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Abstracts are listed in presentation order, beginning with Platform Presentations.

1. **Effect of a Monoclonal Antibody (Mepolizumab) Against Interleukin-5 (IL-5) in Reducing Eosinophilic Inflammation in Children with Eosinophilic Esophagitis (EoE).**

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Background: EoE is a chronic relapsing disorder with prominent esophageal eosinophilia. IL-5 promotes eosinophil development and release from bone marrow. Reducing eosinophils may be an effective option to treat EoE in children.

Design: This was a multicenter, randomized, double-blind, parallel group study sponsored by GlaxoSmithKline (MEE 103219). Mepolizumab, a humanized monoclonal anti-IL5 antibody, was administered to children with EoE ages 2-17 years in three different doses: 0.55 mg/kg (Group 1), 2.5 mg/kg (Group 2), or 10 mg/kg (Group 3). Endoscopy with biopsy of mid and distal esophagus was performed prior to therapy and at week 12 following drug infusion. Eosinophils were counted in all high power fields (hpfs); peak count ≥20/hpf in either esophageal biopsy was an entry criterion. The primary pharmacodynamic endpoint was the proportion of subjects with peak eosinophil count <5/hpf at week 12. Exploratory intragroup comparisons of changes in mean and peak eosinophil counts were made using a paired t test assessed at $\alpha=0.05$ significance level.

Results: Following therapy, 5/59 (8%) of patients had a peak eosinophil count <5/hpf. Eosinophil counts in esophageal biopsies for each group are shown below:

	Mid		Distal	
	Pre Therapy	Post	Pre Therapy	Post
Group 1 (N=19)	Mean ± SD 31.1 ± 25.82	11.0 ± 11.97*	Mean ± SD 42.5 ± 34.50*	14.2 ± 14.19*
	Peak ± SD 82.7 ± 60.26	35.76 ± 44.3*	Peak ± SD 110.1 ± 58.44*	41.9 ± 37.46*
Group 2 (N=20)	Mean ± SD 33.4 ± 31.39	4.9 ± 5.07*	Mean ± SD 38.8 ± 29.57*	6.3 ± 6.28*
	Peak ± SD 101.0 ± 92.31	14.25 ± 16.27*	Peak ± SD 99.7 ± 65.58*	21.5 ± 20.14*
Group 3 (N=20)	Mean ± SD 40.32 ± 29.83	8.08 ± 8.51*	Mean ± SD 45.8 ± 31.01*	9.0 ± 10.13*
	Peak ± SD 94.3 ± 51.27	35.15 ± 29.68*	Peak ± SD 111.3 ± 62.34*	33.7 ± 39.71*

N, number of subjects; SD, standard deviation; *, $P\leq0.01$ compared to pre therapy value

In addition to a reduction in tissue eosinophils following therapy, the presence of eosinophil microabscesses (≥10 eosinophils per focus) was reduced in all treatment groups (% subjects with microabscesses pre- vs post-therapy: mid, 21% vs 6%; distal 17% vs 2%).

Conclusion: Mepolizumab in all doses used in this study causes marked reductions in tissue eosinophilia in biopsies from children who have EoE.

2   Microarray Analysis of Eosinophilic Gastritis (EG) Identifies a Strong Link with Cadherin-like 26 Overexpression.

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Background: The pathogenesis of EG is unknown. Gene microarray analysis identified the cytokine eotaxin-3 as important in the pathogenesis of eosinophilic esophagitis. We sought to identify genes that are dysregulated in EG using microarray analysis.

Design: Samples of gastric mucosa from patients with (EG) and without (nonEG) EG were utilized for microarray analysis, RT-PCR, or histology. For microarray analysis, transcripts differentially regulated between EG and nonEG patients were identified by subjecting microarray data to T-test ($P<0.01$) followed by a 2-fold filter analysis. Microarray and RT-PCR expression values were subjected to the Mann-Whitney test to determine statistical significance ($P<0.05$). Immunohistochemistry (IHC) was performed on formalin-fixed biopsy sections using standard techniques; stain intensity was graded 0-4+, and stained cells in the most intensely inflamed areas at 400X high power field (hpf) were counted to obtain peak values.

Results: The study included 5 EG (4 females, 1 male; mean age 12.6,,b6.2 years), and 5 nonEG (4 females, 1 male; mean age 9,,b7.3 years) patients. Gastric mucosa appeared abnormal (granular/nodular) in all EG patients and normal in nonEG patients. 4/5 EG, and 0/5 nonEG, patients had eosinophilic inflammation in other GI mucosal biopsies obtained concurrently. Peak eosinophil count in EG gastric biopsies ranged from 35-603/hpf (mean 284,,b230/hpf), and in nonEG gastric biopsies from 0-21/hpf (mean 8.6,,b6.7/hpf). Microarray analysis indicated that a conserved set of 28 genes were upregulated and 76 genes were downregulated in EG compared to nonEG biopsies. Cadherin-like 26 (CDH26), a member of a superfamily of transmembrane glycoproteins essential for cell-cell adhesion, was the most overexpressed gene in EG biopsies (20.9-fold, $P<0.01$). RT-PCR confirmed increased CDH26 mRNA in EG biopsies compared to nonEG biopsies (15.3-fold, $P<0.05$). IHC showed 4+ protein expression (Sigma Prestige, St. Louis, MO, rabbit polyclonal anti-CDH26, 1:50) in 3/5, and 1+ in 1/5, EG gastric biopsies compared to nonEG gastric biopsies. CDH26 antibody stained surface and gland epithelial cells, and not nonepithelial cells; the mean of the peak values for stained cells in EG biopsies was 104/hpf (range 0-241/hpf).

Conclusion: EG is characterized by markedly dysregulated gene expression and the most overexpressed gene is CDH26, whose gene product is expressed in gastric epithelial cells.

3 Trisomy 18 is a Consistent Cytogenetic Feature in Pilomatricoma

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Background: Pilomatricoma, a skin adnexal tumor that recapitulates hair growth, is by far the most common epithelial neoplasm of childhood. Its proliferating cells appear uniquely influenced to undergo terminal differentiation and death, perhaps accounting for the tumor's inevitably benign course. Cytogenetic features of pilomatricoma have not been previously explored.

Design: After cytogenetic G-banding analysis on an enlarging subcutaneous neck pilomatricoma from an otherwise healthy 12-year-old girl demonstrated trisomy 18, we investigated this and ten additional cases by fluorescence in-situ hybridization (FISH). Pilomatricomas with a substantial basaloid component were retrieved from the pathology department files at Children's Hospital Boston, Boston, MA. FISH was performed on formalin-fixed, paraffin-embedded, 5 µm-thick tissue sections, using an alpha satellite centromeric probe for chromosome 18 and counting at least 100 cells from various areas of viable tumor and from surrounding nonneoplastic tissue.

Results: In the index patient, 2 of 19 metaphase cells from independent 7-day adherent cultures showed trisomy 18 with the following karyotype: 47, XY, +18[2]. FISH confirmed trisomy 18 in 11 of 400 cells (2.8%; normal range, 0-0.2%) from the index case. Six of the additional 10 cases demonstrated trisomy 18 by FISH. This abnormality was confined to the basaloid component of the tumor and absent from the surrounding nonneoplastic tissue in all cases. In the FISH-positive cases, trisomy 18 was observed in 0.5-2.8% (mean 1.1%) of neoplastic cells.

Conclusion: We report the first cytogenetic alteration in pilomatricoma: trisomy 18. This aberration is a consistent finding in pilomatricoma, occurring in a subset of proliferating tumors and confined to the neoplastic epithelial cells. Our FISH methods likely underestimated the frequency of trisomy 18 due to incomplete representation of whole nuclei in the 5-micron sections, as well as loss of nuclear material in the progression from basaloid cells to "ghost cells." Although trisomy 18 does not occur in every cell of pilomatricoma, it may indeed have a biologic role inducing growth and/or cellular maturation/senescence in neighboring cells by paracrine signaling or direct cell-to-cell interactions. BCL-2, an anti-apoptotic gene involved in the highly regulated growth cycle of hair follicles, is located on chromosome 18 and may be of interest. Understanding what turns off the epithelial proliferation in pilomatricoma could have broad implications for the treatment of other epithelial neoplasms, and recognizing the recurrent cytogenetic abnormalities may be an important first step.

4 Identification of Candidate Genes for Histiocytoid Cardiomyopathy Using Whole Genome Analysis: Analyzing Material from the HC Registry

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Background: Histiocytoid cardiomyopathy (HC) is a rare, but distinctive arrhythmogenic disorder characterized by incessant ventricular tachycardia, cardiomegaly, and often sudden death within the first 2 years of life. Since it was first described by Voth in 1962, the underlying genetic mechanism of HC has eluded researchers. Several mechanisms have been proposed, (X-linked and autosomal recessive inheritance patterns, defect in complex III of the respiratory chain, mitochondrial DNA mutation); however, they have not been verified, and the etiology of HC remains unknown. Thus, molecular analyses using HC lesions and the Purkinje areas from hearts of age-matched controls were performed to reveal the molecular/genetic basis of HC.

Design: Total RNA and genomic DNA were prepared from formalin-fixed paraffin-embedded tissue from 12 cases of HC and 12 age-matched controls. Whole genome DASL (cDNA-mediated Annealing, Selection, extension and Ligation) profiling was performed to identify genes differentially expressed in HC. Changes in RNA expression were confirmed by TaqMan QPCR. Changes in DNA copy number were measured by TaqMan copy number variation analysis.

Results: Analysis of changes in gene expression in HC cases identified two gene sets that were significantly downregulated compared to controls and aligned sequentially along the genome. The first gene cluster consisted of the S100A8, S100A9, S100A12 genes at 1q21.3c and the second gene cluster consisted of IL1RL1 (ST2), IL18R1, IL18RAP at 2q12.1a. We also observed strong decreases in expression of IL-33 compared to controls. The IL1RL1 (ST2) receptor has previously been shown to participate in an IL33-mediated cardioprotective signaling system. Decreases in copy number of the S100A genes were confirmed by TaqMan CNV assays, suggesting that cases were haploinsufficient for these genes. S100A genes are downstream of the p38-MAPK pathway that can be activated by IL33 signaling.

Conclusions: These data suggest that the IL33-IL1RL1/p38-MAPK/ S100A8-S100A9 axis is downregulated in HC and provides several candidate genes on 1q21.3c and 2q12.1a for inherited genomic variations that predispose patients to HC. The discovery of these novel HC genetic markers may lead to diagnostic and screening tools which can be used prenatally as well as in affected patients and family members.

5   G0/G1 Cell Cycle Arrest in Insulin-Producing Islet Cells of Persistent Hyperinsulinemic Hypoglycemia of Infancy: Evidence for Islet Neoformation from Transdifferentiation of Acinar and Ductal Cells

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Background: Diffuse variant of persistent hyperinsulinemic hypoglycemia of infancy (PHHI) is associated with an increased number of insulin-producing cells and hypersecretion of insulin into the systemic circulation. Such a process could involve one or more mechanisms to include cell cycle progression in pre-existing beta cells, neoformation of beta cells from progenitor cells, or transdifferentiation of mature acinar and ductal cells into beta cells. The hyperinsulinemia could involve one or more pathogenetic factors, such as a mass action effect consequent to an expanded population of beta cells, genetic hyper-responsiveness to nutrients, or constitutive activation of signal transduction pathways involved in the synthesis of insulin. We provide histopathologic, immunohistochemical, ultrastructural, cell cycle and molecular/signal transduction data to support the contribution of transdifferentiation of mature acinar and ductal cells into insulin-secreting cells in this entity.

Design: Pancreatic tissue from three patients was analyzed. One with normal histology served as control. The other two are newborns (non-diabetic mothers) with refractory hypoglycemia, one with SUR gene mutation, and the other with a family history of PHHI. Monoclonal antibodies to Ki67, p27Kip1, S phase-specific kinase protein 2 (Skp2) were applied. Sections were processed for transmission electron microscopy (TEM).

Results: Hematoxylin-eosin slides showed neoformation of the islets from ductal elements. Increased number of beta cells could also be seen within acini in a diffuse pattern, and outside any well-defined islets. There was no mass action effect as a consequence of increased number of beta cells. TEM showed acinar cells that contain zymogen granules and endocrine granules. Ki67 expression in PHHI cases was 30.4% and 28.6% in the exocrine and endocrine compartment. There was no nuclear expression of Skp2 in the admixed endocrine and exocrine components. Diffuse nuclear expression of p27Kip1 in these cells was seen. Mitotic index was 1/30HPF.

Conclusion: Diffuse nuclear expression of p27Kip1, a Cdk2 inhibitor, lack of nuclear Skp2 in the admixed exocrine and endocrine component, and a relatively high proliferation rate without a proportionally high mitotic index in PHHI, show that these insulin-secreting cells enter cell cycle, but do not progress to Cyclin E-dependent G1 phase. Our cell cycle, histological, and ultrastructural data support the contribution of transdifferentiation of insulin-secreting cells from exocrine elements in diffuse PHHI.

6 Identification Of Human Papillomavirus (HPV) Genotypes In Pediatric Laryngeal Papillomatosis (PLP)

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Background: PLP is the most common benign neoplasm to affect the larynx in children. Its incidence in the US is estimated at 4.3 per 100,000 children. The etiology is infection of the upper airways by HPV types 6 and 11 with slight predominance of HPV-6 detection. PLP is characterized by unpredictable course varying from spontaneous resolution of papillomas to rapid progression with life-threatening airway obstruction rarely leading to malignant transformation. HPV-11 may be associated with a more aggressive course with higher incidence of airway dissemination, tracheostomy, malignant transformation and mortality.

Design: Our institution files were searched for patients with a histopathologic diagnosis of PLP (younger than 18 years of age), from 1982-2009. Formalin-fixed, paraffin-embedded specimens were obtained for DNA extraction using previously published protocols. To investigate the presence of HPV DNA, we amplified the genetic material using a set of consensus primers (MY09/MY11), followed by nested PCR with GP6/MY11 primers. Beta-globin was amplified as internal positive control. The 190 bp heminested PCR products were subjected to DNA sequencing. The hypervariable 34–50 bp DNA sequence downstream of the MY11 primer site was compared to the known HPV DNA sequences stored in the GenBank using on-line BLAST for genotyping.

Results: 22 patients met our inclusion criteria. The age range was 7 months to 11 years. The ration between male and female was 1:1 (11 males, 11 females). In 7 specimens DNA amplification was not obtained. HPV DNA amplification was obtained in 14 specimens. Of these, HPV-11 was seen in 11 patients (79%) and HPV-6 was seen in 3 patients (21%). No cases revealed co-infection by both viral genotypes or other HPV genotypes. In one case (11 year-old female) amplification of beta-globin was obtained with negative HPV DNA amplification, suggesting an etiologic agent different from HPV infection in this case.

Conclusion: Our results show a different distribution of HPV genotypes from previous studies, with a higher incidence of HPV-11 infection in our population of PLP cases. In addition, we identified one case out of 15 (7%) cases with DNA amplification, which did not show amplification of HPV DNA, suggesting a possible etiology different from HPV infection. These results also show that direct DNA sequencing of paraffin-embedded tissue is an effective method for detecting HPV genotypes in cases of PLP, and may constitute a useful ancillary technique for the initial diagnosis and follow up of these cases.

7 The Utility of Tissue Transglutaminase Immunostaining in Pediatric Duodenal Biopsies: Patterns of Expression & Role in Celiac Disease; Clinicopathologic correlates of 58 cases

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Background: Tissue transglutaminase (tTG) is a ubiquitous multifunctional protein. It is a calcium dependent enzyme involved in cell death, cell adhesion, cell differentiation and matrix stabilization. tTG mediated posttranslational modification of proteins may be a pathogenetic mechanism in diseases including Celiac Disease (CD). CD is an autoimmune enteropathy triggered by gluten and characterized by villous atrophy on biopsy. tTG modifies gliadin peptides, unmasking immunoreactive epitopes paving the way for disease, in genetically susceptible hosts. tTG is a major target of auto antibodies & its expression by immunohistochemistry, in pediatric celiac disease has not been fully examined. We studied the tTG expression in duodenal mucosa of childhood Celiac Disease in 58 cases and correlated with clinical features.

Design: The clinical features, pathology and duodenal tTG expression was studied in 58 patients. Endoscopically obtained biopsies were formalin embedded, cut at 4 microns and immunostained with tissue transglutaminase 2, clone CUB 7402 (AbCam Inc, Cambridge MA) with 1:1280 dilution, citrate antigen retrieval, & polymer detection with DAB (Leica Microsystems, Bannockburn MA). Hematoxylin/Eosin stained sections were also studied. Staining of the surface epithelium & lamina propria glands was examined. Staining was scored as 0 (negative), 1(focal) and 2(diffuse), and strong or weak. H&E sections were examined concurrently. Positive controls were non CD patients without endomysial antibodies.

Results: The patients included 30 females & 28 males. Ages ranged from 6 months to 16 years. 21/22 CD cases had elevated anti endomysial antibodies (EMA) with 1 case each having Down Syndrome and type-1 Diabetes. Endocopically 22/58 CD cases showed erythema & duodenal friability, and histologic features of CD. 33/58 had normal histology, 2/58: active duodenitis and 1/58 had a tubulovillous adenoma. 19/22 CD and 18/33 non CD cases showed variable staining, 3/22 CD cases and 15/33 non CD cases showed no staining. Staining was noted in the brush border, cytoplasm and especially the basement membranes.

Conclusions: tTG expression in pediatric duodenal biopsies of CD does not show a specific pattern. Staining similar to CD is noted in unaffected duodenal biopsies. Surface epithelial staining is more intense in CD, in areas of villous blunting. Externalization of tTG into extracellular matrix was observed. Concentration of tTG in the basement membrane may have a role in deamidation of the toxic fraction of gliadin. Detailed larger studies to map the pattern of expression in CD and the localization and intensity of gamma glutamyl lysine, which reflects the deamidation activity of tTG are required.

8 Decidual Vasculopathy: Location and Association with Ischemic Lesions

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Background: Decidual vasculopathy (DV) describes a number of lesions involving uteroplacental vessels, including persistence of arteriolar smooth muscle, incomplete physiologic conversion, intimal accumulation of foamy macrophages (atherosis), fibrinoid degeneration/necrosis, decidual vasculitis and thrombosis. DV is often seen in preeclamptic placentas and in disorders of underperfusion and is associated with placental ischemia and infarction. Often, these abnormal vessels are difficult to identify, partly because there is debate about where they are most frequently found and if additional special sections are needed to find them. In addition, the question of whether ischemic lesions are more likely to be present based on the location and extent of DV has not been well studied.

Design: To determine the placental locations of DV, the following microscopic sections were submitted: 2 membrane rolls (MR) from the rupture site to the placental edge, 3 full thickness sections including basal plate (FT), and at least 3 sections of the basal plate only (BP). 76 cases with adequate material fulfilled the criteria for the diagnosis of DV. The location(s) of DV was recorded as well as the presence of ischemic change (IC), infarcts (INF) and retroplacental hematoma (RPH).

Results: Overall, DV was found in MR in 67.1%, in FT in 32.9% and in BP in 25.0% of cases (p value = 0.004). DV was present only in MR in 53.9%, only in FT in 14.5% and only in BP in 9.2%. DV was present in 2 locations in 19.7% and in all 3 locations in 2.6%. The presence of DV in each location (MR, FT, BP) was associated with placental IC (p value = 0.019, 0.022, 0.034 respectively) but not with INFs or RPHs. There was no difference in placental lesions between the different locations of DV (MR, FT, BP).

Conclusion: Since severity of DV was not assessed, mild cases were included, which would be less likely to have severe lesions such as INF or RPH compared to just IC. DV was found most frequently in the MR compared with FT and BP. However, in a significant number of cases (23.7%), DV was found only in the other locations. Furthermore, in 9.2% of cases, DV was found only in the extra sections of BP. Therefore, although DV is frequently present in the MR, extra sections of BP specifically designed to identify DV is recommended, particularly in any placenta with a clinical history suggestive of underperfusion.

9 Multifocal Choriangiomatosis

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Background: Multifocal choriangiomatosis (MC) is an uncommon villous capillary lesion sharing some features with villous choriangiosis and placental choriangioma.

Design: We identified 53 cases of MC amongst 5429 consecutively accessioned placentas of >20 weeks gestational age (GA) over a 10 year period. MC was subcategorized as extensive versus patchy based on the size of the largest focus (> than a 2x microscopic field) and severe versus mild-moderate based on the capillary density of affected intermediate/terminal villous units. Two GA-matched controls were selected for each case from the same cohort and a case-control analysis of associated clinical and pathologic features was performed.

Results: MC was more frequently seen in the early preterm (<32 weeks GA) subgroup (1/66 vs 1/110). Affected placentas showed significant increases in associated avascular villi (64% vs 3%), choriangiosis (60% vs 11%), and distal villous immaturity (45% vs 5%). Only one case had a choriangioma. Other common placental findings were concentric narrowing of fetal villous arterioles (23%), villous edema (21%), and dysmorphic villi (11%). The five cases with severe MC were more likely to have avascular and/or dysmorphic villi. Maternal factors significantly associated with MC were age >35 years (22% vs 7%) and gravidity >5 (28% vs 15%). Fetuses with placental MC had a significantly increased prevalence of congenital anomalies (27% vs 3%). Although not achieving statistical significance, MC fetuses also had a higher perinatal mortality rate and an increased prevalence of suspected antenatal growth restriction and birth weight <10th percentile for GA compared to controls. Fetuses with extensive MC (n=27) when compared with patchy MC had a significantly higher prevalence of congenital anomalies (39% vs 15%) and were more likely to be >90th percentile for GA (23% vs 4%). Those with patchy MC were more likely to be <10th percentile for GA (23% vs 9%).

Conclusion: MC is a distinct villous capillary lesion often associated with choriangiosis, distal villous immaturity, and avascular villi. It is most common in early preterm births and has an increased perinatal mortality rate and frequency of SGA infants compared with GA matched controls. Clinical factors significantly associated with MC are advanced maternal age, multigravidity, and fetal anomalies. The dichotomy between cases with extensive versus patchy MC in terms of prevalence of associated fetal anomalies and birth weight suggests that there may be two subgroups of MC.

10 LAIR2, Downregulated in Preeclampsia, Localizes to Sites of Extravillous Trophoblast Invasion

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Background: A global gene expression microarray analysis of surplus chorionic villus sampling (CVS) tissues identified leukocyte-associated immunoglobulin-like receptor 2 (LAIR2) as downregulated in the first trimester of preeclamptic pregnancies, when compared to non-preeclamptic gestations. LAIR2 is the soluble receptor counterpart to LAIR1, an inhibitory receptor shown to bind collagen. LAIR1 is widely expressed throughout the body. LAIR2 expression, in contrast, appears much more restricted, with highest expression in the placenta. No prior work on LAIR1 or LAIR2 has examined the placenta. We therefore conducted localization studies in placental tissues to determine the precise sites of LAIR2 mRNA production and protein binding.

Design: ISH and IHC were performed on FFPE placental samples from each trimester. Localization and intensity of staining were determined for each technique.

Results: In situ hybridization experiments, to detect the site or sites of LAIR2 production, revealed a highly restricted LAIR2 localization. LAIR2 mRNA was found only in the more distal portions of extravillous trophoblast (EVT) cell columns, adjacent to the invading EVT. Immunohistochemical staining detected intracellular LAIR2 staining in these same cells. Extracellular staining of this soluble receptor was found in the fibrinoid between invasive EVT cells distal to the cell columns.

Conclusion: ISH and IHC staining for LAIR2 detected specific, highly localized expression at the leading edge of EVT cell columns in first trimester placentas. This staining likely represents the site of production for this soluble receptor. Following secretion, the receptor appears to bind extracellular material among the invasive EVT. We hypothesize that this soluble receptor coats extracellular ligands to which the LAIR1 receptor on invasive EVT otherwise would bind. LAIR2 thus may prevent the premature inhibition of extravillous trophoblast invasion. Decreased expression of LAIR2 may have physiologic relevance in preeclampsia and in fact may play an etiologic role in this disorder by fostering premature inhibition of EVT. Further study of LAIR2 as a novel biomarker for risk of preeclampsia, growth restriction and/or other implantation problems is indicated.

11 Abnormal Villous Cytotrophoblast Differentiation and Ap-2á Expression in Selected Pathologic Conditions of Pregnancy

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Background: Transcription factor AP-2á has a critical role in the regulation of human villous cytotrophoblast (CT) to syncytiotrophoblast (ST) differentiation. We reported previously that there is a reverse pattern of AP-2á gene expression in CT and ST in placentas of severe preeclampsia (SPE) vs controls, suggesting accelerated villous CT differentiation in SPE. In the present study, we have now broadened our investigation to include other types of high risk pregnancy.

Design: Paracentral sections from grossly unremarkable areas of placentas from patients with SPE, mild pre-eclampsia (MPE), diabetes mellitus (DM), chronic hypertension (HTN), idiopathic intrauterine growth restriction (IUGR) and gestational age-matched control cases (CG) (ten placentas in each group) were double immunostained for AP-2á and E-cadherin. The numbers of AP-2á positive and negative CT and ST nuclei in each section were blindly counted in ten 40x fields (5 different fields counted independently by RS and JS). Statistical differences between groups were determined by one-way ANOVA.

Results: The differences between means±SD of the 100 fields analyzed in each placental group were highly statistically significant ($p<.001$) for each studied parameter.

	Chorionic villi	Syncytial knots	ST	AP-2á positive STCT		AP-2á positive CT
CG	25.5 ± 8.0	10.6 ± 9.0	312.9 ± 183.4	48.7 ± 69.4	32.6 ± 18.0	22.4 ± 16.0
MPE	28.7± 10.0	17.4 ± 10.3	261.7 ± 134.0	15.6 ± 60.0	49.7 ± 25.0	37.5 ± 23.0
SPE	29 ± 7.1	14.6 ± 10.0	312.0 ± 183.2	106.9 ± 140.0	26.4 ± 26.0	22.4 ± 24.2
HTN	24.5 ± 12.0	14.8 ± 11.0	259.4 ± 130.0	14.7 ± 58.4	48.1 ± 23.0	35.4 ± 19.4
DM	23.5 ± 7.5	13.7 ± 9.5	270.6 ± 121.4	12.8 ± 44.0	51.9 ± 35.0	38.7 ± 35.0
IUGR	20.9 ± 7.0	13.8 ± 9.0	233.5 ± 136.3	9 ± 35.4	53 ± 56.5	42.0 ± 57.0

Conclusion: MPE and SPE are characterized by accelerated morphological (smaller villi, i.e. placental hypermaturity) and biochemical villous trophoblast (more AP-2á positivity) maturation as compared with CG. A reverse trend with both morphological (larger villi, i.e. placental hypomaturity) and biochemical lagging behind the CG (less ST AP-2á positivity and more CT AP-2á positivity) was observed in DM and IUGR. The syncytial knotting in all study groups was more prominent than in CG, indicating placental hypoxia. These findings suggest that abnormalities in the AP-2á cascade of transcription factors and signaling molecules may be responsible for different types of hypoxic patterns of placental injury in high risk pregnancy.

12 In Vitro Optical Coherence Tomography of Human Fetal Membranes

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Background: Prior studies on the structure of human fetal membranes have been done in vitro and involved light or electron microscopy with fixation, dehydration, and staining. Recently Optical Coherence Tomography (OCT), an optical imaging technique, has provided high resolution cross-sectional images of living biological tissues, with a penetration of 2-3 mm. We evaluated the use of this technique to examine fetal membranes from elective term cesarean deliveries.

Design: Ten women undergoing elective term cesarean delivery were consented for participation in this IRB-approved study. Immediately after delivery samples of fetal membranes were collected from subjects with uncomplicated pregnancies. For each set of fetal membranes 6 samples were collected: 2 along the surgical site of membrane rupture, one halfway between the rupture site and the placental edge (mid-zone), one from the thickened membrane next to the placental edge (peri-placental zone), one along the axis between the peri-placental zone and the mid zone, and one along the axis between the mid zone and the rupture site. All women underwent a low transverse incision in the uterine membranes. After collection the samples were stretched across customized disks, rinsed in chilled sterile normal saline, and immediately brought to the OCT laboratory for analysis using a bench top spectral domain OCT (SD-OCT) system. Following OCT scan, the samples were put in formalin for histological study. The OCT and histological images were compared.

Results: The OCT images correlated well with the histological findings. We were able to delineate at least 4 layers of the fetal membranes. Of interest, in two cases, lake-like structures were observed in OCT and verified by histology. Both patients were treated with heparin during pregnancy for history of deep venous thrombosis. These structures have been previously described by Stanek et al as microscopic chorionic pseudocysts in the fetal membranes. These authors described such cysts in 4% of all placentas and in 15% of placentas from preeclamptic and diabetic mothers at 24-42 weeks' gestation possibly representing a hypoxia-associated placental lesion.

Conclusion: OCT shows promise as a new technique for real-time clinical histo-pathologic correlation. Furthermore, the technique reaffirms the existence of microscopic chorionic pseudocysts existing in the chorionic laeve of the fetal membranes.

13 Expression of Lymphatic Markers Prox-1 and D2-40 in a Placental Development Series

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Background: The scant data available on placental lymphatic vessels point to the absence of a lymphatic circulation. A recent study on mesenchymal dysplasia (MD), however, identified pathologic lymphangiogenesis using D2-40 lymphatic marker. We sought to detect placental lymphatic vessel development by using specific lymphatic markers in a comprehensive developmental series of placentas.

Design: A human placental developmental series (12 to 39 weeks of gestational age, n=17) with no significant pathology was studied. Serial sections were stained with H&E and with antibodies to the lymphatic markers Prox-1 and D2-40, as well as with the panendothelial cell marker CD31.

Results: No staining with Prox-1 was identified. D2-40 consistently marked stromal cells (fibroblasts and/or Hofbauer cells) of villi at all gestational ages. In addition, perivascular/pericellular extracellular matrix and the chorionic cell layer beneath the amnionic membrane were highlighted by D2-40. No D2-40 positivity of endothelial cells was noted when compared to CD31 staining of endothelial cells.

Conclusion: Our extensive developmental study has established that no lymphatic vasculature is present in the placenta. The absence of Prox-1 expression is confirmatory, as endothelial cells need active Prox-1 to acquire lymphatic fate. Given the absence of preexisting placental "mother" lymphatic vessels, it is difficult to explain the reported pathologic lymphatic growth in MD. However, a reactive process with lymphatic differentiation is a possibility.

14 Review of Stillbirths at a Women and Children's Hospital: Analysis of 72 Cases by Gestational Age. Implications for Prevention in Future Pregnancies

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Background: Stillbirth is unfortunately a very common adverse outcome of pregnancy occurring in 7 out of every 1000 deliveries in the United States. Analysis of causes of death at various gestational ages can provide invaluable information regarding underlying causes of demise and can aid the clinician in terms of recurrence and prevention at different stages of pregnancy. This study evaluates 72 stillbirths at a single institution with respect to gestational age.

Design: The data for this study was taken retrospectively from records of stillbirths delivered at the Kapi'olani Medical Center for Women and Children in Honolulu, Hawaii during a four-year study period (1/20/05-10/10/08). This study analyzes information obtained from clinical case records, autopsy reports, and placental reports of 72 stillbirths. The Tulip classification was chosen for categorizing the causes of death in this study because it incorporates maternal, fetal, and placental entities together and has been reported to have a higher percentage of "known" causes of demise. Causes of death (COD) were classified into six categories per the Tulip system: (1) congenital anomaly (CA), (2) placenta (PL), (3) prematurity/immaturity (PRE), (4) infection (INF), (5) other (O), and (6) unknown (U), and were then further subcategorized per Tulip protocol. The causes of death were then organized into gestational age (GA) categories: previable (PV) (<24 weeks), preterm (PT) (24-36 weeks), and term (T) (37-40 weeks).

Results: The most frequent COD's overall were congenital anomalies (n=16, 22%) and infections (n=16, 22%). Gestational age-specific results are tabulated below:

GA	COD: CA	COD: PLA	COD: PRE	COD: INF	COD: O	COD: U
PV (n=39) 20.5% (n=8)	15.4% (n=6)	17.9% (n=7)	25.6% (n=10)	7.7% (n=3)	12.8% (n=5)	
PT (n=27) 25.9% (n=7)	11.1% (n=3)	0%	22.2% (n=6)	22.2% (n=6)	18.5% (n=5)	
T (n=6)	16.7% (n=1)	66.7% (n=4)	0%	0%	0%	16.7% (n=1)

Among the known COD subclassifications, ascending infections (n=13), non-monogenic syndromes (n=7), placental parenchymal causes (n=5), preterm delivery (n=5), placental bed pathology (n=4), and umbilical cord complications (n=4) were the most common.

Conclusions: Our study revealed a wide spectrum of COD with "unknown" causes comprising less than 20%. Congenital anomalies and infections were the most common COD in the PV and PT categories. Placental COD's were by far the most common in the term category. This data will be useful in building future knowledge and insight toward reducing preventable causes of demise at various gestational ages.

15 Childhood Pheochromocytomas and Paragangliomas Are Frequently Malignant and Carry a High Rate of Germ Line Mutations

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Background: Pheochromocytomas are catecholamine-producing tumors of the adrenal medulla, whereas sympathetic paragangliomas (sPGL) are their extra-adrenal abdominal counterparts. Head and neck PGL are usually non-catecholaminergic and are called parasympathetic PGL. Over the past decade, it has become clear that 6 candidate genes, including RET, VHL, NF1, SDHB, SDHC, and SDHD, are responsible for germline mutations in up to 30% of PCC and PGL patients. However, due to the relative rarity of these tumors, pediatric patients have not been systematically studied.

Design: We retrieved all patients with a PCC or PGL below age 20 in The Netherlands (population 17 million) over the period 1983-2008, through the national automated pathology database (PALGA). Clinical charts and slides were reviewed and tumor and normal DNA were isolated for the anonymous assessment of candidate gene mutations, if this had not already been done.

Results: A total of 73 patients was found in the national pathology archives of which 50 were available for further detailed analysis. There were 31 PCC and 19 PGL, with a slight female preponderance and mostly occurring between 10 and 20 years of age. Successful genetic testing could be performed in 27 patients, of which 18 (67%) had a germline mutation in one of the candidate genes (n=8 VHL, n=3 SDHB, n=2 SDHD, n=5 RET). Furthermore, 4 patients were diagnosed with synchronous malignant disease, defined as histologically proven distant metastases, and 5 more patients were shown to have malignant disease during follow-up (18% composite malignancy rate). Three of these 9 patients (33%) died of their disease.

Conclusions: PCC and PGL are increasingly recognized in the pediatric population and carry a very high rate of germline mutations in one of 6 candidate genes. Genetic testing is imperative if patients do not come from families with known mutations, especially if they have multiple tumors or bilateral PCC. A careful search for signs of malignancy should be carried out both at diagnosis and during follow up, given the rate of malignancy and subsequent death.

16 Proteomic Studies of Anaplasia in Wilms Tumor

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Background: Wilms tumor (WT) is the most common malignant tumor in the pediatric kidney. Anaplasia, focal or diffuse defined by histologic criteria is the most important parameter to guide the clinical treatment plan. Currently, besides no specific biomarker for diagnosing WT, there are no biomarkers to correlate with the histological subtypes. The attempt of this research is to identify and characterize potentially useful biomarkers and provide insight into the peculiar molecular biology of WT with unfavorable histology.

Design: In our pilot study, utilizing iTRAQ labeling technology, coupled with quantitative mass spectrometry (LC-MS/MS), we identified proteins that are differently regulated in different WT histology. iTRAQ technology allows simultaneous quantitative analysis of up to eight samples. In our pilot experiments we selected four WT specimen including two with classic favorable histology, one with focal anaplasia and one with diffuse anaplasia. All four samples were labeled in duplicate as to fully utilize all 8 reporting ions and also to have a statistical distribution of the ratio of the reporter ions that are one-to-one. Total of

256 proteins with a Protein Score >1.0 are identified from all samples (proteins with >90% confidence. Cut-off values are set as 0.67 fold for underexpression and 1.5 fold for overexpression.

Results: Compared with classic favorable morphology: in the focal anaplasia group, we identified 15 proteins underexpressed and 12 proteins overexpressed; in the diffuse anaplasia group, we identified 18 proteins underexpressed and 8 proteins overexpressed. With the 44 proteins involved, the vast majority clearly seem to have a similar regulation pattern; per cut-off values set, none have shown an expression pattern that has gone to the different direction. Some extremely interesting biomarkers have been identified, and they provide new insights into the puzzling biology of WT with unfavorable histology.

Conclusion: though there are biological differences, with unfavorable histology, either focal anaplasia or diffuse anaplasia the protein expression seem to be similarly dysregulated, compared with the favorable histology group. The newly identified potential markers for WT with unfavorable histology include SAA, ORM1 and 2, CCT3, TMSB4X, GAPDH, FGG and FGB etc. These proteins provide new insights into the molecular biology of WT and have many practical implications.

17 Claudin 6: A New Marker of Non-CNS Malignant Rhabdoid Tumors and Atypical Teratoid/Rhabdoid Tumors

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Background: Malignant rhabdoid tumors (MRT) are highly aggressive childhood neoplasms which occur in the central nervous system as atypical teratoid/rhabdoid tumors (AT/RT), the kidney and extrarenal locations. These tumors are defined by mutations or deletions of the SMARCB1 gene, resulting in loss of INI1 protein expression. Recently, increased mRNA and protein expression of claudin 6, a component of tight junctions in fetal tissues, was shown in AT/RTs. We report claudin 6 expression by immunohistochemical staining in a large group of MRT.

Design: A tissue microarray containing 35 AT/RT and 15 non-CNS MRT from The Children's Hospital of Philadelphia was stained using an antibody against claudin 6. Cores were scored for both percent of tumor with positive staining (0-100%), and intensity of expression on a semiquantitative scale (0-3). These scores were then multiplied to generate a final immunohistochemical score (0-300) per core which was averaged between cores. The pattern of claudin 6 expression was also characterized for each core as cytoplasmic, membranous or both.

Results:

Table 1. Intensity of staining

	1-100	101-200	201-300	Total
AT/RT	4 (12%)	12 (34%)	19 (54%)	35/35 (100%)
Non-CNS MRT	1 (7%) 2 (13%)	12 (80%)	15/15 (100%)	

Table 2. Pattern of expression

	Cytoplasmic	Membranous	Both
AT/RT	14 (40%)	4 (11%)	17 (49%)
Non-CNS MRT	6 (40%)	2 (13%)	7 (47%)

Conclusions: Claudin 6 expression was seen in all MRT studied. The pattern of expression was similar between the AT/RT and non-CNS MRT with a combination of cytoplasmic and membranous expression being the most common in both. Variability in staining intensity was seen between the two groups with AT/RT showing lower levels of protein expression; however, this difference was not statistically significant. Further study is required to determine the significance, if any, of claudin 6 in the pathogenesis of MRT and to better characterize its expression in other pediatric brain tumors. However, these findings raise the possibility that claudin 6 may have utility as an additional marker that can be used in establishing the diagnosis of MRT.

18 PAX5 Immunohistochemical Staining as a Fusion Gene Marker in Rhabdomyosarcomas: Too Good to be True?

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Background: The PAX5 immunohistochemical stain is a marker of B-cells and B-cell derived malignancies. Its expression has also been documented in Merkel cell carcinomas, urothelial tumors, and neuroendocrine carcinomas. A recent study investigating PAX5 expression in pediatric round blue cell tumors found positive nuclear staining in 2/3 of the 51 tested alveolar rhabdomyosarcomas (RMS) but none of the 55 embryonal RMS. In this series 7 of the alveolar RMS had PAX gene fusion testing available. The 5 fusion positive cases exhibited PAX5 staining whereas the 2 negative cases did not, suggesting that PAX5 staining could be a fusion gene marker. A subsequent study of 24 RMS cases, all with PAX gene fusion testing available, demonstrated positive PAX5 staining in 60% of the alveolar cases but none of the embryonal cases. In contrast to the previous study, PAX5 staining did not correlate with fusion gene status; staining occurred in 6 of 7 fusion positive and 3 of 8 fusion negative alveolar RMS cases.

Design: We searched the Seattle Children's Hospital (SCH) surgical pathology database for all RMS diagnoses over the last 10 years. Representative paraffin blocks from the cases were stained with the PAX5 immunohistochemical marker. The presence or absence of nuclear PAX 5 staining was then correlated with tumor morphology (alveolar vs. embryonal), intensity of myogenin staining, and PAX gene fusion status (when available).

Results: There were 112 surgical pathology specimens from 63 patients. Available tumor material from pre-treatment or recurrent tumor off therapy yielded 56 cases including 27 alveolar RMS, 24 embryonal RMS, 1 mixed and 4 with indeterminate subtype. Of the 27 alveolar RMS, 21 had fusion gene testing available of which 9 were PAX fusion gene positive. Positive nuclear PAX5 staining was identified in only 6 cases, all of which were alveolar RMS. One of the six cases had a t(1,13) translocation identified by RT-PCR; 2 were fusion negative, and 3 were without fusion gene testing. Positive PAX5 staining did not correlate with intensity of myogenin staining.

Conclusions: Our series confirms the observation that positive PAX5 staining occurs only in the alveolar subtype of RMS. The lack of PAX5 staining in 8 of our 9 PAX gene fusion positive cases however indicates that the PAX 5 stain is not in any way specific for the presence of a fusion gene. The overall low number of PAX5 positive cases in our series was disappointing and puts in question the diagnostic utility of PAX5 staining in the diagnosis of alveolar RMS.

19 Utility Of SALL4 Immunohistochemistry In Evaluating For Foci Of Yolk-Sac Tumor In Immature Teratomas

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Background: The presence of a malignant germ cell tumor component (most commonly yolk sac tumor) within immature teratoma is cause for potentially more aggressive intervention. Recently, SALL4, a member of the OCT3/4 family of transcription factors, has been shown to be a sensitive marker of yolk-sac tumor in gonadal and extra-gonadal sites. Thus, SALL4 may potentially be able to detect subtle foci of yolk-sac tumor within otherwise immature teratomas. In this study, we sought to evaluate whether SALL4 immunohistochemistry identified unsuspected foci of yolk-sac tumor in previously diagnosed immature teratomas.

Design: Eleven cases of immature teratomas diagnosed without a yolk-sac component (IT) and eight cases diagnosed with a yolk-sac component (IT-YST) were retrieved from the pathology archives of Children's Medical Center, Dallas, TX. Five of the eight mixed germ cell tumor cases displayed unequivocal presence of YST on H&E stains. The remaining three were diagnosed as being "suspicious" for YST, due to the small size of the focus or absence of AFP staining in the areas of interest. SALL4 immunohistochemistry was performed on selected sections from each case. We defined positive SALL4 staining as any amount of nuclear labeling within any cell type. The diagnoses and clinical outcomes were revisited in light of the SALL4 staining.

Results: In all five cases of unequivocal IT-YST, SALL4 strongly labeled the nuclei of the YST component, which was otherwise clearly identifiable by its growth pattern, nuclear pleomorphism, and presence of nucleoli and mitotic figures. In the three cases of IT with suspected YST component, none showed SALL4 staining of the suspect foci. In three of 11 cases initially diagnosed as IT, SALL4 highlighted small foci of atypical cells. Re-review of the H&E slides from these cases confirmed the presence of small atypical foci that raised the possibility of YST. In each instance, the patient was treated with surgery alone, and is alive without evidence of YST (follow-up: 6, 8, 96 months, respectively). In all cases, SALL4 labeled a subset of immature neuroepithelial cells and immature glands.

Conclusion: SALL4 is a useful marker of YST that can help confirm the presence of a mixed IT-YST. It may also highlight small foci that are suspicious for YST in otherwise typical immature teratomas. The significance of the latter finding requires further investigation.

20 Lin28 a Novel, Lineage-Specific, Marker of Germ Cell Neoplasia

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Background: The histopathology of germ cell tumors (GCT) is remarkably diverse, reflecting the pluripotency of germ cells. Distinction between the various subtypes without ancillary markers presents a diagnostic challenge. This is particularly true of yolk sac tumor (YST) since it may have unusual patterns of growth, e.g. papillary or solid, differentiate along lines of primitive "intestinal" or "endometrioid-like" glands, mimic other tumors such as immature teratoma, sex cord-stromal tumor, embryonal carcinoma, and serous and clear cell carcinoma, or just be an inconspicuous component of a mixed GCT. Oct3/4 (OTF3/POU5F1) has been established as a useful marker in GCT since it is strongly expressed in dysgerminoma / seminoma and embryonal carcinoma. Oct3/4, however, is not expressed in YST.

Design: Lin28, an essential upstream regulator of Blimp1/Prdm1 necessary for primordial germ cell development, has recently been shown to be associated with GCTs (Nature 2009;460:909-913). We herein studied pediatric germ cell tumors of various types and a cohort of pediatric non-germ cell neoplasms for the immunohistochemical expression of Lin28 and Oct3/4.

Results: Tumor cells of all intratubular germ cell neoplasia, unclassified (ITGCNU) (n=6), seminomas/dysgerminomas (n=7) and ECs (n=6) demonstrated strong immunoreactivity for Lin28 (nuclear and cytoplasmic) and for Oct3/4 (nuclear). Tumor cells of all YSTs (n=8), either the pure form or as a component of a mixed GCT, showed strong immunoreactivity for Lin28 (nuclear and cytoplasmic), while none were immunoreactive for Oct3/4. Choriocarcinoma (n=3) was focally, weakly immunoreactive for both Lin28 (cytoplasmic only) and Oct3/4 (nuclear). Lin28 was not expressed in mature or immature teratomas (n=8), sex-cord stromal tumors (n=2), hepatoblastomas (n=2), neuroblastomas (n=2), adrenal cortical carcinomas (n=2), Wilms tumors (n=2), or rhabdomyosarcomas (n=2).

Conclusion: 1. Like Oct3/4, Lin28 is strongly expressed in ITGCNU, seminoma, dysgerminoma, and embryonal carcinoma; In YST, however, Lin28 is a sensitive and specific marker, in contrast to Oct3/4 in which it is not expressed. 2. Lin28 is useful in recognizing unusual variants or morphologic patterns of YST and to differentiate them from their mimics. 3. In conjunction with Oct3/4, Lin28 is useful to distinguish YST from EC and to delineate areas of transition between these 2 components.

21 Germline DICER1 Mutations Are Common in Both Hereditary and Presumed Sporadic Pleuropulmonary Blastoma

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Background: Pleuropulmonary Blastoma (PPB) is a neoplasm that arises in the developing lung. PPB is a feature of a cancer predisposition syndrome. Affected children and their family members are at increased risk for rhabdomyosarcoma, cystic nephroma and other neoplasms. A recent linkage study showed affected individuals shared a common 7 Mb segment on 14q31-32. One gene, DICER1, was considered a strong candidate as mice with conditional inactivation of DICER1 in epithelium develop cystic lungs. Initial candidate gene sequencing identified germline loss-of-function mutations in 11 families (Science 2009).

Design: Family history information, blood and/or saliva was collected from 68 families affected by PPB. DICER1 sequencing was performed on germline DNA from all children with PPB. Immunohistochemical staining was performed using an anti-DICER1 rabbit polyclonal antibody (Sigma, St. Louis, MO) and an automated stainer.

Results: Thirty-six of 68 (53%) of children with PPB have heterozygous germline loss-of-function mutations in DICER1; these include 19 frameshift insertion-deletions and 16 nonsense mutations. An additional 3 splice junction mutations and 4 missense mutations are being evaluated for functional consequences. 26 children with PPB had no mutations by sequence analysis. Tests for large deletions are in process. Immunohistochemical analysis shows loss of DICER1 staining in tumor-associated epithelium over cambium layers suggesting the wild-type allele is lost in tumor tissue. Laser-microdissection of tumoral epithelium with DICER1 sequencing is in progress.

Conclusion: Germline DICER1 mutations are a major predisposing factor in the development of pleuropulmonary blastoma. Further investigations will shed light on the heritability, penetrance and expressivity and the apparently unique pathogenesis of this pediatric cancer predisposition syndrome.

22 Role of the Wnt/β-catenin Pathway in Pediatric Desmoids Tumors

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Background: Desmoid fibromatosis is a rare neoplasm with fibroblastic/myofibroblastic differentiation arising in children and young adults. Desmoids do not metastasize but are locally aggressive and prone to frequent local recurrence. The behavior of desmoids is difficult to predict based solely on histological features. CTNNB1 (the gene for β-catenin) mutations and β-catenin nuclear accumulation have been identified in sporadic desmoid tumors. Desmoids may also be associated with germline mutations of the APC gene seen in familial adenomatous polyposis (FAP). We evaluated the incidence of CTNNB1 and APC mutations in a series of pediatric desmoids to gain insight into their molecular pathogenesis.

Design: 42 desmoids in pediatric patients were identified in the archives of Texas Children's Hospital and UT-MD Anderson Cancer Center Departments of Pathology (1995-2009). Clinical charts were reviewed including history of FAP or other hereditary cancer syndromes. APC gene mutations were previously determined on blood samples from patients presenting with FAP. DNA was extracted from archival paraffin blocks of desmoids and control dermal scars and assessed for CTNNB1 exon 3 mutations by Sanger sequencing. Immunohistochemistry for β-catenin was also performed on desmoids and control dermal scars.

Results: CTNNB1 mutations were observed in 27 of 42 (64 %) desmoids. Three mutations previously described in adult desmoids were identified: T41A (63%), S45F (30%), and S45P (7%). APC mutations were present in 7 of 42 (17%) desmoids. No mutation was identified in either gene in 8 of 42 (19%) desmoids. β-catenin nuclear staining was observed in 28 of 31 (90%) tested cases. Of 28 desmoids with β-catenin nuclear staining, 82% showed mutations in either CTNNB1 (20) or APC (3).

Conclusion: A high percentage of desmoids (90%) showed nuclear localization of β-catenin, implying constitutive activation of the Wnt signaling pathway. Overall 81% of desmoids showed mutations in either CTNNB1 or APC. Our findings point towards Wnt signaling activation as a major pathway in the development of pediatric desmoids. The relative high percentage (17%) of desmoids associated with APC mutations has significant implications for patient management, as referral for genetic counseling should be recommended for children diagnosed with desmoid tumors lacking CTNNB1 mutations.

23 Complex Congenital Heart Defects in Association with Maternal Diabetes and Partial Deletion of the A2BP1 Gene

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Background: Various types of congenital heart defects were reported in association with maternal diabetes, most commonly as single defects. At least in animal models, maternal diabetes induces congenital heart defects by altering the expression of several genes involved in cardiovascular development. Mutations or abnormal expression of the A2BP1 gene (FOX-1 gene) in chromosomal region 16p13.2 have been previously described in patients with epilepsy, mental retardation, and autism and have not been found in association with congenital heart defects.

Design: We report a case of complex congenital heart disease in a term female infant with maternal diabetes who eventually died of sepsis and post surgical complications at 20 days of age. Tissue from the autopsy was processed for regular cytogenetics, microarray-based comparative genomic hybridization (aCGH) and polymerase chain reaction (PCR).

Results: The autopsy revealed micrognathia, scoliosis, hemivertebrae, unilateral left renal agenesis and dextrocardia with cardiomegally. The complex heart findings included a single common atrium, a single ventricle and atrioventricular valve, atresia of the pulmonic valve and ascending pulmonary artery, and total anomalous pulmonary venous drainage. The infant had normal female chromosomal karyotype. Microarray aCGH and PCR revealed a partial 162 Kb interstitial deletion within chromosomal region 16p13.2 that was mapped to the A2Bp1 gene.

Conclusion: This is the first description of A2BP1 gene deletion in association with complex congenital heart anomalies. Analysis of this case and review of the literature demonstrate the teratogenic effect of maternal diabetes on the developing fetal heart, also resulting in dysregulated gene expression. Our observation emphasizes the importance of aCGH in scanning the human genome in neonates born with congenital anomalies.

24 FOXL2 And SOX9 Distinguish The Lineage Of The Sex Cord-Stromal Cells In Gonadoblastomas

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Background: Gonadoblastomas (GB) are rare mixed germ cell-sex cord stromal tumors that almost exclusively arise in dysgenetic gonads containing the testis-specific protein Y-encoded gene. GBs are composed of immature germ cells and sex cord-stromal cells of unknown differentiation. The sex cord-stromal cells are often described to resemble Sertoli-like cells and immature granulosa cells, or are designated as "sex cord-stromal cells of other type". FOXL2, a member of the forkhead-winged-helix family of transcription factors, is one of the earliest expressed genes during the development of the female gonad and is required for proper differentiation of granulosa cells during folliculogenesis. The transcription factor SOX9 is an intermediate downstream target of SRY and required for testis development by formation and maintenance of (pre-)Sertoli cells. The aim of our study was to further characterize the differentiation of the sex cord-stromal cells by evaluating the expression of these two counteracting transcription factors.

Design: Archival paraffin embedded material of 6 GBs, 4 of which were overgrown by dysgerminoma, were examined by IHC for the expression and localization of FOXL2 and SOX9. Underlying disorders of sex development included Turner, Swyer and Frasier syndromes.

Results: The sex cord-stromal cells revealed strong nuclear staining for FOXL2 in 6/6 cases and were negative for SOX9 expression. Germ cells in the GB (0/6) and in the dysgerminoma (0/4) components were devoid of FOXL2 and SOX9 expression. Areas of transition between GB and dysgerminoma revealed nests with gradual reduction of FOXL2 expressing supportive cells and increased germ cell proliferation.

Conclusion: Based on consistent FOXL2 expression, our results support that the sex cord-stromal cell component of GBs is of granulosa cell origin. In addition, FOXL2 appears to be a useful marker for evaluation of overgrowth by dysgerminoma and to identify the transition zone and nests of "dysgerminoma in situ" in which FOXL2 demonstrates gradual loss of sex cord-stromal cells to complete absence, respectively.

FOXL2 and SOX9 also seem to be useful to distinguish GB from carcinoma in situ (CIS). CIS and GB are thought to be a continuum, and it is suggested that the absence of functional Sertoli cells leads to female differentiation and subsequent GB, whereas CIS requires a certain level of testicular differentiation. This is in concordance with our findings that the sex cord-stromal cells in GBs are of female origin. As FOXL2 and SOX9 are differentially expressed, presence of one factor and absence of the other can help to differentiate CIS with a Sertoli cell component from GB with a granulosa cell component.

25 Serotonin Transporter (SERT) of Lung Endothelium Promotes Perinatal Pulmonary Vascular Adaptation and Its Defective Expression Characterizes Alveolar Capillary Dysplasia/Misalignment of Pulmonary Veins (ACD/MPV)

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Background: SERT promotes pulmonary vasodilatation by controlling bioavailability of serotonin, a potent vasoconstrictor. The SERT promoter has a length polymorphic region (SERT-LPR) with a long (L) and a short (S) allele. The S allele is said to lower the transcription rate of SERT, thus reducing gene activity, while the L allele provides high SERT gene activity. We hypothesize that 1) SERT function is unique to intact pulmonary endothelial cells (EC); 2) SERT plays a role in pulmonary vascular remodeling, ensuring proper adaptation to extrauterine life 3) SERT expression is defective in ACD/MPV lungs resulting in maladaptive vascular changes leading to lethal pulmonary hypertension. 4) Defective SERT expression is due to SERT promoter length polymorphism.

Design: We studied 1) SERT EC specificity/sensitivity in tissue arrays of 4 autopsy lungs (4 days and 1, 5, and 11 years of age); 2) temporo-spatial expression of SERT in normal antenatal (all trimesters, n=17) and postnatal lungs (1 &10 years); 3) SERT expression in ACD/MPV lungs (n=12) and in lungs of patients with pulmonary hypertension of varied etiology (n=14) by immunohistochemistry. 4) SERT-LPR of ACD/MPV patients was assessed by PCR and confirmed by DNA sequencing.

Results: SERT expression was observed only in pulmonary EC; no other organs showed EC positivity. Expression of EC SERT began at about 30 weeks gestational age, gradually increased to become strongly diffuse near term and continued at high level postnatally. ACD/MPV lungs showed no or very minimal EC SERT reactivity; SERT antibody diffusely marked pulmonary ECs in congenital diaphragmatic hernia (n=5), congenital alveolar dysplasia (1), bronchopulmonary dysplasia (3), and primary pulmonary hypertension (5). Amplicon of SERT-LRP of all ACD/MVP lungs showed homozygosity for the L allele.

Conclusion: SERT is a highly specific and sensitive EC marker of perinatal and adult lung. The late gestational expression peaking at birth implies that SERT plays a role in pulmonary vessel remodeling near term by lowering serum serotonin level. Because of its defective expression, SERT may be a diagnostic marker for ACD/MPV and thus can be utilized in pediatric pathology practice. We tested SERT promoter length polymorphism as a possible explanation for the lack of SERT expression. All ACD/MPV patients had the L allele, excluding this possibility. Further studies of upstream regulators of SERT promoter region and mutational analysis of SERT gene may unravel key aspects of the pathomechanism that leads to ACD/MVP.

26 Prox-1 is Expressed in Respiratory Epithelium During Development and Identifies Cells with Neuroendocrine Fate

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Background: Prox-1, a prospero-related homeobox gene is well known to play a key role in establishing lymphatic endothelial cell fate. Recent studies, however, have indicated that Prox-1 also controls liver, pancreas, retina and brain morphogenesis. We were interested in determining if Prox-1 contributes to lung airway development.

Design: Sections of human fetal lung (representing all three trimesters, n=7), and postnatal lung (1 and 10 years) with no significant lung pathology, as well as lung sections of patients with neuroendocrine hyperplasia of infancy (NEHI, n=4) were selected and stained with anti-Prox-1, bombesin and serotonin antibodies.

Results: Prox-1 was expressed in respiratory epithelial cells throughout gestation (starting as early as 12 weeks of gestation age) and remained positive in postnatal lung. Prox-1 positive cells were either single (scattered) or in small cell clusters and were more numerous in the early stage of gestation. When compared with the localization of bombesin and serotonin positive cells, Prox1 virtually identified the same cells. Prox-1 picked out the bombesin positive hyperplastic cell clusters of airways that characterize NEHI lungs.

Conclusion: We have established that Prox-1 is expressed in respiratory epithelium during gestation and that these Prox-1 positive respiratory epithelial cells belong to the highly specialized pulmonary neuroendocrine cell system (PNEC). Our results may open up new, Prox-1 signaling related research avenues to gain more understanding of PNEC development. Prox-1, as a nuclear marker for PNEC can be utilized in the pathologic diagnostic workup of NEHI.

27 Screening And Characterization Of Spontaneous Porcine Congenital Heart Defects For Gene Identification And Models Of Human Disease

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Background: Rodent models of human congenital birth defects have been instrumental for gene discovery and investigation of mechanisms of disease. However, these models are limited by their small size making practiced intervention or detailed anatomic evaluation difficult. Swine have similar anatomy and physiology to humans, and their size at birth is comparable to a human neonate.

Design: In a pilot screen, we developed methodology to conduct a now ongoing large scale screen for specific structural birth defects in piglets that are either stillborn or die in the first 48 hours of life or are euthanized for other research purposes. Piglets are from research herds that are non-genetically modified, relatively inbred, and have known pedigree information. Piglets are screened on site for the presence of cleft palate, myelomeningocele, megabladder/hydroureter/hydronephrosis, and congenital diaphragmatic hernia. Hearts are excised and sent to the University of Rochester for detailed examination by pathologists.

Results: Materials from over 950 piglets have been examined and structural birth defects have been identified including cleft palate and megabladder with hydroureter and hydronephrosis. Of significant interest, examination of 623 hearts from one herd revealed 8 congenital heart defects. Of these, 6 had perimembranous ventricular septal defects (VSD), one had an atrioventricular canal defect, and one had a bicuspid pulmonary valve. Two of these piglets were born alive. The six piglets with perimembranous VSD were highly related and shared common ancestors making an autosomal recessive inheritance likely. Two of these had identical defects with marked trabecular extension, and these were farrowed by sister sows and sired by boars derived from a common ancestor.

Conclusion: We have implemented a large scale screen for spontaneous genetic congenital heart defects in swine. To date, we have identified 15 congenital heart defects including one family with potentially heritable perimembranous VSD. Recently available porcine genetic tools will allow for susceptibility locus mapping and candidate gene identification. The detailed anatomic pathological review of inbred animals with newly published genomes is a novel approach to developing genetic models of human disease.

28 The Anatomy of the Posterior Urethra by Optical Projection Tomography

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Background: Optical projection tomography (OPT) is a form of three-dimensional light-based microscopy that employs computerized reconstruction of images acquired at hundreds of levels throughout a specimen. As such, the technique offers several advantages to the study of normal and abnormal development: Specimens up to 1.5 cm in maximum dimension can be examined at a maximum resolution of 3 microns; tissue can be unstained or stained with a wide variety of histochemical stains to further characterize the tissue; specimens remain intact throughout imaging and are thus available for additional forms of examination; OPT is faster, less expensive, and offers higher resolution than micro MRI and can image larger specimens than confocal microscopy.

Thus far, OPT has received little attention by fetal pathologists, but its application has great potential to expand the morphologic understanding of a number of congenital disorders. Bladder outlet obstruction (or lower urinary tract obstruction, LUTO) is a prime example. When fetal urine cannot be expelled from the urinary bladder, oligo- or anhydramnios ensues, setting off a cascade of urinary anomalies and eventual prune belly syndrome. Associated pulmonary hypoplasia is generally lethal in the perinatal period. Anatomic diagnosis of the obstructing lesion is important, in part in order to confirm prenatal diagnoses and determine pathogenesis, but also to permit accurate parental counseling (some forms of bladder outlet obstruction can be familial). Such counseling is often based upon diagnoses reached by prenatal ultrasound, an approach that, while valuable from a clinical viewpoint, is less precise than careful anatomic delineation.

Design: We present a series of normal male urethras collected from mid- to late-gestation and compare the images to specimens associated with bladder outlet obstruction, i.e., urethral atresia or posterior urethral valves. As part of the OPT technology, formalin-fixed specimens were scanned under UV light to capture autofluorescence; images were reconstructed using NRecon software (Skyscan, Belgium) and viewed as virtual sections or in three dimensions using Bioptonics Viewer v2 software.

Results: The efficacy of three-dimensional imaging is apparent, as sites of obstruction are visualized readily. Urethral atresia and posterior urethral valves can be visualized without disrupting the specimen by gross dissection. This is an important issue, as dissection can alter the original anatomy of a valve or valves.

Conclusions: The use of optical projection tomography offers substantial benefits in both research and clinical arenas.

29 Cystic Dysplasia of the Epididymis: a Disorder of Mesonephric Differentiation Associated with Renal Maldevelopment

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Background: The occurrence of congenital epididymal malformations with a cystic component has not been fully characterized; most epididymal cysts occur later in life and are likely acquired. In addition, congenital malformations of the male excretory system are extremely uncommon in fetuses and neonates. Well known examples of excretory duct anomalies include congenital absence of the vas deferens and cystic dysplasia of the rete testis (CDRT), among others, and are frequently associated with renal and/or urinary malformations, mainly renal agenesis. However, epididymal dysplastic changes have not been reported in these cases.

Design: 110 fetal and neonatal autopsies diagnosed with epididymal anomalies and/or renal malformations were reviewed. In addition, an orchiectomy specimen from a patient with renal agenesis was also selected. H&E slides containing testis and epididymis were reviewed for each case, and the morphological findings recorded.

Results: 20 specimens (ranging from 27 weeks of gestation through 10 days of life for the 19 autopsy cases and 4 years-old for the surgical specimen) shared the same spectrum of histological findings in the epididymis characterized by cystic ductal dilation, resulting in dysplastic ducts of variable diameters and irregular shapes, with ill-defined walls. Some cases showed diffuse lesions involving the entire organ, whereas in others the involvement was segmental. Efferent ductules also showed dysplastic features. Except for 1 case with associated CDRT, other anomalies of the male excretory system were not present, and the vas deferens was patent in all cases. 18 cases had either renal and/or urinary tract anomalies, including renal dysplasia (8), pelvicaliceal dilation (8), renal agenesis (4) and hypoplasia (1), ureteral agenesis (2) and hypoplasia (1), urethra and bladder agenesis (2), prostate agenesis (2) and autosomal recessive polycystic renal disease (2).

Conclusion: Our observations led to the recognition of a peculiar congenital lesion characterized by architectural distortion due to aberrantly shaped, cystically dilated epididymal ducts, in a pattern not previously described. Similarly to what is observed in other male genital system anomalies (including malformations of the rete testis, vas deferens and seminal vesicles), most lesions occurred in association with renal and/or urinary tract malformations, suggesting a spectrum of congenital malformations. The shared embryological origin of these structures may explain their simultaneous occurrence, possibly related to disrupted mesonephric duct development. We propose the term cystic dysplasia of the epididymis for this anomaly.

30 Pulmonary Vascular Growth (VG) in Lungs with Developmentally Compromised Pulmonary Blood Flow (BF)

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Background: Proper BF is thought to provide key governing signals for VG. The mature lung receives its blood supply from two vascular systems (pulmonary and systemic) that are interconnected by several preexistent precapillary anastomoses. We aimed to characterize VG in pulmonary sequestration (PS) and pulmonary atresia (PA), conditions that are known to have developmentally compromised pulmonary BF.

Design: Eight PS and 3 PA cases were selected. Review of clinical history with image studies was performed. VG was studied by analysis of gross anatomy and microscopic architecture by HE stain and by immunohistochemistry using specific pulmonary endothelial cell markers such as angiotensin converting enzyme (ACE) that is expressed at 12 weeks of gestation age (WG) and serotonin transporter (SERT) that is active at 30 WG. CD31, SMA and elastic-trichrome (ET) stains were also used. Lymphatic vessel (LV) growth was assessed by Prox-1 and D2-40 immunostains.

Results: The PS cases consisted of intralobar (n=2), and extralobar (4 chest/mediastinum, 2 intraabdominal) sequestrations. All PA was part of tetralogy of Fallot. All 11 lungs had diminished pulmonary BF and prominent systemic vasculature. Based on features related to abnormal VG (malaligned vessels, thick large and/or small arteries, abundance of capillary pericytes, presence/absence of alveolar capillaries and expression of ACE/SERT) we found that VG was most affected in conditions that were located the furthest away from the lungs. VG in abdominalPS were more severe than those in the chest/mediastinum, while VG was less sever in intraPS, and VG in PA lungs were the least affected. LV disturbance (presence of numerous dilated lymphatics) showed a similar trend. The severity of VG was also related to airway development; the more maldeveloped the airways were (back-to back airways with diminished smooth muscle wall) the more defective the pulmonary VG was.

Conclusions: Congenital absence of pulmonary flow in PS and PA provides a unique model to study pulmonary VG. In this model we have identified a tendency that shows a relationship between VG severity and distance of conditions from the lung. This may reflect the availability pulmo-systemic anastomoses (the furthest the condition is the least number of anastomoses are present), which substantiates the need of proper BF for intact VG. The relationship between VG and airway development is not surprising because growth factors secreted by normal airway epithelium (i.e. vascular endothelial growth factor) plays a crucial role in pulmonary VG. Based on the minimal VG abnormality seen in PA, systemic BF appears to substitute pulmonary blood flow to provide signals for adequate pulmonary VG.

Poster Presentations:

31 Geometric Approaches for Cancer Detection and Classification in Pediatric and Adult Lesions Based on Nuclear Structure

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Background: Visual interpretation of histopathology images is the most common method for making accurate diagnoses in surgical pathology. Many lesions, both benign and malignant, can be difficult to distinguish from one another based solely on histomorphological grounds and expensive studies may be required. Image analysis using nuclear features has yielded variable results in the ability to assist the pathologist in distinguishing certain lesions with definitive accuracy. The aim of this study was to explore whether novel mathematical and geometric approaches are superior to feature-based methods for discriminating between differential diagnoses in certain pediatric liver tumors and thyroid tumors based solely on nuclear structure (size, shape, chromatin pattern) from digital images.

Design: Analysis was performed comparing 5 cases of fetal-type hepatoblastoma with normal liver and another analysis between 5 cases each of follicular adenoma of the thyroid, 5 follicular carcinoma of the thyroid, and normal thyroid. Representative fields of Feulgen stained sections from routinely processed tissues were imaged at 1000X magnification. Nuclear segmentation from digital images was performed using a hybrid level set and graph cut method. Classification is performed by quantifying similarities in a group of nuclei to groups of nuclei in a trained database. Group (distribution) distances are computed using novel geometric approaches (based on optimal transportation distances) as well as more commonly used numerical features.

Results: Our geometric approaches are able to automatically classify this data with 100% accuracy using both case-based and randomized blindfolded cross validation strategies. Forty nuclei are needed for perfect classification accuracy. These methods allow for the graphic representation of nuclei in each population (normal vs. hepatoblastoma) over the most discriminating geometric path (provided by the optimal transportation metric). Using these methods we show it is uncommon for hepatoblastoma nuclei, for instance, to have chromatin concentrated around the edge of the nucleus.

Conclusion: We propose a novel computer-assisted method for cancer detection and classification based on quantifying distributions of nuclei over mathematical geometries. Validation of our approach with the test data available demonstrated that our methods can distinguish between normal liver and hepatoblastoma and follicular adenoma and follicular carcinoma with 100% accuracy. In addition, our approach can be used to define unique nuclear "signatures" for individual tissues and lesions.

32 Immunohistochemistry for H2ax Can Help Distinguish Emperipolesis in Rosai Dorfman Disease from Hemophagocytosis in Reactive Lymph Nodes.

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Background: One of the histologic hallmarks of Rosai Dorfman disease (RDD) is emperipolesis, the phenomenon where cells traffic through the cytoplasm of a larger host cell without damage. Hemophagocytosis is when an activated macrophage engulfs other cells ultimately resulting in cell death. Some reactive lymph nodes show sinus histiocytosis and activated macrophages with cells in their cytoplasm. Determining if these cells represent recent hemophagocytosis or emperipolesis is difficult to distinguish on morphologic grounds. The macrophages in RDD express S100. However, some activated macrophages in reactive conditions may also express S100. Therefore, the differential diagnosis of partial involvement of a lymph node by RDD or sinus histiocytosis with hemophagocytosis may be difficult to discern even with the help of the standard immunohistochemical (IHC) markers. A recent marker of apoptosis that works well in standard IHC is H2AX, unlike the other tests, such as the TUNEL assay for apoptosis. H2AX is a member of the histone H2A family, is involved in the nucleosome of compacted chromatin, and is necessary for the maintenance of genomic stability. Therefore, this double stain is useful in identifying DNA damage and apoptotic cells within macrophages.

Design: 10 cases of RDD and 4 cases of hemophagocytosis from Children's Hospital of Philadelphia were reviewed and stained with CD163, H2AX and double stained with CD163/H2AX. A double stain with CD163 and H2AX was used to help distinguish hemophagocytosis from emperipolesis and further evaluate the integrity of the cells in emperipolesis. CD163, the hemoglobin scavenger receptor, is a macrophage marker.

Results: The 10 cases of RDD show only rare H2AX positive cells in the macrophages in 1 of the 10 cases. All other cases of RDD showed no evidence of cell damage in the emperipoletic cells. The HLH cases show evidence of H2AX staining in the hemophagocytosed cells within the macrophages on the double stain.

Conclusion: Emperipolesis is a phenomenon where cells traffic through the cytoplasm of a larger host cell with virtually no damage to the cells. The cells that are hemophagocytosed by macrophages show evidence of cell damage and apoptosis as evidenced with the H2AX stain. A double stain using CD163 and H2AX may be useful in differentiating hemophagocytosis from emeripolesis and is simple to perform unlike other complicated apoptosis assays.

33 Immunophenotypic Comparison of Peripheral Blood (Pb) Versus Bone Marrow (Bm) Blasts in Pediatric Acute Leukemias

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Background: Recent advances resulted in growing reliance on immunophenotyping in the diagnosis and classification of hematopoietic neoplasms. Some AL are now more defined by their molecular genetics than morphologic features, and are being diagnosed primarily based on the immunophenotype of blasts regardless of source (BM, PB or even body fluids). Furthermore, with the advent of targeted therapy, the presence of a single marker may dictate a specific course of management. These advances, while relegating morphology and BM blast percentage to a lesser relevance, appear also to have elevated expectations with regards to obtaining full work-up from specimens other than a BM. Especially in dealing with the very sick or young child, the pathologist is often asked to render a complete diagnostic and prognostic work up on a PB sample. Behind this trend is a desire to spare the patient a procedure, and an intuitive assumption that PB and BM blasts- in the same patient, at a given point of time - are identical. Our review aims to evaluate the immunophenotypic aspects of this assumption.

Design: Records were searched for cases of AL that had immunophenotyping of both PB and BM at the time of diagnosis. Utilizing similar gates for the same patient, blasts positivity values for each of our AL panel markers were compared.

Results: Reviewing data from the past ten years (more than 300 new AL) generated 4 patients who had concurrent immunophenotyping performed on both PB and BM at diagnosis (2 AML and 2 ALL cases). A review of the percentages for positive markers was conducted and Δ values (PB vs. BM) were obtained. A difference of 10 percentage points or more was defined as significant this study. Significant differences were seen in several myeloid, lymphoid and platelets markers in all patients, as follows:

 Patient 1 (AML): CD2, CD7, CD11b, CD11c, CD33, CD34, CD41, CD42b and CD61
 Patient 2 (ALL): CD3, CD33, and HLA: DR
 Patient 3 (AML): CD11b, CD11c, CD19, CD33, CD34, CD45, CD61 and HLA: DR
 Patient 4 (ALL): CD10, CD22, CD41 and TdT

The largest difference was in patient 1 (Δ = 80.8% for CD61).

These differences created challenges in interpretation, which were ultimately resolved by incorporating other findings.

Conclusions: - Blasts from simultaneous PB vs BM source show significant immunophenotypic differences, likely due to more factors than the "wear and tear" of circulating PB blasts. We hypothesize that PB blasts are a subset of BM blasts
- These differences can be big enough to change the interpretation (positive vs. Negative) resulting in diagnostic difficulties. They will be more relevant as future treatments focus on a single marker. A prospective study will help in further elucidation.

34 Hypermethylation and Epimutations at the DLK1-MEG3 Imprinted Domain in Hepatoblastomas

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Background: Hepatoblastoma is the most common hepatic malignancy of early childhood and infancy. These tumors are postulated to arise from hepatoblasts, bipotential hepatic progenitor cells that normally differentiate into hepatocytes and cholangiocytes. Although long recognized as aggressive lesions, particularly those presenting with advanced stage of disease and the small cell-undifferentiated histologic type, no prognostic or predictive molecular markers for hepatoblastomas currently exist. Hepatoblastomas are more common in children with Beckwith-Weidemann syndrome, a disorder of genomic imprinting, and are 40-fold more common in infants born at extreme prematurity. We have previously reported high levels of DLK1 (delta-like homolog 1), an imprinted gene within the Notch family of ligands, in hepatoblastomas. Here we sought to characterize the imprinting status and CpG methylation profile within the DLK1-MEG3 imprinted domain in hepatoblastomas.

Design: Five hepatoblastomas (HB8, HB9, HB25, HB31 with mixed small cell/embryonal and HB40 with teratoid histology) and paired adjacent control non-neoplastic liver were chosen. All specimens were obtained under the approved guidelines of Baylor College of Medicine Institutional Review Board protocol H-13999. Genomic DNA was extracted from snap-frozen tissue, treated with sodium bisulphite to convert unmethylated cytosines to uracils, and the CG4, CG6 and CG7 differentially methylated CpG islands located on chromosome 14q32.2 were amplified by PCR. The amplicons were cloned and 10-15 clones from each specimen were subjected to sequencing. CpG methylation analysis was performed using BiQ analyzer.

Results: All five hepatoblastomas demonstrated striking hypermethylation of the CG4 intergenic differentially methylated island. Average CpG methylation in tumors was 90.2 +/- 4.4% and was significantly different from paired normal liver at 72.8 +/- 5.5% (p = 0.001; two-tailed paired Student's t-Test). More notably, informative SNP genotyping within CG4 revealed biparental hypermethylation instead of maternal hypomethylation in HB8 and HB25. The highest methylation levels were noted in HB25 (95%), a small cell/embryonal hepatoblastoma that was refractory to multiple chemotherapeutic regimens.

Conclusion: Aberrant CpG methylation of the DLK1-MEG3 imprinted domain is common in hepatoblastomas. The effects of hypermethylation and loss of parental-specific methylation signatures on DLK1 gene expression and tumorigenesis need to be addressed.

35 Incidental PTLDs in Surgically Resected Allograft Bowel Specimens

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Background: The early diagnosis of posttransplant lymphoproliferative disorder (PTLD) is important clinically for the successful management of allograft recipients. The usual sequence of events is a rejection episode leading to increased immunosuppression and subsequent rising EBV titers. In most cases the diagnosis of PTLD is made clinically and radiologically with the pathologist's role restricted to subtyping the PTLD for treatment purposes. At Children's Hospital of Pittsburgh (CHP)PTLD was found incidentally in six cases by pathologic examination of surgically resected allograft bowel specimens from intestinal transplant recipients (ITx).

Design: Between 2002 and 2009, 91 stoma revisions or segmental resections were performed at CHP in the ITx population. Incidental PTLD was identified on 5/91 (5.5%)cases in a surgically resected specimen; 3 were stoma revisions. A sixth case, documented a higher grade PTLD than what was previously diagnosed. The demographics of the six patients were reviewed, including age at transplant, EBV status at time of transplant, amount of time to development of PTLD, the type of PTLD, and documentation of rejection episodes prior to the development of PTLD.

Results:

Case	Age	Tx type	EBV status time of tx	Time to PTLD	EBV load at PTLD dx (copies/mL)	PTLD type	Specimen assoc w/ PTLD	Hx rjxn
1	11.5 y	Sml bwl/ colon	Neg	3 m, 17 d	16,000	Mono	Resection	Yes
2	2.1 y	Sml bwl	Neg	3 y, 8 m, 12 d	560,000	Spindle cell tumor	Resection	Yes
3	10.8 y	Sml bwl	Neg	2 m, 22 d	14,000	Poly	Stoma rev	Yes
4	2 y	Multivisc	Neg	7 m, 24 d	12,000	Poly	Stoma rev	Yes
5	7.7 y	Sml bwl	Neg	3 m, 17 d	14,000	Poly	Stoma rev	Yes
6	6.5 y	Sml bwl	Neg	6 m, 4 d	12,000	Mono	Resection	Yes

Of note, while serial EBV PCR studies revealed increasing EBV titers in all six patients, a review of biopsy specimens obtained prior to the PTLD diagnoses identified EBER-positive cells in only two of the six patients.

Conclusions: Posttransplant lymphoproliferative disorder may be discovered incidentally in allograft surgical resection specimens without prior clinical or radiologic documentation of the disease. This study shows that this can be especially significant in patients who have had higher grades of acute cellular rejection early in the transplant course. Appropriate sampling of any allograft resection specimen, especially stoma revisions, is necessary to avoid missing such an important unexpected finding.

36 Focal Form of Congenital Hyperinsulinism: Adenomatous Hyperplasia of Endocrine Cells Inside or Outside the Pancreas. Immunohistochemical Analysis of P57 Expression.

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Background: Congenital hyperinsulinism (CHI) is a rare genetically heterogeneous disorder characterized by profound hypoglycemia due to inappropriate insulin secretion. Two histologically and genetically forms are recognized due to ATP-sensitive K-channel defects: a diffuse form, which involves the whole pancreas and a focal form, with an area of adenomatous hyperplasia within an otherwise normal pancreas. Focal CHI is due to a somatic K-channel mutation on chromosome 11p15.5, generally inherited from the father, and a loss of heterozygosity of the corresponding region on maternal chromosome. In this maternal region resides the P57kip2 gene. This gene is involved in regulation, as inhibitor, of cell proliferation. Focal CHI is curable with limited pancreasectomy.

Design: We present 3 cases of focal CHI: 2 inside the pancreas and 1 with the aspect of a polyp on the posterior surface of the pancreas, simulating an ectopic CHI on F-fluoro-L-Dopa PET. In all cases we analyzed the P57 expression on immuhistochemistry.

Three children, 2 males (10- and 13-month-old) and 1 female (31-month-old) showed a clear areas of increased F-fluoro-L-Dopa PET uptake within the head (2 cases) and, apparently, outside the pancreas, respectively. On the surgery, two children needed several intraoperative frozen sections to ensure complete excision; while in the third case the adenomatous hyperplasia was an exophitic polyp easily identified on naked eye, confirmed on frozen section. Immunohistochemistry for P57 was performed on formalin-fixed paraffin-embedded tissue.

Results: All lesion were composed of large endocrine cells with dispersed abnormal nuclei. In two cases there were satellite lobules in the nearby normal pancreas. On immunohistochemistry, in all samples P57 expression was lost in the lesion, whereas it was normal outside the islets of Langerhans.

Conclusion: CHI is a rare disease. Preoperative diagnosis with F-fluoro-L-Dopa PET distinguishes between diffuse and focal CHI and allows correct surgery. Focal form can be either inside or outside the pancreas. Absence of P57 immuno-staining is consistent with a 11p15.5 loss as the cause of the focal islet cell adenomatous hyperplasia.

37 Altered Cytokine Profile in a Rat Model of Fetal Alcohol Spectrum Disorders

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Background: Alcohol consumption during pregnancy causes fetal alcohol spectrum disorders (FASD), which is linked to early pregnancy loss, intrauterine growth restriction, and impaired placentation. Alcohol impairs the immune response, but its effects on the complex interactions between the feto-placental unit and the maternal immune system, which are necessary for placentation, are largely unknown. In this study, we examined the effects of chronic gestational exposure to alcohol on cytokine expression profiles in placenta, mesometrial triangle (MT-implantation site), amniotic fluid, and maternal serum.

Design: Pregnant Long-Evans rats were fed with isocaloric liquid diets in which alcohol comprised 0% (N= 4) or 24% (N= 8) of the caloric content, starting on gestational day (GD) 6. Blood, amniotic fluid, placentae, and MT were harvested on GD 18. The samples were subjected to a bead-based multiplex ELISA, which included measurement of 11 pro-inflammatory cytokines, 5 anti-inflammatory cytokines, 5 anti-inflammatory chemokines, 1 pro-inflammatory chemokine, and 2 growth factors. Data were analyzed using the Mann-Whitney test.

Results: 24-plex analysis revealed that, in controls, the levels of nearly all analytes measured, except GCSF, IL-1beta, and TNF-alphafz were in higher in placenta as compared to the MT. Gestational alcohol exposure significantly reduced expression of 9 cytokines in the MT, 7 of which were pro-inflammatory in nature (IFN-gamma, IL-1beta, IL-2, IL-6, IL-17, IP-10, and MCP-1). The 2 anti-inflammatory cytokines reduced by ethanol exposure were IL-5 and eotaxin. In contrast, VEGF was significantly elevated in the MT of alcohol-exposed dams. Alcohol exposure had no significant effect on cytokine expression profiles in placental tissue, amniotic fluid, or maternal serum.

Conclusion: Chronic gestational exposure to alcohol alters cytokine expression profiles at the site of implantation. Alcohol-mediated reductions in pro-inflammatory cytokines, which are needed to promote trophoblast implantation, could represent a critical factor in the pathogenesis of impaired placentation in FASD. Moreover, increased VEGF expression could reflect persistence of non-converted maternal vessels associated with ethanol exposure.

38 Diagnostic Utility of Glypican 3 Immunohistochemistry in Identifying Hepatoblastoma Subtypes.

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Background: Hepatoblastomas (HB) are the commonest malignant liver tumors in childhood. Broadly they are classified into epithelial (fetal (F), fetal with mitoses or crowded fetal (CF) and embryonal (E)), mesenchymal, mixed, small cell undifferentiated (SCU) and teratoid. Pure fetal histology is associated with good prognosis while SCU has a poor prognosis and hence are important variants to identify for treatment purposes. In most instances, varying proportions of the above subtypes are intermixed and can pose problems especially differentiating fetal from CF; CF from embryonal and embryonal from SCU and neuroepithelium of teratoid HB. Glypican 3 is a heparan proteoglycan that is overexpressed in hepatoblastomas and hepatocellular carcinomas as shown by us and others and has been shown to be an useful immunohistochemical marker. We believe that glypican 3 staining pattern can be a useful tool in identifying different components of HB.

Design: We reviewed 90 cases of hepatoblastomas that included both biopsies and post-therapy cases. Glypican 3 staining was done in all of them using the Ventana automatic stainer with adequate positive and negative controls. The staining intensity and pattern was graded as negative to 3+, and the pattern was noted as fine granular pericanalicular (apical) or coarse, pericanalicular and diffuse cytoplasmic.

Results:

All fetal and embryonal components showed strong staining in all cases while the staining was negative in small cell component as shown below. In post-treatment cases, it identified any residual epithelial component.

HB subtype	Pattern and intensity of Glypican 3 staining
Fetal areas without mitoses	3+; Fine granular pericanalicular only
Crowded fetal (with mitoses)	3+ Coarse pericanalicular &/or diffuse cytoplasmic
Embryonal	3+ Coarse diffuse or intense homogeneous cytoplasmic
SCU	Negative
Mesenchymal	Negative
Biliary/cholangiolar	Negative
Macro-trabecular	Variable fine or diffuse, either F or E pattern

Conclusions: The pattern of glypican 3 staining appears to differentiate areas of fetal HB without mitoses from fetal HB with mitoses (CF) and embryonal areas; and embryonal from SCU and neuroepithelial areas of teratoid HB. This is of particular use in small biopsies where typing can be difficult and where identification of SCU areas may be important prognostic indicator. Identification of pure fetal (fetal without mitosis) subtype at resection would also eliminate the need for additional chemotherapy. We recommend Glypican 3 be made an essential component of any IHC panel for diagnosis of HB.

39 Development of a Web Based, Internationally Certified 'Encyclopedia' of Pediatric Heart Disease.

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Background: Structural study & classification of heart specimens are essential in Pediatric Cardiology and anchor the understanding and management of heart disorders. A reproducible nomenclature is crucial in the description of the various cardiac diseases and allows meningful analysis. We describe the effort of the International Society for Nomenclatures of Pediatric and Congenital Heart Disease (ISNPCHD) to acquire images illustrative of pediatric cardiac disorders and organized using the various coding terminologies available to the ISNPCHD.

Design: At the 2008 annual meeting, the ISNPCHD installed the Archiving Working Group (AWG) to develop a web based 'encyclopedia' utilizing the International Pediatric and Congenital Cardiac Codes (IPCCC). Over the past decade, heart specimens from All Children's Hospital & other Florida institutions, have been examined and catalogued primarily under the direction of one of the authors (DS). In the AWG's editorial process, images are identified and linked to appropriate IPCCC codes. When available, a set of short concise definitions are also linked. These are organized using the IPCCC structure and subsequently reviewed for accuracy, diagnostic value and quality by a group of international cardiac pathology, surgery and imaging experts. Once the certification process is completed, the images are posted to the AWG web site (ipccc-awg.net) for review.

Results: Progress in management of pediatric heart disorders, has been made possible by understanding the impact of form on function. As imaging techniques continue to improve, it has become increasingly useful to understand the often confusing & complex anatomic relationships in order to develop an appropriate therapeutic plan. This remains rooted on the impact of abnormal anatomy on cardiac physiology, which requires examination of all available heart specimens. This web based encyclopedia accomplishes these goals.

Conclusion: The use of a common terminology is mandatory to understand the outcomes of diagnostic & therapeutic interventions. This project is international in scope and inclusive of all who are interested in childhood cardiac diseases. By combining pertinent specimen images with appropriate nomenclature, a comprehensive and certified encyclopedia will advance the understanding, diagnosis and classification of congenital and acquired forms of pediatric cardiac disease.

40 Pediatric Insulinomas and Focal Congenital Hyperinsulinism: Two Distinct Beta-Cell Proliferations Both with LOH of Chromosome 11p

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Background: Insulinoma is a rare cause of hypoglycemia in older children, while congenital hyperinsulinism (HI) is more common and leads to persistent hypoglycemia in infants. In HI the histologic pattern, focal or diffuse, is related to the inheritance pattern of the mutation in ATP-sensitive potassium channels in abnormal beta cells. Focal HI is associated with maternal loss of genes on chromosome 11 in the region of p57. Although both focal HI and insulinoma are beta cell proliferations associated with pediatric hypoglycemia, their histologic, immunohistochemical and molecular features have rarely been directly compared.

Design: Eight cases of pediatric insulinoma were identified at our institution from 1971-2008. These tumors and 8 cases of focal HI were examined and histologic features recorded. All lesions were immunostained for chromogranin, synaptophysin, insulin, glucagon, somatostatin, pancreatic polypeptide (PP), Ki67 and p57. LOH was confirmed via microsatellite marker analysis of tumor versus normal tissue for markers surrounding the KATP locus on chromosome 11p.

Results: Insulinomas showed various histologic patterns with loss of the normal lobular architecture of the pancreas. In contrast, focal HI retained a lobular architecture and included intralesional ducts and acini. Other features of insulinomas but not focal HI included a fibrous capsule and prominent fibrous bands (7 of 8) and rare mitotic figures and amyloid (2 of 8). Scattered nucleomegaly, noted in all focal HI cases, was seen in 2 insulinomas. Both lesions were primarily composed of beta cells. Focal HI also demonstrated alpha, delta, and PP-positive cells at the edges of the lobules, while insulinomas contained only rare cells of other types. Insulinomas had variable Ki67 immunoreactivity (0-10%); focal HI had consistently low levels of proliferation (<1% Ki67+ cells). All cases of focal HI and all testable insulinomas (7 of 7) were negative for p57. All insulinomas tested (6 of 6) also showed LOH for markers on 11p by microsatellite analysis.

Conclusion: Insulinoma, a neoplasm, and focal HI, a developmental/inherited lesion, are clinically and morphologically distinct. However, both focal HI and insulinoma demonstrate negative p57 immunostaining and LOH on chromosome 11p, suggesting a possible common pathogenetic bond between these two beta cell proliferations.

41 Pathology of the CNS in Hypoplastic Left Heart Syndrome (HLHS)

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Background: In HLHS oxygen delivery to the brain is dependent on retrograde blood flow from the patent ductus arteriosus through the hypoplastic aortic arch to reach the carotid arteries. Restricted flow through the hypoplastic aortic arch may result in decreased cerebral blood flow, and cause hypoxic/ischemic injury during the prenatal and/or postnatal period. Central nervous system pathology has not been extensively described in HLHS. The purpose of this study was to characterize central nervous system hypoxic/ischemic injury in infants with HLHS, and determine whether these injuries occur in the prenatal period, postnatal period, or both.

Design: The study included 15 infants with HLHS (10 males; 5 females; mean age of 122 days, with a range of 0 to 210 days). 13 were delivered prematurely with a mean gestational age of 34.5 weeks (range: 33 to 36 weeks). The first stage of the 3- stage hybrid repair had been performed in 8 patients, and 3 other patients had undergone the second stage repair. Three of the patients had chromosomal abnormalities: del9q, Turner's and 45 XO/XY (Mosaic Turner's). 10 standard sections of the brain were examined for chronic changes such as gliosis, subacute changes such as mineralization, and acute changes such as pyknotic/eosinophilic neurons and choroid plexus hemorrhage. The sections were examined for the various stages of periventricular leukomalacia. The presence of a neuronal migration disturbance (nests of primitive cells in the cerebral white matter) was also assessed. To assess gliosis we used the following scheme: mild, <25% reactive astrocytes/region on glass slide; moderate, 25–75% reactive astrocytes/region on glass slide; severe, >75% reactive astrocytes/region on glass slide. A GFAP immunostain was performed in the patients with less than 15 day survival.

Results: Gliosis of the frontal white matter was found in all infants (mild to moderate in 11, severe in 4). Diffuse subpial gliosis of the midbrain colliculi was seen in 14 and subependymal gliosis in 3 infants. Additional pathologic findings included perivascular calcification of basal ganglia or cerebellum (n=7), choroid plexus hemorrhage (n=4) neuronal migration disturbances (n=7), and periventricular leukomalacia (n=3). Acute change in the form of pyknotic neurons were seen in 8 cases. Recent subdural hemorrhage was seen in 1 and subarachnoid hemorrhage in 2. In 5 infants that died within 15 days of birth, there was severe frontal gliosis in 2, subpial gliosis in 4, perivascular calcification in 1, migration disturbance in 3, and heterotopic cerebellar grey matter in 2. None of the cases showed spinal cord abnormalities.

Conclusions: Chronic hypoxic ischemic injury in the form of gliosis was found in all infants with HLHS. The presence of chronic hypoxic ischemic injury in infants less than 15 days of age, in addition to the presence of neuronal migration disturbance in 3 of these infants, provides evidence of an injurious process that likely began intrauterine. Acute hypoxic ischemic injury was seen in 53% of the cases. Reduced cerebral blood flow resulting from the hypoplastic aortic arch likely predisposes to both the acute and chronic neuronal injuries seen in cases with HLHS.

42 A Premature Stop Codon Results In Fibulin-4 Deficient Monozygotic Twins With Cutis Laxa, Arterial Tortuosity, And Multiple Fractures

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Background: These are male infants of a size consistent with the EGA of 31 weeks. The mother of the twins was a 27-year-old G4P3003 with limited prenatal care who presented for cesarean delivery. Resuscitation efforts were initiated and continued until the infants became asystolic. Postmortem radiographs showed innumerable fractures of the limbs, thorax, and head. These fractures were in various states of healing with callus formation. Despite the fractures the growth of the long bones was not stunted. The radiographic findings were initially thought to represent osteogenesis imperfecta type IIC. However, there were also vascular anomalies not explained by this phenotype. Grossly, all arteries were elongated, thickened, and tortuous. The carotids, descending aorta, and iliac arteries were redundant to such an extent that they produced corkscrew patterns. There was also cutis laxa with loose, redundant skin over the entire body.

Design: Analysis of cultured fibroblasts showed that all Collagen genes were normal. Dasouki et al. (2007) published a case with similar findings and a missense mutation in the fibulin-4 gene. Fibulin-4 gene sequencing on the present case was then undertaken.

Results: A homozygous mutation, deletion of a single G in exon 1, resulted in a premature termination codon (PTC) in exon 2. This mutation leads to an efficient nonsense-mediated mRNA decay which results in a homozygous "null" phenotype. No fibulin-4 protein is produced.

Conclusions: It seems clear that the main cause of this phenotype is the absence of fibulin-4 in the extracellular matrix. This phenotype is observed in homozygous missense mutations. One of the major consequences of this genetic defect is the absence of elastic fibers seen in multiple tissues after performing elastic staining in our case. Further investigation is needed to understand how the deficiency in fibulin-4 or the lack of elastic fiber development results in bone fractures. This represents the first reported cases and corresponding complete autopsy findings in fibulin-4 "null" phenotypic infants.

43 SF-1 Gene Mutation Related 46,xy Disorder of Sexual Differentiation: Pathological Report of 2 Cases

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Background: 46,XY DSD (male pseudohermaphrodism) is an extremely heterogeneous and challenging group of DSD. 46,XY DSD may be related to androgen biosynthesis or androgen receptor deficiencies. Steroidogenic Factor-1 (SF-1), an orphan nuclear receptor is essential for adrenal and gonadal development.

Design: We report the pathological datas of two SF-1 mutation related 46,XY DSD.

Results: The first case, a 15 month old girl was addressed because of clitoromegaly. Clinical examination showed hypertrophy of the clitoris, fused genital folds with one orifice and gonads in the inguinal region on the right side and in the genital fold on the left side. Carytype was 46;XY. Genitography and US showed a vagina without uterus. On biological tests, AMH, inhibine B, FSH, cortisol and ACTH were within normal range whereas testosterone level was low with a low response after HCG stimulation suggestive of testicular dysgenesis. Surgical reduction of the clitoris and bilateral gonadal excision were performed. Pathological examination showed 2 symmetrical normal developed testis measuring 1 cm each. Under the microscope, they contain numerous seminiferous tubules with normal immature Sertoli cells and rare germ cells. The presence of large, vacuolated, interstitial, Leydig cells suggested a blockade of testicular steroid synthesis. Genetic studies showed a heterozygous non sense mutation in exon 5 of SF-1 gene.

The second case, a 22 month old girl was addressed because of moderate clitoris hypertrophy and normal vulva. US showed an uterus and a vaginal cavity. Caryotype was 46;XY. On biological tests, AMH was low, FSH elevated, cortisol and ACTH in normal range. Testosterone was low. Vaginoplasty and bilateral gonadectomy were performed. Pathological examination showed 2 symmetrical testis measuring 1,5 cm. Histologically, numerous seminiferous tubules with normal immature Sertoli cells and no germinal cells were observed. In the interstitum, large, clear, vacuolated Leydig cells suggested a metabolic storage disease consistent with abnormal testosterone biosynthesis. Genetic testing identified a heterozygous SF-1 mutation in exon 6.

Conclusion: Numerous, large, clear, vacuolated Leydig cells in the interstitium of normal immature testis in a context of 46,XY DSD with Müllerian structures even with normal adrenal function should suggest a defect in testosterone synthesis and should oriented the genetic screening for SF-1 mutation.

44 Anti-stabilin-1 Immunohistochemical Staining in Juvenile Xanthogranuloma, Dermatofibroma and Other Lesions

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Background: Stabilin-1 is a scavenger receptor present on lymphatic and sinusoidal endothelium. Anti-stabilin-1 (STAB1) immunohistochemical staining has been reported to be highly sensitive and specific for juvenile xanthogranuloma (JXG). JXG is a non-Langerhans cell histocytosis with varying morphologic features that may mimic other lesions including dermatofibroma (DF). A commonly used immunohistochemical marker, anti-factor XIIIa, is reactive with both JXG and DF while macrophage rich DFs, like JXG, may appear CD68 positive making the distinction between the 2 lesions difficult. Our study systematically tested the utility of STAB1 in discriminating JXG from DF and other lesions.

Design: We reviewed the histology of cases signed out either as JXG or in which JXG was considered in the differential diagnosis. JXGs were classified into early (lesions with small cells with moderate eosinophilic cytoplasm) or late (rich in xanthomatous or spindle cells). All cases were stained with STAB1 (Sigma Prestige Antibodies, St Louis, MO), factor XIIIa and CD68. STAB1 staining was considered positive if greater than 10% of lesional cells revealed finely granular cytoplasmic staining. Factor XIIIa and CD68 were considered positive when they showed coarsely granular cytoplasmic staining. Staining was further assessed to be weak, moderate or strongly positive (1+, 2+, or 3+, respectively). Staining in JXGs was compared to non-JXGs.

Results: 15 cases were JXG while 13 were DF, 1 a hypertrophic scar and 1 a cellular blue nevus. The findings of the comparison are summarized in Table 1.

Table 1. Results of STAB1, Factor XIIIa and CD68 staining in lesional cells of JXG, DF and other lesions

	JXG (n=15)	DF and others (n=15)
STAB1	15	0
Factor XIIIa	14	3
CD 68	15	0

STAB1 appeared to be slightly more sensitive for JXGs when compared to Factor XIIIa . However, STAB1 appeared to also stain scattered macrophages non-JXGs lowering its specificity. The 3 factor XIIIa positive non-JXG were all DFs with STAB1-negative lesional cells. Six late JXGs showed much stronger (2+ to 3+) STAB1 staining and weak (1+) factor XIIIa staining. Although no DFs had CD68-positive lesional cells all 13 cases had many scattered CD68-positive macrophages.

Conclusion: STAB1 immunohistochemical staining appears to be a sensitive marker for JXG that may be particularly useful in late cases with weak or equivocal factor XIIIa staining. Specificity, however, is somewhat lower due to reactivity with macrophages in non-JXG cases. A larger study will determine if STAB1 is a good discriminator of JXG from non-JXG.

45   Immunohistochemistry For Phosphorylated Histone H3 And Cleaved Caspase3 As A Tool For Assessment Of Mitosis-Karyorrhexis Index In Neuroblastomas

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Background: Mitosis-Karyorrhexis Index (MKI), the number of mitoses and karyorrhectic nuclei per 5000 neuroblastic cells, is used in pathology prognostic classification of neuroblastomas. It is calculated by counting 5000 cells on routine H&E-stained sections, a laborious and time-consuming process. Phosphorylated histone H3 (PHH3) and cleaved caspase3 (CC3) are protein markers of mitosis and apoptosis, respectively, and can be detected by immunohistochemistry (IHC). Cells highlighted by IHC stains may be easier to count and lend themselves to automated counting. The purpose of this study is to correlate IHC analysis for PHH3 and CC3 with conventional MKI in neuroblastomas.

Design: Sixteen neuroblastomas (12 poorly differentiated and four differentiating) were retrieved from the pathology files of Children's Medical Center, Dallas, TX. All H&E-stained sections of each case were reviewed and a representative section selected for the study. IHC for PHH3 and CC3 was performed by standard immunoperoxidase methods on an automated immunostainer (Ventana, Tucson, AZ). Counting for conventional MKI and manual counts of PHH3 and CC3 were performed in areas away from necrosis, calcification, hemorrhage, and lymphocytic infiltrates. In the same areas, automated counts of PHH3 and CC3 were performed using an image analysis system (ACIS III, Dako, Carpinteria, CA). Pearson's correlation test was used to compare conventional MKI with manual and automated counts of PHH3 and CC3.

Results: Conventional MKI showed significant positive correlation with manual PHH3 count (r=0.93, p<0.0001), automated PHH3 count (r=0.85, p<0.0001), manual PHH3+CC3 count (r=0.93, p<0.0001), and automated PHH3+CC3 count (r=0.87, p<0.0001). Conventional MKI showed significant but weaker correlation with manual CC3 count (r=0.78, p=0.0004) and automated CC3 count (r=0.53, p=0.038).

Conclusion: IHC for PHH3 and CC3 shows strong significant positive correlation with conventional MKI. We also demonstrate that automated counting of PHH3 and CC3 stains is feasible and shows strong significant positive correlation with conventional MKI. These findings should be validated in larger studies. Automated analysis of IHC-stained sections may reduce inter-observer variability and may become especially useful as electronic storage and transmission of pathology images play greater roles in diagnosis and research.

46   Eosinophilic Infiltration in the Duodenal Mucosa of Russian Pediatric Patients Presenting with Recurrent Abdominal Pain.

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Background: There is a relative dearth of data on the normal number of eosinophils in the pediatric duodenum. The development of accurate threshold values for the abnormal is complicated by variations that may occur due to geography and the industrial development of a country. The purpose of this study was to document the average number of eosinophils in the duodenum in northern Russia and compare it with the already known values in the US.

Design: Duodenal mucosal biopsy specimens from 115 children presenting with recurrent abdominal pain to St. Petersburg pediatric hospital between 2003-2004 were retrospectively reviewed. Clinical information was obtained for each patient. Slides were evaluated for the villous to crypt ratio, lymphoid hyperplasia, composition of inflammation in the lamina propria, and eosinophilic inflammation, including eosinophil peak count, average number of eosinophils in the lamina propria per 10 high power fields, eosinophil degranulation and epithelial damage.

Results: The mean age of children (65M, 50F) in this study was 10.4 ± 4.5 years. The working clinical diagnoses prior to biopsy were celiac disease (27), malabsorption (26), chronic duodenitis (14), superficial gastroduodenitis (8), Crohn's (1), normal (3), and recurrent abdominal pain (36). Morphologic diagnoses after biopsy were normal duodenum (66), mild chronic non-specific inflammation (24), active chronic duodenitis (5), celiac (3) and increased eosinophils (17), including two cases of eosinophilic duodenitis. The average number of eos/hpf was 6.1 ± 7.1 with a median of 4.33 and range between 0 and 69.

Conclusion: Only two cases among all analyzed showed unequivocal eosinophilic duodenitis. Our preliminary impression is that of lower prevalence of eosinophilic entities in northern Russia in comparison with the US. Our findings of 6 eos/hpf in the Russian pediatric population represent a discrepancy in relation to the US number of 16 eos/hpf, which emphasizes the need to tailor the diagnosis of eosinophilic duodenitis by region.

47 CD31 And Ki-67 Are Useful Markers to Distinguish Well-Differentiated Fetal Hepatoblastoma From Normal Liver

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Background: Hepatoblastoma is the most common malignant liver tumor in early childhood. It is composed of epithelial and mesenchymal elements in varying proportions. The epithelial element recapitulates hepatocyte development from the primitive blastema through embryonal hepatocytes to fetal hepatocytes. Fetal hepatoblastoma most closely resembles normal liver and sometimes it is difficult to distinguish the two, especially in small biopsy samples. Few previous reports indicate an increased staining for vascular markers in hepatoblastoma. Hepatoblastoma, being a neoplastic process should have a higher proliferative activity, which can be useful to distinguish fetal hepatoblastoma from normal liver tissue. We studied the utility of vascular marker CD31 along with Ki-67 in diagnosis of hepatoblastoma.

Design: Archived H&E stained slides of hepatoblastoma diagnosed at our institute were selected. Total 35 cases were rewieved. Representative blocks with tumor including fetal component (N=30), embryonal component (N=26) and the adjacent normal liver (N=24) were immunohistochemistry stained with CD31 and Ki-67. For CD31 stain, the number of positive sinusoids were counted at 40x objective and reported as number of positive sinusoids per high power field. For Ki-67, the areas with highest nuclear staining were counted and reported as percentage. All slides were reviewed by three pathologists independently. The results were compared by unpaired t test.

Results: CD31 stain was absent to minimal in normal liver sinusoids (2.83 ± 1.24, Mean±SE), but significantly increased (uniform staining patten) ($P<0.0001$) in fetal (28.07 ± 1.76) and embryonal components types (33.00 ± 3.00) compared to normal liver. Ki-67 was low in the normal liver ($2.21\pm0.41\%$), and significantly higher ($P<0.0001$) in both fetal ($21.23\pm3.74\%$) and embryonal components ($58.65\pm4.03\%$). Though there were 5 cases of fetal component with minimal Ki-67 activity.

Conclusion: CD31 stain is uniformly overexpressed in the tumor sinusoids compared to normal liver. Tumor cell proliferative index as assessed by Ki-67 is significantly increased in hepatoblastoma, especially in embryonal component. CD31 and Ki-67 are both useful markers to distinguish between normal liver and fetal component hepatoblastoma, and CD31 is a better marker, since Ki-67 may not increase in fetal component of a few cases of hepatoblastoma.

48   Long Terms Effects of Biologic Therapies on Clinical and Histological Scores in Children

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Background: There is much controversy over the ideal therapy for inflammatory bowel disease. There has been some indication that the new therapies will lead to complete mucosal healing. It is proposed that achieving mucosal healing will allow for a greater chance of changing the natural course of the disease and reducing the risk of development of fistulizing disease, surgery and cancer. Many studies have been done looking at clinical remission with various therapies but there are limited studies evaluating the long-term effect of therapies on histological healing. Many studies have confirmed good clinical response and few adverse effects with long term use Infliximab. The aim of this study was to review the effects of Infliximab on histological changes seen in children with inflammatory bowel disease after at least two years of therapy.

Design: This was a retrospective review of patient charts and histology slides from endoscopic biopsies. Charts were reviewed for all those children with inflammatory bowel disease treated with Infliximab at our institution for more than two years. The charts were further reviewed to focus on those children who had intestinal biopsies before starting Infliximab and than again at least two years from starting Infliximab therapy. Clinical disease activity scores were determined, using a standardized scoring system before Infliximab therapy and again at least two years after starting Infliximab. The pathology slides were assessed by a board certified pediatric pathologist and scored according to a previously reported scoring system.

Results: There were 16 patients who met our inclusion criteria. The average disease activity score at diagnosis was 39. The average disease activity score was 9.5 at after an average of 50 months of Infliximab therapy. All patients received scheduled Infliximab every 4 to 8 weeks during the time period between biopsies. The histological scores were compared by section. The colon and terminal ileum showed significant improvement ($p=0.009$ and $p=0.023$, respectively). The duodenum, stomach and esophagus had improvement in most patients but the difference was not significant.

SITE	n	BEFORE	AFTER	p
Colon	15	176	81	0.0009
TI	7	55	5	0.023
Duodenum	12	28	6	0.212
Stomach	12	773	43	0.057
Esophagus	11	11	7	0.24
TOTAL SCORE		347	142	

Conclusion: We conclude that prolonged therapy with Infliximab in children with inflammatory bowel disease allows for significant clinical and histological improvement in disease activity. A randomized prospective study would be ideal to confirm these conclusions.

49   Does Developmental Relationship between Arterial and Neuronal Growth in the Lung Exist?
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Background: Ample studies have targeted airway smooth muscle innervation but little data exist on the relationship between pulmonary arterial and nerve development. Recently vascular endothelial growth factor (VEGF) has been proposed to act as neuroprotective and neurotrophic factor supporting neuronal growth and regeneration. The aim of this study was to analyze the relationship between pulmonary arteries and nerves during lung development and in pathologic conditions where pulmonary vascular development is interrupted.
Design: Cases of ante- and postnatal lungs (n=10) with no significant pathology, as well as eight cases of pulmonary sequestration (PS) were selected. All sections were stained with H&E and with antibodies to neuron specific markers such as S-100 and PGP 9.5.
Results: S-100 and PGP 9.5 have identified mainly single cells around the developing airways as early as 12 weeks of gestational age (WGA). At about 18 WGA small nerve plexi mostly located around large airway were seen. Smaller nerve units were noted mainly in the collagen collar of developing arteries with a rare unit inside the arterial smooth muscle wall. This pattern is maintained through gestation and postnatally. In PS S100 and PGP 9.5 highlighted multiple enlarged nerve units around arteries and diminished staining was seen around the airways.
Conclusion: Our results indicate a relationship between developing pulmonary arteries and nerves because in PS where vascular development is known to be interrupted, neural growth was also affected and abnormally developed nerves showed close architectural relationship with pulmonary arteries. We hypothesize that in PS improper VEGF signaling plays a role in defective vascular growth in one hand and in abundant neural growth (by failing to provide appropriate axonal guidance) in the other. Targeted studies with VEGF and its receptors are needed to further explore a developmentally regulated relationship between nerves and vessels in the lung.

50   Extension of Calretinin Positive Neural Fibers into the Aganglionated Zone of Hirschsprungs™ Disease Cases

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Background: Hirschsprungs disease (HD) is a congenital malformation that is almost exclusively detected in the neonatal period. HD is a disorder of the motor neurons, in which there is a defect in the migration pathway of neural crest cells to colonize the myenteric and submucosal plexuses. This subsequently leads to aganglionated segments of colon, which is frozen in a hypertonic state resulting in obstruction. Recent publications have utilized immunoshistochemical (IHC) staining for Calretinin in order to reliably identify ganglion cells and their neural fibers in the setting of suction rectal biopsies demonstrating sensitivity and specificity. Here, we propose to examine the transition zone and the extent of Calretinin positive fibers in lamina propria the submucosal/myenteric plexuses to determine if calretnin immunoreactivity mimics the presence ganglion cells.

Design: Our initial study included a total of 7 recent full or partial colonic resection cases for HD, which were selected from the files at the University of Virginia Department of Pathology. IHC staining for Calretinin (DAKO, Clone:DAK-calret1) was performed on the transition zone sections as well as proximal and distal sections. The sections were examined for the presence and extent of calretinin positive neural fibers and ganglion cells. Internal staining of mast cells along with the ganglionated portion of the specimen served as controls.

Results: Calretinin stained sections were examined from the selected cases and demonstrated intense staining of the ganglion cells as well as the neural fibers in close proximately to the ganglion cells. Calretinin staining of neural fibers was maintained but decreased in intensity as one progressed away from the ganglionated portion of the specimen. Specifically, the lamina propria was the first portion to lose staining with Calretinin positive neural fibers extended an additional 9.4mm(mean), range 5-17mm, beyond the last identified ganglion cell, while the Calretinin positive fibers within the submucosal/myenteric plexus extends an average of 22.5mm (range 14-33mm).

Conclusion: Our results support the usage of Calretinin as a marker for ganglion cells and their fibers. However, we demonstrate that the Calretinin positive neural fibers can extend up to 33mm into the aganglionated portion of the specimen. This may prohibit the use of rapid IHC to intraoperative identify a transition zone or potentially complicate the utilization of Calretinin IHC stain for examination of ultrashort Hirschsprungs™ cases

51 Measurement of Bombesin Using A Competitive Fluorescent Microsphere Immunoassay

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Background: Neuroendocrine Cell Hyperplasia of Infancy (NEHI) is a recently described pediatric lung disease of unknown pathogenesis. Histologic diagnosis of the disorder relies on the only consistent morphologic finding seen on lung biopsy, an increased proportion of neuroendocrine cells within the epithelium of distal airspaces, best demonstrated by bombesin and serotonin immunohistochemistry. Objective quantification of this increase by morphometric analysis is time-consuming and difficult to translate to general surgical pathology practice. In order to facilitate the diagnosis of NEHI, we have begun to develop a competitive fluorescent microsphere immunoassay (cFMI) to measure bombesin in limited quantities from biological specimens.

Design: Fluorescent microspheres were coated covalently with purified bombesin and used to compete for binding to bombesin antibody. Strepavidin R-phycoerythrin was used as the fluoresecent reporter and the intensity of the fluorescent signal was quantified using the Luminex100 flow analyzer. The assay was calibrated with known concentrations of purified bombesin, and then used to measure the bombesin concentration in protein extracts from tissue samples and urine.

Results: A linear relationship was obtained between fluorescence and known concentrations of bombesin, which ranged from 10 pg/ml to 1000 pg/ml. The concentrations of bombesin in protein isolated from a ganglioneuroma and 2 non-NEHI lung samples ranged from 50 to 500 ng/mg protein, which is comparable to the quantity of bombesin reported in biological samples by other methods. Bombesin (500 ng/mg of creatinine) was detected in the urine of a patient with neuroblastoma. Consistent with immunohistochemical studies, no bombesin was detected in the liver.

Conclusion: cFMI appears to be a sensitive and specific method to measure bombesin from small amounts of tissue and urine, and likely serum. cFMI may provide a rapid, less invasive method to diagnosis NEHI, which could be widely implemented. Unlike the conventional ELISA the Luminex system has the capacity to measure multiple analytes from one sample, providing a valuable tool to interrogate potential pharmacologic targets for this disorder.

52 The Pathology of Metachondromatosis

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Background: Metachondromatosis (MC) is a rare hereditary exostosis/ enchondromatosis syndrome first described by Maroteaux in 1971. The exostotic component, unlike osteochondroma (OC), is more common in the digits, oriented on an axis towards the vicinal joint, and may regress spontaneously. The endostotic component most often occurs in the iliac crest and long bones. We have recently mapped MC to a locus that does not overlap with EXT1&2, genes responsible for OC. The histopathology has not been detailed and has been described as identical to OC.

Design: We identified 16 MC lesions (MCs) excised from 3 patients followed at our institution in the last 20 years. Diagnoses were clinically established and all patients lacked EXT1/2 mutations. Clinical histories, imaging, and pathology were reviewed and the pathology was compared with 50 sporadic and hereditary OCs from age-matched controls.

Results: The majority (11/16) of MCs were excised from the digits of the hands and feet and 5 were from the long bones of the lower extremity. Intact lesions ranged from 0.9-5 cm. Unlike metaphyseal-based OC, excised exostotic MCs (15/16) involved the metaphyseal-epiphyseal junction (11/15), or were exclusively epiphyseal (4/15) with nearly all subperiosteal. The 1 endostotic MCs was from the distal femoral epiphysis. All patients had additional endostotic and exostotic lesions that that were not removed.

The histologic features of MCs were unique. All exostotic lesions were covered by a fibrous capsule and most (14/15) had only a partial hyaline cartilaginous cap. Like OC, the cap was more disorganized than a normal growth plate with prominent hypertrophic chondrocytes and incomplete zonation. Unlike OC, the majority of cartilage in MCs (14/15) was a core of disorganized hypertrophic chondrocytes. Mild hypercellularity and binucleate chondrocytes were common, but significant atypia and clustering were not. Possible entrapped growth plate was found in some cases. Myxoid degeneration of the matrix with areas of necrosis was common in MCs and focally present in the "metaphyseal" zone of OC. Endochondral ossification occurred at the periphery of the lesions, similar to that which occurs in enchondroma. The endostotic lesion appeared similar to the core of the exostotic MCs, but with more prominent peripheral endochondral ossification.

Conclusion: MC is clinically, genetically, and pathologically distinct from multiple OC syndrome and enchondromatosis. The highly unusual pathology incorporates features reminiscent of both osteochondroma and enchondroma.

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