular RCCX haplotypes containing different combinations of 4 consecutive long or short C4 genes on a chromosome. Statistically significant differences were also observed on comparison of C4A gene dosage between the two populations ($p=0.004$). The frequency of long and short C4 genes was 71% and 28%, respectively. Two (3.6%) subjects had complete C4B deficiency. In addition, rearrangements of the neighboring CYP21 and TNX genes were observed in 3 (5.4%) individuals. Asian Indians have a higher number of C4A genes that play an important role in the clearance of immune complexes. Higher C4 gene dosages may also have conferred Asian Indians greater protection against autoimmune disorders and infectious diseases.

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IS SEXUAL MATURITY OCCURRING EARLIER AMONG U.S. CHILDREN?

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We compare race- and sex-specific onset and completion of puberty and median ages of entry for Tanner stages among U.S. children across national surveys: NHES (1966–70), HHANES (1982–84) and NHANES III (1988–1994). SUDAAN was used to calculate proportions of entry into a stage. Medians and selected percentiles for ages at entry for each stage were estimated from probit analysis. There were no differences in the ages for sexual maturity indicators for black boys between NHES and NHANES III. The white boys in NHANES III were in stage 2 or greater for genital development and they entered stage 3 genital development and stages 3 and 4 pubic hair earlier than the white boys in NHES but were significantly later in their ages at entry into stage 5 genital development. The Mexican-American boys in NHANES III were also in stage 2 or greater for genital development, and they entered stages 3 and 4 genital development earlier than the HHANES boys, but the entry into stage 5 for genital and pubic hair development was not significantly different in the HHANES-NHANES III comparisons. There were no differences in the ages for sexual maturity indicators for black girls or white girls between NHES and NHANES III. The white girls in NHANES III enter stage 5 pubic hair later than the NHES white girls. Mexican-American girls in NHANE III were in stage 2 or greater for breast and pubic hair development than the HHANES girls and had earlier ages at entry into stage 4 breast and pubic hair development than the HHANES girls. The NHANES III Mexican-American girls had ages at entry into stage 5 pubic hair later than the HHANES girls. In conclusion, sexual maturation for black boys and white boys and black girls and white girls has not become earlier over the past 30 years. Sexual maturation in Mexican-American boys and Mexican American girls has not become earlier in the past 10 years. For white boys and Mexican-American boys and girls, the onset of puberty and its progression are earlier than in the past, but the completion of sexual maturation is later. Supported by HD-38356, NIH.

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DISCORDANT SEXUAL MATURATION IS RELATED TO BODY WEIGHT AND BMI AMONG U.S. CHILDREN.

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Purpose: This study investigates the extent to which weight, height, and BMI is associated with the degree of discordance between stages of sexual maturity indicators. Methods: Weight, height, BMI and Tanner stages from 2105 boys and 2106 girls aged 8–19 years from the National Health and Nutrition Examination Survey III (NHANESIII, 1988–1994) were analyzed. This survey followed a complex, stratified, multistage probability cluster design. Data were divided into sex-specific groups according to the degree of discordance of their assessed stages of sexual maturation within the same individual: genitalia (or breast) stage equal to pubic hair stage and genitalia (or breast) stage more or less advanced than pubic hair stage by one or two stages. Weight, height, and BMI were compared separately by sex and race between discordant groups using analysis of covariance adjusted for age. SUDAAN software was used to incorporate the complex sampling design of NHANESIII. Results: In boys, mean weight and BMI values were smallest in those whose pubic hair stage was less advanced than their genital stage, higher for boys with concordant pubic hair and genital stages, and largest for boys with pubic hair more advanced than their genital stage. There were no significant differences in height between the discordant groups for boys. In girls, mean weight and BMI was smallest for those with pubic hair stage more advanced than their breast development stage, larger for those with concordant stages, and largest for those whose breast stage was more advanced than their pubic hair stage. There were no significant differences in height between the discordant groups for girls. The larger the discordance in stages between the indicators, the greater the difference in weight and BMI for boys and girls. The extent of these differences in weight, BMI and height between the discordant groups was present for each racial group: non-Hispanic white, non-Hispanic black, and Mexican-American. Conclusion: Discordance in stages of sexual maturation, irrespective of age, is related to growth in weight and BMI. Supported by HD-38356, NIH. Email: christine.schubert@wright.edu

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IMPACT OF DEXAMETHASONE TREATMENT ON ENTEROCYTE DIVISION IN THE NEONATAL PIGLET.

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Background: Little is known about the development of intestinal mucosa in preterm infants. Increased enterocyte replacement time (7–10 days) has been reported in newborn piglets as compared to 3 week old piglets (3 days) (Moon et al., Proc Exp Biol Med, 137:151, 1971). Decreased enterocyte turnover may result from decreased rate of enterocyte division. This maybe an explanation for the increased risk of intestinal perforation in neonates treated with dexamethasone (dex). **Objective**: The goal of this study was to utilize immunohistochemical staining with murine antibodies for bromodeoxyuridine (BrdU, a thymidine analog) and proliferating cell nuclear antigen (PCNA, a DNA polymerase enzyme produced during cell division) to determine the percentage of enterocytes dividing in the small intestinal crypts of suckled piglets treated with dex as compared to control animals from birth to 11 days. We hypothesized that the percentage of dividing enterocytes would increase with age and that dex treated animals would exhibit a lower percentage of dividing enterocytes than control animals at all time points sampled. **Design/Methods**: 11 suckled piglets (1.48+0.44 kg birth weight) from a single litter comprised the control group. 13 suckled piglets (1.64+0.23 kg birth weight) from 2 litters received daily i.m. injections of 1mg/kg dex. All piglets were injected with 50 mg/kg BrdU 1h prior to sacrifice. Two piglets from each group were sacrificed on days 0,3,5,7,9, and 11 of life. The small intestine of each piglet was harvested and fixed in Carnoy's solution. Cross-sectional tissue sections from the duodenum, jejunum, and ileum (mean of 173 ± 75 crypts analyzed per piglet) were examined for BrdU and PCNA labeling. The ratio of labeled to total crypt cells was determined. **Results**: All piglets remained healthy as indicated by a mean weight gain of 215±4 g/day. In control animals, BrdU labeling revealed that the fraction of dividing crypt cells was 26.3±6.3, 28.2±5.3, and 31.9±5.7, in the duodenum, jejunum, and ileum respectively with no change over time. Consistently, PCNA labeling in control animals suggested that the fraction of dividing crypt cells was 32.8±7.2, 36.1±6.6, and 35.6±6.9 in the duodenum, jejunum, and ileum respectively with no change over time. In piglets treated with dex, BrdU labeling revealed similar results in that the fraction of dividing crypt cells was 32.6±5.0, 35.8±6.2, and 34.2±6.5 in the duodenum, jejunum, and ileum respectively with no change over time. **Conclusions**: Enterocytes in the intestinal crypts of neonatal piglets appear to divide at a constant rate during the first 11 days of life. Dex treatment does not effect this rate.

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ACETYLATION STATUS OF IKBA DETERMINE ITS LOCALIZATION.

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Neuroblastoma is a heterogeneous pediatric cancer derived from the sympathetic nervous system. Children over 1 year of age with stage IV NB tumors respond poorly to treatment, whereas low stage tumors in infants frequently undergo spontaneous remission. Failure to undergo programmed cell death is a putative mechanism for cancer cell resistance to chemotherapy, while in contrast spontaneous remission is thought to be a result of increased apoptosis. Neuroblastoma cells display two predominant phenotypes identified as N and S type in cultures. Previous studies had shown that NF-κB activity and function differ according to NB tumor cell phenotype. Constitutive NF-κB activity is essential for the survival of S-type cells whereas in doxarubicin induced N-type cells NF-κB activation induces cell death. In the absence of external stimuli NF-κB is present in the cytoplasm as a complex with its inhibitory protein IκBα. In presence of external stimuli such as cytokines, uv radiation or viral infection IκBα is phosphorylated by kinases and this activates the gene transcription. Histone acetylases (HATs) and histone deacetylases (HDACs) regulates the gene expression of the transcription factors. In order to understand the role of NF-κB activation in NB pathogenesis we have studied the acetylation of IκBα.We have mapped the acetylation site of IκBα to two lysine residues at amino acid 87 and 98 of IKBα. We have carried out mutational analysis and studied the role of the double lysine mutant K87, 98R compared to the wild type IκBα. The fractionation experiment clearly indicated that the double mutant in which both the acetylation sites are mutated localizes to the nucleus where as the wild type IκBα is predominantly cytoplasmic. When treated with TNFα the double mutant does not undergo degradation even after 2 hours treatment with TNF where as the wild type IκBα undergoes degradation after 1 hour treatment with TNF. Functional analysis using reporter showed that the double lysine mutant activity is similar to that of the supper repressor. The above studies clearly indicate the role of HATs and HDACs in regulating the NF-κB, that plays an important role in NB pathogenesis.

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AXONAL PROJECTIONS IN THE MOUSE SUBTHALAMIC NUCLEUS AND SUPERIOR COLLICULUS REQUIRE *PITX2* FOR PROPER DEVELOPMENT.

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Central nervous system (CNS) development proceeds through a coordinated series of events that transform the relatively simple neural tube into a complicated structure consisting of highly regionally specific populations of neurons and glia. These developing neurons form complex, evolutionarily conserved networks of axons that guide development of future CNS structures, and encode information important for regional specialization of the developing brain. Transcription factors play an important role in early CNS regional specialization, through anatomically distinct expression patterns that dictate activation and repression of genes necessary for proper neuronal network development. *Pitx2*, a paired-like homeodomain transcription factor, is expressed in the embryonic neural tube in a pattern suggestive of a role in CNS regional specialization. In zebrafish, *Pitx2* participates in left-right asymmetry, and in mouse and human it is essential for normal development of multiple organs, including the pituitary gland, eyes, heart, and abdomen. Complete loss of *Pitx2* in mice ($Pitx2^{-/-}$) is associated with embryonic lethality by ~e15 due to cardiac defects, and our earlier work implicates a role for *Pitx2* in regulating embryonic CNS gene expression. *Pitx2* is expressed in discrete populations of postmitotic neurons in the embryonic mouse brain, including GABAergic neurons of the thalamus and superior colliculus. In this study, we analyzed the effects of PITX2 deficiency on neuronal projections in two areas of high PITX2 expression, the embryonic mouse ventrolateral thalamus and superior colliculus. We used injections of the lipophilic dye, 1,1'-dioctadecyl-3,3,3',3'- tetramethylindocarbocyanine perchlorate (dil) to retrogradely label neuronal axons and cell bodies. We found loss of subthalamo-tegmental neuronal projections in e13.5 $Pitx2^{-/-}$ embryos, and reduced rostral projections in developing superior colliculus. Our results suggest a role for *Pitx2* in regulating regionally specific axonal outgrowth in the developing ventrolateral thalamus and midbrain, perhaps through interactions with other transcription factors or signaling molecules.

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ANTECEDENT HYPOGLYCEMIA DOES NOT ALTER MUSCULAR INSULIN RESISTANCE DURING EPINEPHRINE INFUSION IN HEALTH SUBJECTS.

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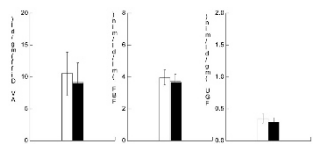
Antecedent hypoglycemia has been shown to increase β-adrenergic sensitivity in healthy control subjects as demonstrated by increased heart rate during isoproterenol infusion. It is unknown whether it alters metabolic responses to epinephrine. I therefore studied how hypoglycemia on the day prior affected forearm glucose utilization during epinephrine infusion in 6 healthy control subjects between the ages of 18 and 40 years of age. Each subject was studied twice, once with antecedent hypoglycemia (50 mg/dl, Ante Hypo) and once with antecedent euglycemia (90 mg/dl, Ante Eu) on Day 1 of the study. Hypoglycemia was induced using insulin infusion (40 mU/m^2/min) from 0800 to 1000 and from 1200 to 1400. With Ante Eu similar insulin infusions were used but 20% dextrose was infused to maintain normal glucose concentrations. On Day 2 a 4 hour insulin clamp was performed with epinephrine (0.015μg/kg/min) infused over the last 3 hours. Arterial plasma glucose levels, measured via a radial artery catheter, were held at 90 mg/dl. Venous plasma glucose levels and forearm blood flow (FBF, occlusion plethysmography) were measured in the dominant arm so that forearm glucose uptake (FGU) could be determined via the Fick Principle, FGU=[arterial-venous glucose (AV diff)]*FBF.



The figure compares AV diff, FBF, and FGU at the end of the epinephrine infusion between Ante Hypo (open) and Ante Eu (solid) studies. There were no differences present. These results demonstrate that antecedent hypoglycemia does not alter muscular glucose utilization to physiologic epinephrine infusion in healthy control subjects.

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ARRHYTHMIAS IN PATIENTS AFTER CONGENITAL HEART SURGERY.

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Background: Arrhythmias are often associated with congenital heart surgery. The incidence and predictors of these arrhythmias have been incompletely described in this population.

Methods: We prospectively followed 141 pts with a mean age of 3.7 ± 7.3 years undergoing congenital heart surgery at our institution between 1/7/03 and 6/7/03 via telemetry reviewed every 24 hours from surgery until they were discharged from the hospital (mean = 12.3 days ± 14 days). Risk factors evaluated included age, cardiac defect, ventricular function and AV valve regurgitation, cardiopulmonary bypass, and cross clamp times.

Results: The incidence of arrhythmias was 27% (n=38). These included frequent (> 10/min) PVCs (n=20), JET (n=10), non-sustained VT (n=10), and SVT (n=4). One pt developed transient complete heart block. Pts who developed post-op arrhythmias were more likely to be on inotropes (p=0.001), had surgical modified ultrafiltration (p=0.029), longer ischemic time (p=0.007), clamp time (p=0.023), pump time (p=0.011), days in hospital (p=0.001) and showed a trend to more poor outcomes (death or transplant) (n=4, p=0.085). Age, gender, cardiac function, residual AV valve regurgitation, and presence of pre-op arrhythmias did not predict post-op arrhythmias. Surgeries with the highest incidence of arrhythmias involved the LV outflow tract (48%) and TOF (40%). Majority (55%) of the pts had an arrhythmia occur on the first post-op day. Amiodarone was the most frequently used antiarrhythmic (n=9) for a mean duration of 1.7 days (range 1–6 days).

Conclusions: Pts who developed post-op arrhythmias were more likely to have undergone certain types of cardiac surgery, require inotropes, have had modified ultrafiltration, spent more time with ischemia, longer clamp and pump times, and to have spent longer in the hospital. This data may allow selection of high risk groups for prophylactic treatment of arrhythmias.

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PHARMACOLOGICAL TREATMENT OF HYPERINSULINEMIA OBESITY SYNDROME IN CHILDREN.

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INTRODUCTION: Obesity is a significant and growing problem in children in the United States. Childhood obesity carries with it risk for other morbidities including hyperinsulinemia and type 2 Diabetes Mellitus. The purpose of this retrospective study was to compare the efficacy of a combination drug therapy among three different age groups of children with hyperinsulinemia and obesity. **METHODS:** All subjects were referred to the Pediatric Endocrinology Clinic at Advocate Hope Children's Hospital for treatment of childhood obesity. The subjects were started on a combination drug therapy using amphetamine multi-compounds (Adderall XR®) and metformin (Glucophage XR®). Data was collected by chart review on all subjects, including demographics, treatments, and outcomes (body mass index- BMI, and insulin levels). Subjects were divided into 3 groups to evaluate response by age group, Group I: < 11 yrs. Group II: 11–14 yrs., and Group III: >14 yrs. Response to treatment was defined as a decrease in BMI between baseline and the 12 months evaluation. **RESULTS:** Thirty-three subjects completed 12 months of treatment at the time of this report. There were 15 males and 18 females. The racial distribution consisted of 21 Caucasians, 6 Hispanics, 1 African American, and 5 others. *Fasting and 2-hour post-prandial blood glucose levels were normal in all subjects at baseline and at 12 months of treatment. Median values for blood chemistries evaluated at baseline were within normal limits. The baseline mean insulin levels for 18 subjects for which data was available were elevated at 35.2 IU/ml fasting and 106.8 IU/ml 2-hour post-prandial. These values decreased to 15.4 IU/ml fasting ($p=0.005$) and 44.5 IU/ml 2-hr post-prandial ($p=0.031$) by 12 months of therapy.* The percentage of subjects who responded to treatment was 82%, 67%, and 85% for Groups I, II, and III, respectively. The mean BMI of those subjects that responded in Groups I-III was 30.7, 36.2, and 41.8 at the baseline, which decreased to 27.7 ($p=0.004$), 32.2 ($p=0.001$), and 37.4 ($p=0.000$) by the 12th month of treatment. **CONCLUSION:** Combination drug therapy with amphetamine compounds and metformin can induce significant sustained weight loss and improve insulin resistance in obese children.

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ANTIBIOTIC COATED CENTRAL VENOUS CATHETERS IN CRITICALLY III CHILDREN.

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Background: Compared to non coated catheters (NAC), antibiotic coated catheters (AC) have decreased rates of catheter colonization and BSI in adults. Few studies have evaluated AC in children. **Objective:** To determine if use of central venous catheters (CVC) coated with antibiotics decreases the prevalence of bloodstream infections (BSI) in children. **Design/Methods:** We studied children admitted to the University of Illinois PICU who required a CVC from 1/99 - 12/01. Patients received AC or NAC. AC were polyurethane multi-lumen bonded with minocycline and rifampin. Information on chronic conditions, hospital course, medications, and reasons for and location of CVC placement/removal was recorded. **Results:** Data for 218 CVC (from 157 patients, total 2233 catheter days) were analyzed. 35% were AC. Mean days per catheter was not significant (NS) between AC and NAC. Patients were 1 to 248 months old (mean 49). Mean age for AC and NAC was 27.4 and 60.6 months, respectively. CVC were placed in femoral (68%), subclavian (12%), jugular (19%), or other (1%) sites and there were no differences in BSI or contamination rates based on site or type of CVC. Only 10% of CVC were removed because they were possibly infected. Of 218 CVC, BSI developed in 23 and CVC contamination developed in 15. The BSI rate per 1000 catheter days was 12.9 (AC) and 7.8 (NAC) (not significant). CVC contamination rate per 1000 catheter days was significantly different between AC (2.8) and NAC (10.4) [Table1]. The longer the CVC were in, the greater the risk of contamination and BSI, regardless of CVC type (corr coeff = 0.23). Organisms responsible for BSI: Coag neg staph (10), Candida sp (6), E faecalis (5), E coli (5), Enterobacter sp (2), S. aureus (2), P. aeruginosa (1), other (3). Of BSI caused by candida, 5 patients had AC and 1 had NAC ($p = 0.012$). **Conclusion:** In our population there was no protective effect from BSI with use of AC but risk of contamination was decreased. With increasing days per catheter the risk for CVC contamination and BSI increased, regardless of type of CVC.

No. of Days Per CVC	Contam AC/Total AC (%)	BSI AC/Total AC (%)	Contam NAC/Total NAC (%)	BSI NAC/Total NAC(%)
<10	1/34 (2.9)	6/34 (18)	3/106 (2.8)	4/106 (3.8)
11-30	2/38 (5.3)	6/38 (15.8)	8/32 (25)	5/32 (15.6)
>30	0/4 (0)	2/4 (50)	1\4 (25)	0/4 (0)

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ANALYSIS OF INTENSIVE INSULIN MANAGEMENT OF TYPE 1 DIABETES: INSULIN PUMP WITH OR WITHOUT PRECEDING USE OF MULTIPLE DAILY INJECTIONS.

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The current ideal treatment of Type 1 diabetes (DM-1) is to mimic the insulin delivery of the non-diabetic. Currently, this optimally is done with the use of intensive insulin regimens using continuous subcutaneous insulin infusion (CSII) via an insulin pump or via multiple daily injections (MDI). Both approaches utilize a basal-bolus (b-b) insulin regimen and require pts to count carbohydrates and bolus insulin appropriately. Purpose: To assess whether outcomes were different in pediatric DM-1 pts on CSII who utilized MDI prior to CSII (Gr 1) vs. those on CSII who did not first transition to b-b with MDI (Gr 2). Methods: This was a retrospective chart review of DM-1 pts on CSII followed within a University-based pediatric endocrine office. Pts are generally followed q 3 mos. We report the mean of the interval HbA1c during the 1st and 2nd 6 mos intervals while on CSII +/- MDI. Results: 95 pts DM-1 on CSII were identified (62 F/33M; Cauc =89.5%, AfrAm=7.4%, Other=3.3%); 88 had data for presentation (Gr 1 n=18; age 12.5±4.0yrs, 13F/5M; Gr 2: n=70; age 14.3±3.0yrs, 46F/24M. Duration of: DM prior to MDI= 2.0±2.2yrs (Gr 1), CSII=4.8±3.5 yrs (Gr 2); MDI prior to CSII=0.5±0.3yrs (Gr 1); CSII=1.3±1.0yrs (Gr 1), 2.7±1.4yrs (Gr 2). 5 pts stopped CSII after 1 yr (Gr 1=1, Gr 2=4) due to ↑ HbA1c +/- QoL issues associated w/wearing the pump.
Conclusions: While DM-1 pediatric pts on CSII had a trend towards ↓ HbA1c values at 6-mos when preceded by Rx with MDI, this trend did not reach statistical significance, given the small n. There were no significant differences in HbA1c w/in or between Grps. Selection bias cannot be discounted. Basal insulin requirements changed little from MDI to CSII and during CSII in both Grps, while maintaining fairly good glycemic control. 65% of pts on CSII were female which may have social considerations when considering CSII. Future analysis will include additional pts and may affect outcomes.

	HbA1c (%±SD)				Mean Basal insulin (U/kg/day) ±SD			
	Pre-MDI	Pre-CSII	$CSII_{6mos}$	$*CSII_{12\ mos}$	At MDI	Pre-CSII	$CSII_{6mos}$	$*CSII_{12mos}$
Gr 1	8.4±1.7	7.7±0.5	7.6±0.8	8.0±0.9	0.4±0.2	0.5±0.2	0.4±0.1	0.4±0.2
Gr 2	NA	8.1±1.3	8.1±1.2	8.2±1.4	NA	NA	0.4±0.1	0.4±0.1
P		NS	NS	NS			NS	NS

*Gr 1 n=10; Gr 2 n=60

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MANAGEMENT OF AIRWAY OBSTRUCTION IN CHILDREN WITH MEDIASTINAL MASSES.

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Newly diagnosed mediastinal masses in children can potentially lead to fatal cardiorespiratory compromise because of their external compression on the airway or major vessels. This is especially a concern during the initial diagnostic work-up when a patient undergoes sedation or general anesthesia. A multidisciplinary approach to guide this process is essential to minimize complications. This case report presents JR, a 7 year-old white male, who presented to The Cleveland Clinic with left cervical lymphadenopathy. He denied any respiratory symptoms. CXR showed an anterior mediastinal mass, bilateral hilar adenopathy, minimal narrowing of the trachea due to mass effect, and small bilateral pleural effusions. When undergoing chest and abdominal CT, JR developed mild dyspnea while supine. He subsequently went to the OR for lymph node and bone marrow biopsy under general anesthesia. Anesthesia was induced with an inhalational technique, using sevoflurane and nitrous oxide. After documentation of the ability to ventilate, the level of anesthesia was deepened and he was intubated with a 5.0 endotracheal tube. Intraoperatively, he developed bronchospasm and self-extubated. He was reintubated with a 4.0 reinforced endotracheal tube after receiving 20mg of succinylcholine. Postoperatively, he developed a significant Alveolar-arterial oxygen gradient and was on 100% FiO2 with saturations ranging from 88% to 90%. He was unable to be extubated and had bilateral chest tube placement to drain the pleural effusions, which had rapidly progressed. The biopsy confirmed Nodular Sclerosing Hodgkin's Lymphoma. He received multiple chemotherapy agents to shrink his tumor mass and was extubated after four days without further complications. JR's case exemplifies the fact that the severity of respiratory symptoms is not a reliable indicator of the degree of airway compromise. Based on a literature search, an algorithm was developed to guide the initial management of children with mediastinal masses. Close coordination among oncology, anesthesia, surgery, radiology, PICU and ENT must be in place to ensure proper airway management during any diagnostic or therapeutic procedure. CXR may provide the first indication of airway obstruction and CT scans of the thorax, using small contiguous cuts, can confirm obstruction. Echocardiography should be performed to assess the level of involvement by the tumor of the heart and great vessels. Pretreatment of the mass, either by radiation or chemotherapy, may rarely be required prior to biopsy in order to minimize potential airway compromise.

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RADIATION-INDUCED OSTEOSARCOMA FOLLOWING OSTEOSARCOMA AND LI-FRAUMENI SYNDROME.

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Osteosarcoma is the most serious late complication following ionizing radiation to bone in children. The majority of secondary osteosarcomas occur following radiation of other solid tumors: Ewing's sarcoma, rhabdomyosarcoma, neuroblastoma, Wilms tumor and Hodgkin's disease. Children with reintoblastoma have a genetic predisposition to the development of secondary osteosarcoma. These second tumors almost always occur in the irradiated bone, as opposed to the usual sites of predilection for osteosarcoma in long bones. The tumors are also associated with prior alkylating and anthracycline therapy. Latent periods can be long from 6 – 20 years. Secondary osteosarcoma has a poor prognosis. This is a case report of a 10-year-old girl with a secondary osteosarcoma following treatment for a rhabdomyosarcoma. She presented at age 3 with pain and a mass in the right thigh. Biopsy revealed an alveolar rhabdomyosarcoma. CT of chest, abdomen, pelvis and bone scan were negative for metastatic disease. The head of the biceps femoris was excised and she was treated with chemotherapy on an IRS protocol. She received radiation to the right posterior thigh for a total of 45 Gy in 2/96. Six years and 9 months after initial tumor she again developed a painful mass in right thigh and knee. Risk of secondary osteosarcoma has been shown to be 0.5% at 5 years following first cancer to about 1% at 10 years follwing initial tumor. Imaging and biopsy confirmed the diagnosis of osteosarcoma in the previously irradiated bone. This case is unique also because of the strong family history of unusual tumors including breast cancer and osteosarcoma in a male relative. This case may likely represent Li-fraumeni syndrome (LFS). LFS, shown to be associated with germline mutations in TP53, involves a wide range of malignancies including breast cancer, brain tumors, acute leukemia, soft tissue sarcomas, bone sarcomas, and adrenal cortical carcinomas with a strong predilection within first degree relatives. This case clearly meets clinical criteria for LFS.

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SILENT CELIAC DISEASE IN PATIENTS WITH ENDOCRINE DISORDERS.

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Celiac disease (CD) is a disorder in which dietary gluten and related proteins trigger an immunologic response leading to small bowel inflammation and autoantibody (Ab) production. The early diagnosis and treatment of CD can prevent complications including malabsorption, lymphoma, squamous cell carcinoma, osteopenia and infertility. Anti-endomysial IgA Ab by immunofluorescence has a sensitivity of 94–100% and specificity of 93–100 % for diagnosis of CD. A human recombinant anti-tissue transglutaminase ELISA has 92–98.5% sensitivity and 95% specificity for CD diagnosis. Diagnosis of CD is confirmed by histopathologic exam of mucosa distal to the duodenal bulb. The reported prevalence of CD in the US is 0.4%. In patients with Type I Diabetes Mellitus (T1DM) the prevalence has been reported from 3.9% to 13.5%. Five percent of patients with Turner syndrome, 8% with Down syndrome, and 7.7% with autoimmune thyroid disease are reported to have CD. Our aim is to determine the prevalence of positive CD Ab in our patients with one or more of the following endocrine disorders: T1DM, autoimmune thyroid disease (Graves or Hashimoto), short stature or delayed onset of puberty. We also want to compare the prevalence of CD Ab in our population to that reported in other geographic locations. This is a retrospective study of 277 patients who presented to the endocrine clinic at Cleveland Clinic Children's Hospital with the listed endocrine disorders from April 2002 till April 2003; and who were screened for IgA Ab to tissue transglutaminase or endomysium. Confirmatory small bowel biopsies were done for most patients with positive Ab screening. Chi-square or Fisher's exact tests were used to compare patients with endocrine disorders on the prevalence of positive CD Ab. The CD Ab were significantly more prevalent only in T1DM ($P=0.009$). The table below shows the positive CD Ab prevalence in our patients with the studied endocrine disorders. Our numbers are too small to assess the significance in patients with thyroid disease, Turner, or Down syndrome.

Condition	Total	CD Ab Pos	Biopsy Pos	Biopsy Neg.	Biopsy pending	Ab Prevalence
T1DM	137	11	3	3	5	8.0%
Thyroid disease	34	2	1		1	5.8%
Growth disorder	101	3	1	1	1	2.9%
Turner syndrome	4	1	1			25.0%
Down syndrome	8	1	1			12.5%

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A CASE OF MUNCHAUSEN'S SYNDROME BY PROXY: CHILD ABUSE PERPETUATED BY PHYSICIANS.

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The diagnosis of Munchausen's Syndrome by Proxy (MSP) in a 15-month old Caucasian male was confirmed by marked and rapid improvement of his failure to thrive and developmental delay following removal from his mother's care. Although the diagnosis was considered many months earlier, only during an extended admission was the diagnosis pursued and reported. Munchausen by proxy is distinct from other types of factitious illness in that the motivation of the fabricated illness is the need of the caretaker, almost always the mother, for the attention of the healthcare provider(s). By participating in the fiction, it is the child's physicians who may perpetuate the abuse. As in this case, such abuse occurs through ordering extensive laboratory or radiologic testing, performing endoscopic or surgical procedures, empirically prescribing unnecessary medication, or prescribing specialized enteral or parenteral nutrition. In many cases, it may first appear that the mother is loving and devoted, always participating in the child's care, and never leaving the child's bedside when in the hospital. A closer look often reveals true maternal neglect and deprivation. In its simplest form, the mother falsely reports symptoms or signs. It its most serious form, the mother produces illness. In the case presented, it was strongly suspected that the mother administered medication to the child resulting in profound hypotension and bradycardia, falsely reported patient symptoms to medical staff, and withheld oral and gastrostomy feeds necessary for the child to grow. MSP is a form of child abuse. It is the responsibility of all healthcare providers to recognize the syndrome and to learn how to report it effectively. A team effort is always necessary, including social service and legal support. The stress of dealing with these cases is significant. Disbelief by some staff can cause serious controversy to occur with extremely high stress for everyone involved in the care of the child. As a tertiary referral center, we attract cases of MSP due in part to access to the multitude of sub-specialists available. MSP may present as the difficult case which "doesn't fit" or respond to usual therapy or can be a complex case that has mystified previous physicians. These are the presentations of MSP, which prompt referral to our center.

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PREVALENCE OF ENDOSCOPIC AND HISTOLOGIC LESIONS OF THE UPPER GASTROINTESTINAL TRACT IN CHILDREN WITH INFLAMMATORY BOWEL DISEASE: A PROSPECTIVE STUDY.

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The value of routine upper endoscopies as part of the initial evaluation of children with suspected inflammatory bowel disease (IBD) has not been well established. Literature search reveals few pediatric studies that looked into the prevalence of disease specific and nonspecific lesions in children with IBD. The aim of this study is to establish the prevalence of disease specific (e.g. non-caseating granulomatous inflammation) versus nonspecific (diffuse inflammatory changes, peptic ulceration and H.pylori) of the upper gastrointestinal (GI) tract in children with IBD, in comparison to patients without IBD. This will further characterize the role of upper endoscopy in the initial investigation of children suspected to have IBD. A prospective chart review of all children undergoing endoscopy as part of their evaluation at our institution from May-September 2002, as part of their evaluation for GI symptoms is currently under way. The collected data includes patients' age, gender, duration of symptoms, medication, imaging studies as well as visual and histopathologic results of esophagogastroenteroscopy and colonoscopy. 236 patients have been review, 113 had no significant diagnostic findings on endoscopy or histopathology. Twenty new cases of IBD were identified and 15 cases were diagnosed with unspecific colitis. Additionally 15 patients with known IBD under went endoscopy during the study period. Most patients with Crohn's (N= 23) underwent upper and lower endoscopy, only one patient had a disease specific lesion in the upper GI tract. Patients with ulcerative colitis showed similar disease nonspecific lesions to those with Crohn's disease. No Helicobacter pylori infection was identified. IBD medications did not correlate with lack of upper intestinal findings. Our study is currently in progress to further delineate the prevalence of upper GI lesions in children with IBD.

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PERSISTENT LEFT-SIDED CERVICAL LYMPHADENOPATHY AS A PRESENTATION OF KIKUCHI-FUJIMOTO DISEASE.

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This is a case report of KR, a 14 year- old African American female who was admitted to the Cleveland Clinic Foundation because of persistent fevers and painful cervical lymphadenitits. KR had been managed on an outpatient basis for her symptoms with 2.5 weeks of broad-spectrum antibiotics without any improvement. In the hospital KR was treated with Unasyn and continued to have spiking fevers and worsening of her cervical lymphadenitis. Laboratory studies demonstrated a WBC of 5.15, H/H 12.3/38.8, PLT 214 with differential of 70 neutrophils, 20.6 lymphocytes, and 9.0 monocytes. ALC was 1.06. EBV titers were consistent with prior infection. ESR was elevated to 54. Pediatric ENT was consulted and a lymph node biopsy was performed. Biopsy was diagnostic for Kikuchi- Fujimoto disease. KR was started on IV solumedrol and sent home the following day on a 9-day steroid taper. Kikuchi-Fujimoto disease, also known as histiocytic necrotizing lymphadenitis, is a rare condition of unknown etiology that usually presents as fever and cervical lymphadenopathy in young women. This disease was first recognized in the early 1970's and has since been reported throughout the world. The majority of cases are benign, although there have been case reports of fatalities during the acute phase of the illness. Kikuchi-Fujimoto disease is diagnosed via lymph node biopsy. Findings on biopsy include patchy or confluent areas of necrosis, varying amounts of nuclear debris in affected areas, aggregates of histiocytes, presence of medium- to large-sized transformed lymphocytes and plasmactyoid T cells, and absence of neutrophils and eosinophils. Patients with Kikuchi-Fujimoto disease in the past have been misdiagnosed as having lymphoma. There also have been case reports of patients initially diagnosed with Kikuchi-Fujimoto disease later found to have SLE. The mean age of presentation is 30 years and the male to female ratio has been estimated to be 1:4. Most cases of Kikuchi-Fujimoto disease are treated symptomatically with NSAIDS. Patients whose clinical courses have been prolonged with persistent fever and adenopathy may benefit from more aggressive treatment with prednisone. Prednisone has also been useful in patients with recurrent disease. An approach to lymphadenitis in children will be reviewed.

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RITUXIMAB TREATMENT FOR PEDIATRIC AUTOIMMUNE HEMOLYTIC ANEMIA.

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The case of a two month old African American female with refractory idiopathic autoimmune hemolytic anemia (AIHA) is described. Based on a literature review, her case is unusual with respect to age at presentation, illness severity and lack of response to initial therapy, as well as an unusual expression of isohemagglutinins at the age of two months. She presented to the pediatric intensive care unit of the Cleveland Clinic with a one day history of poor feeding and increased work of breathing. Initial physical exam findings were characteristic of a baby experiencing high-output heart failure; it was noteworthy for marked paleness, respiratory distress, and lethargy. Cardiac examination revealed a 2/6 systolic flow murmur, thready peripheral pulses, capillary refill of 2–3 seconds, and a palpable liver edge 3cm below the right costal margin. Initial laboratory findings showed a hemoglobin level of 1.0 g/dL and hematocrit of 6%. Coombs test was strongly positive for IgG erythrocyte antibodies. To rule out the possibility that the hemolysis was a result of an antibody produced by a maternal immunocompetent clone that crossed to the baby's circulation, Short Tandem Repeat Polymorphism PCR analysis was performed on the baby's and the mother's mononuclear cells. It showed no evidence of maternal cells in the baby's circulation. Management included initial intubation with mechanical ventilation and resuscitation with blood transfusions and intravenous fluids. Medications used in therapy included Prednisolone (1–3mg/kg/day), intravenous immunoglobulin IVIG (1gram/kg/day x 5 days) and cyclosporine A (2–10mg/kg/day) aiming at serum levels of 200ng/mL. Hemoglobin levels repeatedly fell to 5–6 g/dL despite these treatment measures, making her erythrocyte transfusion-dependent. This refractoriness prompted a trial of Rituximab, a monoclonal antibody targeted against the CD20 antigen of B-lymphocytes and plasma cells. It was initiated 44 days after initial diagnosis and was administered in six weekly doses (375mg/m2/dose), during which time corticosteroids and cyclosporine A were tapered and discontinued. Hemoglobin levels increased gradually and normalized at 11 g/dL, 15 weeks after diagnosis. She became transfusion – independent at 7 weeks after starting Rituximab therapy. Our patient did not develop acute infusion reactions secondary to Rituximab. As hypogammaglobulinemia is a side effect of Rituximab therapy, she received monthly IVIG infusions (400 mg/kg). A review of the current literature regarding treatment for refractory AIHA in the pediatric population is presented.

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IN-UTERO MECONIUM AND ADVERSE OUTCOMES IN VERY LOW BIRTH WEIGHT INFANTS.

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Purpose of study: To determine if the *in-utero* passage of meconium is associated with a greater need for resuscitation and adverse outcomes among very low birth weight infants (VLBW). **Methods Used:** Placentas of 431 infants born at <1350gm that had "brown pigment" identified on routine H&E staining underwent Prussian blue staining and fluoroscopy to identify hemosiderin and meconium, respectively. Slides were examined at 400x using a Zeiss Axioskop 2 plus microscope with a custom filter: excitation 405/30 nm bandpass filter; dichroic mirror 440 nm and emission 550 nm long pass. Placental abruption, infarction, and chorioamnionitis were noted. Clinical variables including resuscitation performed at birth and the most severe injury on cranial ultrasound were collected from the medical record. Statistical analysis included the use of Chi-square analysis, binary logistic regression, Student t-test and Kruskal-Wallis, as appropriate. A p value of <0.05 was considered significant. **Summary of results**: The 70 infants (16.2%) for whom *in-utero* meconium passage was identified were younger, weighed less, and were more likely to be delivered by C-section and to have chorioamnionitis than infants without meconium. Gender, race, antenatal steroids and other pregnancy complications did not differ. Infants who passed meconium *in-utero* were more likely to receive all forms of resuscitation than were control infants and to have lower Apgar scores. They more often were intubated in the delivery room (62.9 vs. 47.7%, p=0.02), received cardiopulmonary resuscitation (17.1 vs. 6.4%, p=0.003), and were given early volume resuscitation (32.9 vs. 15.5%, p=0.001). These infants were more likely to have grade III-IV intraventricular hemorrhages (13.2 vs. 5.0%, p=0.011) and bronchopulmonary dysplasia (56.7% vs. 41.5%, p=0.022) than were infants who did not pass meconium. The difference in grade III-IV intraventricular hemorrhage remained significant in regression models including gestational age, birth weight, antenatal steroids, and chorioamnionitis. Respiratory distress syndrome, necrotizing enterocolitis, periventricular leukomalacia and death did not differ between the infants that passed meconium *in-utero* and those that did not. **Conclusions reached:** Fluoroscopy is a sensitive method for detecting the *in-utero* passage of meconium by VLBW infants. Preterm infants that pass meconium are more likely to receive resuscitation at delivery and have an increased incidence of grade III-IV intraventricular hemorrhages.

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TRENDS IN THE INCIDENCE OF RETINOPATHY OF PREMATURIY.

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Background: Severe Retinopathy of Prematurity (ROP) is a major morbidity in very preterm infants. The incidence of severe ROP varies between centers. Vascular Endothelial Growth Factor (VEGF) has been implicated in the pathogenesis of ROP and is regulated by hyperoxia, hypoxia and oxidant stress. At our center, since 2001, we have changed our clinical practice, in infants <32 weeks (BW<1,500 g), as follows: (1) Prevent hyperoxia in the first 6–8 weeks (Sao_2 limits 85–95%); (2) Prevent hypoxia after 6–8 weeks (Sao_2 limits 94–98%); (3) encourage breastfeeding in this group of infants.

Purpose of the study: This study was done to compare the trends in the incidence of ROP at our center to Vermont-Oxford Network (VON) Centers. **Methods:** This is a retrospective chart review from July 1997-June 2003. Infants <32 weeks (BW<1.500g) and who survived to discharge were the subjects. The infants were cared for at Hospital A, an inner city academic medical center, or Hospital B, a community hospital. The trends in incidence at our center were compared with centers from VON using the chi-square test.

Results: **Hospital A**: In 1997, 35/38 survived, 34% with stage 3–4 and 6% laser (p=0.37 overall outcome of ROP compared to VON). However from 1998–2000, with similar number of survivors each year, stage 3–4 was 23–24%, Laser was 16–26% (p=<0.0001, much worse compared to VON). In 2001stage 3–4 was 19% and laser 12% (p=0.05, better than VON). In 2002, stage 3–4 was 7% and laser 0%, a dramatic decline in severe ROP. When comparing the type of feeds (BM or formula) there was no difference in % who developed stage 1–2 or Stage 3–4, laser (p=0.60).

Hospital B: 1997–2001, number of survivors ranged from 22–61 each year with % survivors ranging from 85.7–100%. The incidence of stage 3–4 ranged from 5–13% and laser 3–7% (p=0.09–0.78- similar to VON. Again in 2002 stage 3–4 was 6% and laser 6%. When comparing type of feeds (BM or formula), the % who developed Stage 3–4 or needed laser was significantly higher when formula fed (p=0.04).

Conclusions: The trend of severity of ROP among Hospital A infants was worse than VON from 1998–2000 and since 2001–2002 shows a dramatic decline. However at Hospital B the trend has been similar to VON from 1997–2000 and is unchanged in 2001–2002. There is a trend towards protective effect from breast milk as seen in Hospital B infants.

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EVIDENCE FOR THE INVOLVEMENT OF ENDOTHELIN IN NEONATAL MORPHINE TOLERANCE.

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Purpose: Management of neonatal opioid tolerance and withdrawal symptoms remains a major challenge in neonatal intensive care units. Numerous neuromodulators have been demonstrated to be involved in the mechanism of tolerance-dependence to opiates, but none has been utilized clinically to reduce the development of tolerance to opioid analgesics. The purpose of this study is to investigate if central endothelin (ET) mechanisms are involved in the development of morphine tolerance in neonatal rats. **Methods:** Pregnant rats were divided into two groups and rendered tolerant to placebo and morphine pellets, respectively, and rat pups were delivered by cesarean section. Neonatal rat brains were dissected and used for analysis. The effect of morphine tolerance on characteristics of ET receptors was determined by using [^{125}I]ET-1 binding and G-protein stimulation was determined by using [^{35}S]GTPγS binding assay in the brain of both placebo and morphine tolerant neonatal rats. **Results:** The affinity (Kd) and density (Bmax) of [^{125}I]ET-1 binding was found to be similar in placebo and morphine tolerant neonatal rats. In the [^{35}S]GTPγS binding assay, morphine produced significantly lower ($P<0.05$) maximal stimulation in morphine tolerant neonatal rats (33.10%) when compared to placebo treated neonatal rats (90.90%). Maximal stimulation produced by ET-1 in morphine tolerant neonatal rats (41.26%) was also significantly lower ($P<0.05$) as compared to placebo treated neonatal rats (92.23%). Further, we studied the role of BMS182874, an ET_A receptor antagonist, and IRL1620, an ET_B receptor agonist, on G-protein stimulation in morphine tolerance. BMS182874 produced significantly higher ($P<0.05$) maximal stimulation of G-proteins in morphine tolerant neonatal rats (86.07%) as compared to placebo tolerant neonatal rats (29.59%). However, IRL1620 produced similar maximal stimulation of GTP binding in placebo and morphine tolerant neonatal rats. **Conclusion:** Morphine tolerance did not alter characteristics of ET receptors but ET-1-induced GTP stimulation was significantly altered. We conclude that ET is involved in neonatal morphine tolerance, mainly through ET_A receptors. It can be speculated that ET_A antagonists may play a role in neonatal morphine tolerance.

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SURFACTANT PHOSPHOLIPID POOLS IN PATIENTS WITH CHRONIC LUNG DISEASE.

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Background: Stable isotope methods to measure pulmonary surfactant metabolism require acquisition of surfactant from tracheal suctioning. However, in preterm infants with lung injury due to chronic lung disease, non-surfactant phospholipids may also be present in the tracheal effluent. **Objective:** To determine if infants with chronic lung disease have separate pools of phospholipid in tracheal effluent.

Methods: 5 infants < 28 weeks gestation at birth and at least 2 weeks of age who were continuously ventilated from birth received a 24-hour infusion of stable isotope [1-$^{13}C_1$] acetate. Total phospholipid (TPL) was extracted from sequential tracheal aspirates. Each sample was divided into two aliquots: one had no further processing and the second was subjected to osmium tetroxide oxidation to yield disaturated phospholipids (DSPL). The amount of phospholipid in each sample was measured with quantitative gas chromatography (GC).

Results: The amount of DSPL was significantly less than TPL (80±19 vs 216±47 nmol, P=0.04, mean±SEM) and the percent of palmitate in each fraction was significantly higher, as anticipated, in the DSPL fraction (77±6 vs 57±1%, p=0.05). Despite these differences in phospholipid content, there were no differences in FSR (16.5±3.5 vs 16.0±3.6%/day, DSPL and TPL, respectively, P=0.31) or FCR (67.1+10.1 vs 61.0+8.2%/day, DSPL and TPL, respectively, P=0.39).

Conclusions: The disaturated phospholipid pool, which is mainly derived from pulmonary surfactant, comprises approximately 40% of the total phospholipid pool in preterm infants who require prolonged mechanical ventilation. Although at least 2 pools of phospholipid are present, the non-surfactant pool appears to be turning over at the same rate as the surfactant pool.

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NITRIC OXIDE SYNTHASES AND NITROTYROSINE: MARKERS OF NITRIC OXIDE PRODUCTION AND ACTIVITY IN THE DEVELOPING NEONATE AND CHRONIC LUNG DISEASE.

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Background/Purpose: Nitric Oxide (NO) is synthesized from L-arginine by three isoforms of the enzyme nitric oxide synthase (neuronal nNOS; inducible iNOS; and endothelial eNOS). It is unknown to what extent preterm birth and the development of chronic lung disease (CLD) of prematurity alters the normal ontogeny of the expression and function of the NO pathways. One key pathway of NO metabolism is the free radical reaction between NO and super oxide, producing reactive nitrogen species, with the end result being nitrotyrosine, a stable, measurable marker of this NO pathway. Animal studies of the NOS ontogeny differ in where NOS isoforms are found and when in development each is most expressed and comparable knowledge is lacking in the human neonate **Objective:** To assess, by semiquantitative immunohistochemistry (IHC), NOS isoforms and nitrotyrosine at different airway and vascular tree levels in the lungs of neonate at different gestational ages, and to compare results to those obtained for infants with CLD. **Methods:** A library of postmortem lung tissue from neonates was accessed. Formulin fixed, paraffin mounted tissue blocks were cut and the sections were prepared for IHC, using antibodies to each NOS isoform and nitrotyrosine. The airway and vascular tree were evaluated at five and two levels respectively, for staining varying from no staining to intense staining (0–3 scale). The controls were infants of postconceptional age 22–42 weeks who died in <48 hours or from non-respiratory complications. Results were compared to gestational matched infants who had varying levels of CLD based on a respiratory SCORE. **Results:** In the control and CLD groups, staining for all three NOS isoforms was found in the airway epithelium from the bronchus to the most distal levels. The abundance or distribution of eNOS staining in the airways did not show statistically significant correlation with gestational age. Intense eNOS (avg grade 2) and moderate iNOS (avg grade 1.5) staining was found at all vascular levels. nNOS was not as consistently stained in vascular endothelium. CLD did not cause reduction or change the distribution of eNOS. Evaluation of nitrotyrosine staining is underway currently. **Conclusions:** All three NOS isoforms are detectable by IHC early in lung development. NOS ontogeny shows no significant changes in abundance or distribution with advancing gestational age nor is altered in infants dying with CLD. Nitrotyrosine will serve as a marker for NO presence at different ages or levels of severity of CLD.

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RESIDENT AUTONOMY: DOES OUR CURRENT TEACHING ENVIRONMENT ALLOW IT?

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Background: The process of becoming a physician is an arduous one, both in the commitment of time and effort to learn a large body of knowledge and adapt the skills and attitudes requisite of a professional. The overreaching principle of the development of a professional is the concept of autonomy, or being independent in mind or judgment and self-directed. The acquisition of autonomous behavior is made through progressive autonomy, or the gradual increase in responsibility given to a trainee as they become more competent and knowledgeable. The acquisition of autonomy is affected by many factors, including supervisory style, trust of the supervisor in the learner, and the perception of control of the learner on their environment. The specific aim of this study was to assess the factors that affect the ability of residents to attain autonomy and ultimately become independent physicians competent in the care of children.

Methods: The study population consisted of first to fourth year residents recruited from the Pediatrics and Medicine-Pediatrics Residency Programs at the University of Michigan. The study was conducted using a web-based format, utilizing several survey instruments that measured empowerment, trust, and perceived environmental control. The grouped responses were analyzed for relationships between these factors and autonomy and job satisfaction using regression analysis techniques.

Results: While residents rated themselves as having a fair degree of autonomy, they felt that they did not have the degree of autonomy that they expected to have. This result was found across all areas of service: wards, ICU settings and the emergency room. In addition, the residents perceived that the attendings trusted them, but not to the degree that they expected. This finding correlated to their perception of autonomy and their job satisfaction. Residents felt that they had little control over their environment, but this did not seem to correlate with their perception of autonomy or with their job satisfaction.

Conclusions: While residents in the University of Michigan Pediatrics and Medicine-Pediatrics residency programs perceive a fair degree of autonomy and trust, it is not to the degree that they expect they should have at their particular training level. This can impact on their job satisfaction and potentially their competence and confidence as practicing physicians. The information gathered here may help educators improve training programs and facilitate faculty development aimed at achieving competent and confident physicians.

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OUTCOME OF ACUTE MYOCARDITIS IN AFGHANI CHILDREN.

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Background: Acute myocarditis is associated with significant morbidity and mortality worldwide. It is the third most common cause of death among Afghan refugee children in Pakistan, surpassed only by sepsis and pneumonia.

Methods: We retrospectively reviewed 39 children (mean age 2.4 ± 2.5 yrs; range 0.5–12) presenting with acute myocarditis at the Aga Khan University Hospital, Pakistan over a 2 year period.

Results: Acute myocarditis was most prevalent in infants (n=10, 26%) and children aged 1 to 5 yrs (n=26, 66%). Mortality was 28% (n=11), and correlated significantly with ventricular arrhythmias (p=0.001), lack of ACE inhibitor therapy (p=0.001), hypotension at presentation (p=0.02), and severe malnutrition (p=0.01). These patients were also more likely to require mechanical ventilation and cardiopulmonary resuscitation. Age, gender, symptom duration prior to presentation, and cardiac function did not predict mortality. A seasonal trend was noted with almost half the pts (n=19) presenting in February or March. Findings on presentation included fever and cough (84.6%), tachycardia (94.6%), hepatomegaly (92.3%) and tachypnea (89.7%). Elevated cardiac enzymes and EKG and ECHO abnormalities, although widely prevalent, were not predictive of mortality.

Conclusion: Our study suggests a more aggressive approach in treating ventricular arrhythmias, hypotension, nutritional status, and early initiation of ACE inhibitors may favorably affect survival in children with acute myocarditis. The higher incidence of myocarditis in the winter months may be related to the increased incidence of viral infections during this period.

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COST EFFECTIVE HIGH RISK NEONATAL FOLLOW UP PROGRAM.

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Poor patient compliance with appointments in the post discharge follow-up of infants from Neonatal Intensive Care Units (NICU) is a major problem in inner city hospitals, as it increases risk developmental delay without early intervention and longterm care cost. Some major causes of this noncompliance are parental stress, lack of education/resources on early intervention, limited social security benefits, insurance issues, and the inability of primary care physicians to spend time on non-medical issues.

In an attempt to better understand and meet the special needs of this population, a one-year pilot program was implemented where patients were helped with the transition from the NICU to outpatient services with the help of dedicated social worker. More specifically, a controlled randomized study was conducted to test whether care management by a social worker providing education, access to resources, supportive counseling, coordination of care, discharge planning, help with insurance issues, and assistance when first year benefits are denied would make a difference in follow up compliance and quality of outcome. The social worker was available at any time to families via a pager and cellular phone after their child's discharge, to more continuously provide the same services as in the NICU. Hospitalization, maternal health-related quality of life, parental stress indexes, impact on family scale, ages and stages scales, and CRISYS were the tools used to measure quality of life and parent's understanding of issues. 42 families were randomized into control (8) and intervention (34) groups. Both groups were similar in maternal age, race, level of education, and household income. *Parenting Stress Index showed a higher rate of stress in intervention group (78 vs. 68)-they were less defensive; they were sent to therapy.*

Overall, families in the intervention program were found to be more compliant with clinic appointments and follow up with sub specialists (85% vs. 50%) compared to the patients in corresponding pre-study period. It can be concluded that the continuity of care with dedicated primary care follow up program coordinated by social worker starting in NICU through the first year of life is a better way to deliver medical care.

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IDENTIFICATION OF PUTATIVE ACUTE OTITIS MEDIA VIRULENCE FACTORS IN STRAINS OF NON-TYPEABLE HAEMOPHILUS INFLUENZAE.

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Nontypeable strains of Haemophilus influenzae (NTHi) are an important cause of acute otitis media (OM). The pathogenic processes by which NTHi strains cause OM is poorly understood. In order to identify specific virulence factors important for OM pathogenesis, genomic subtraction of the NTHi middle ear isolate G622 against the NTHi throat isolate 23221 was conducted.

Subtractive hybridization identified 45 subtraction PCR (sPCR) fragments unique to the middle ear strain G622. These sPCR fragments were used as probes in dot blot hybridization assays to screen 47 NTHi middle ear isolates and 91 NTHi nasopharyngeal (NP) and throat isolates from healthy children to identify genes found more frequently among middle ear isolates. The isolates used in this study were collected from sites in Minnesota, Ann Arbor, Michigan, Battle Creek, Michigan and Bardstown, Kentucky between 1980 and 2001. sPCR fragments of interest underwent DNA sequence analysis and genetic homologues were identified in the GenBank database.

Of the 45 probes, 6 were significantly more prevalent among middle ear strains compared to nasopharyngeal/throat strains, $p < .001$. The homologous proteins of these six probes are ABC transporter, ATP-binding protein (OppD); Hemin receptor (HemR); Hemoglobin/hemoglobin-haptoglobin protein B (HgpB); High molecular weight adhesin (HMW-A); Hypothetical protein; Histidinol dehydrogenase. These results identify six *H. influenzae* chromosomal regions that are more prevalent in middle ear NTHi isolates compared to nasopharyngeal NTHi isolates from healthy children, suggesting that the proteins encoded by these regions undergo positive selection among strains causing acute otitis media, and, thus, may be important in the pathogenesis of this infection