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

1 TLE1 Immunohistochemistry for Diagnosis of Pediatric Synovial Sarcomas
   AF Lee, CH Lee, LM Sullivan, O Popescu. University of British Columbia, Vancouver General Hospital, and Children's and Women's Hospital of British Columbia, Vancouver, BC; The Children's Hospital of Philadelphia, Philadelphia, PA

   **Background:** Synovial sarcoma (SS), a high-grade sarcoma seen in young adults and adolescents, is characterized by t(X;18)(p11.2;q11.2). SS can be monophasic or biphasic in appearance. The monophasic variant has a histologic differential including a wide range of spindle and small round blue cell neoplasms. TLE1 was found in gene-expression profiling studies to be highly upregulated in SS and initial reports showed it was a sensitive and specific marker for SS. However, a subset of rhabdomyosarcoma (RMS) and peripheral nerve sheath tumors are also immunoreactive. Its expression in pediatric tumors is not fully characterized. We therefore performed TLE1 immunohistochemistry on various pediatric spindle/round cell tumors to address TLE1 immunoreactivity in this patient population.

   **Design:** Pathology files at Children's and Women's Hospital of BC, Vancouver General Hospital, and The Children's Hospital of Philadelphia were searched for formalin-fixed, paraffin embedded pediatric tumors to create 3 tissue microarrays containing 24 SS (19 molecularly confirmed), 83 RMS, 21 Ewing sarcoma, 4 infantile fibrosarcoma, 17 desmoid fibromatosis, 6 digital fibromatosis, 9 fibrous hamartoma of infancy, 3 fibromatosis coli, 13 hepatoblastoma, 7 myofibroma/myofibromatosis, 11 neuroblastoma, 5 superficial fibromatosis, and 25 Wilms tumor. Whole mount sections from 5 cases of molecularly confirmed SS were also obtained. All cases and an external positive control (molecularly confirmed adult SS), were stained oncurrently with anti-TLE1 antibody. Immunostaining was scored as 0 (no nuclear staining); 1+ (weak-moderate staining in < 50% of tumor nuclei); 2+ (weak-moderate staining in 50% or greater of tumor nuclei) and 3+ (strong staining in > 10% of tumor nuclei). Cases with 2+ or 3+ TLE1 staining were considered positive.
Results: Table 1. Pediatric mesenchymal tumors with positive TLE1 immunoreactivity.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>2+ or 3+ Staining</th>
<th></th>
<th>3+ Staining Only</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>Number</td>
<td>%</td>
<td>Number</td>
</tr>
<tr>
<td>Synovial Sarcoma</td>
<td>71</td>
<td>17/24</td>
<td>63</td>
<td>15/24</td>
</tr>
<tr>
<td>Monophasic</td>
<td>91</td>
<td>10/11</td>
<td>91</td>
<td>10/11</td>
</tr>
<tr>
<td>Biphasic</td>
<td>57</td>
<td>4/7</td>
<td>29</td>
<td>2/7</td>
</tr>
<tr>
<td>Unclassified</td>
<td>50</td>
<td>3/6</td>
<td>50</td>
<td>3/6</td>
</tr>
<tr>
<td>Rhabdomyosarcoma</td>
<td>7</td>
<td>6/83</td>
<td>4</td>
<td>3/83</td>
</tr>
<tr>
<td>Alveolar</td>
<td>13</td>
<td>5/38</td>
<td>5</td>
<td>2/38</td>
</tr>
<tr>
<td>Embryonal</td>
<td>2</td>
<td>1/42</td>
<td>2</td>
<td>1/42</td>
</tr>
<tr>
<td>Unclassified</td>
<td>0</td>
<td>0/3</td>
<td>0</td>
<td>0/3</td>
</tr>
<tr>
<td>Ewing sarcoma</td>
<td>5</td>
<td>1/21</td>
<td>0</td>
<td>0/21</td>
</tr>
</tbody>
</table>

TLE1 was negative in infantile fibrosarcoma, desmoid fibromatosis, digital fibromatosis, fibrous hamartoma of infancy, fibromatosis colli, hepatoblastoma, neuroblastoma, myofibroma/myofibromatosis, superficial fibromatosis and Wilms tumor.

Conclusions: TLE1 identifies the majority of pediatric SS with 3+ staining, but at a lower rate than reported for adult SS. While 2+ staining was occasionally observed in alveolar/embryonal RMS and Ewing sarcoma, it appears that 3+ staining is preferentially seen in SS. TLE1 staining may be useful in cases where tissue is limited. Rare alveolar/embryonal RMS also show 3+ staining, which is an important pitfall of TLE1 staining that must be recognized in the pediatric population.
Prevalence of Hepatic Steatosis in Stillborns Delivered to Women with Diabetes Mellitus

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Background: Maternal diabetes is a known risk factor for pregnancy complications including stillbirth, fetal macrosomia and congenital anomalies. We have observed prominent hepatic steatosis in stillbirth autopsies complicated by maternal diabetes, a finding not previously reported. In this case review we aim to determine if hepatic steatosis is associated with maternal diabetes.

Design: Autopsy reports (2005-present) from both institutions were searched for cases of stillbirth occurring in the setting of maternal diabetes including: late onset diabetes mellitus (DM), early onset insulin dependent diabetes mellitus (IDDM) and gestational diabetes mellitus (GDM), with IRB approval for the exchange of data. Data collected included body and organ weights, clinical complications and major diagnoses at autopsy. Maternal ethnicity, BMI, hemoglobin A1c levels and placenta findings were recorded when available. HE and ORO (when available) liver slides were reviewed. Hepatic steatosis was assessed as microvesicular/macroversicular/mixed, periportal/pericentral/non-zonal and graded as Grade 0:0-5%, Grade 1: 6-33%, Grade 2: 34-66% and Grade 3: >66%. Control cases belonged to stillborns delivered to women without any history of diabetes and were matched for gestational age and degree of maceration. The control cohort was enriched for maternal obesity as well as fetuses who measured large for gestational age and with similar primary cause of demise as the diabetic cohort. Data was analyzed using descriptive statistics and clinico-pathological correlation was obtained.

Results:

Cohort characteristics:

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
<th>Gestational age(wks) range(mean)</th>
<th>CHL(cm) range(mean)</th>
<th>Body weight(%) range(mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CASES(n=23)</td>
<td>16</td>
<td>7</td>
<td>22-39(35)</td>
<td>85-141(110)</td>
<td>92-230(156)</td>
</tr>
<tr>
<td>CONTROLS(n=18)</td>
<td>10</td>
<td>8</td>
<td>21-40(34)</td>
<td>71-118(106)</td>
<td>35-166(121)</td>
</tr>
</tbody>
</table>

Hepatic steatosis was identified in 91% diabetic cases and 28% controls (p<0.001), which was confirmed in a subset by ORO. In both cases and controls, steatosis was macrovesicular with a variable combination of large and small droplets.

Characterization of the steatosis:

<table>
<thead>
<tr>
<th>STEATOSIS</th>
<th>GRADE</th>
<th>LOCATION</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>CASES(n=23)</td>
<td>21</td>
<td>3</td>
</tr>
<tr>
<td>CONTROLS(n=18)</td>
<td>5</td>
<td>1</td>
</tr>
</tbody>
</table>

The presence or degree of steatosis did not correlate with type/control of maternal diabetes and in both groups did not correlate with BMI, cause of fetal demise or presence of macrosomia or hepatomegaly. Although limited numbers, there was a trend of less steatosis in younger age fetuses.

Conclusions: Fetal hepatic steatosis is frequent in stillborns delivered to women with diabetes in pregnancy. Although hepatic steatosis is seen within adults having insulin resistance, the etiology of fetal steatosis in maternal diabetes and potential relationship to stillbirth is unclear.
Gestational Diabetes is Associated with Gender-Based Differences in Placental Efficiency and Increased Trophoblastic Cell Death

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**Background:** Nearly one-third of reproductive-age women are obese, and many of these pregnancies are complicated by gestational diabetes (GDM), which significantly increases the risk of stillbirth. The mechanisms underlying these increased risks are poorly understood, but we have recently shown that the ratio of newborn weight to placental weight (placental efficiency) is significantly decreased in GDM, especially if the neonate is a male. We have also shown using an obese non-human primate model that significantly reduced placental blood volume is associated with increased chorionic villous cell death. In the current study, we tested the hypothesis that GDM in women may lead to abnormal placental growth and apoptosis (cell death).

**Design:** Retrospective analysis of 518 singleton placentas with available maternal metrics, including pre-pregnancy body mass index, glucose tolerance tests, gestational age at delivery, birth weight, placental weight, and neonatal gender. Gestational diabetes was diagnosed in 42 cases (22 males and 20 females). Immunohistochemical analyses (Ki-67 and apoptosis [TUNEL assay and Caspase 3]) was restricted to term GDM cases (n=22; 8 males and 14 females) and age-matched normal term controls. Positive staining for each marker was scored by averaging the number of positive nuclei in ten high power fields for each case. Data were analyzed by ANOVA with Bonferroni correction.

**Results:** Birthweight and placental weight were increased in both genders from mothers with GDM, but placental efficiency was significantly decreased if the mother was obese and the newborn was a male (P<0.01). We observed a trend for less stromal cell death in GDM compared with controls. However, males from obese GDM mothers showed a significant increase in relative trophoblastic cell death and decrease in Ki-67 staining compared to females and controls (P<0.05).

**Conclusions:** GDM is associated with significant gender effects in placental efficiency that may be related to differences in chorionic villous growth and death.
Monocytopenia As a Diagnostic Clue to Pediatric "Aleukemic" B-Lymphoblastic Leukemia

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**Background:** B-lymphoblastic leukemia is the most common childhood cancer. Occasionally, circulating blasts in the peripheral blood (PB) are rare (<1%) and may be missed, even when flow cytometric immunophenotyping (FCI) is performed. A negative result may provide a false sense of security or delay the performance of a bone marrow examination.

**Design:** All patients with a new diagnosis or relapsed B-lymphoblastic leukemia at Children's Healthcare of Atlanta were reviewed over a 3 year period (Jan 2009-Dec 2011). Of these 171 cases, 130 had PB flow performed at our institution. Of these, 15 had a blast count of 1% or less by FCI, and 14 of these had data that could be included in this study. The blast counts ranged from 0.08-1.0%. In all cases, a bone marrow biopsy confirmed the diagnosis. The percentage of monocytes, as determined by gating for CD33 and CD64, in these cases were compared to all patients whose PB was sent for FCI due to at least one lineage cytopenia in 2011 (n=36).

**Results:** The monocytes from the leukemia patients averaged 0.9 %, and were statistically lower than the non-leukemic group, which averaged 7.1% by the Wilcoxon test (P<0.001). 10 of the 14 (71%) patients with leukemia had monocyte counts that were less than 1%, compared to only 3 (8%) of the non-leukemic group. Those 3 patients had bone marrow examinations consistent with aplastic anemia.

**Conclusions:** In patients presenting with cytopenias, assessment of percentage monocytes may be a diagnostic clue in determining the presence of underlying bone marrow pathology. If FCI is performed, acquisition of more events, up to 100,000, is necessary to adequately assess for leukemia. If monocytes are less than 1% in the setting of cytopenias, a bone marrow examination is recommended, even with a negative PB FCI.
Clonal but Benign. Reactive Follicular Hyperplasia with Giant Follicles (RFHGF) Occurring in Adolescent Boys as Peri-Parotid Lymphadenopathy

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**Background:** Reactive Follicular Hyperplasia with Giant Follicles (RFHGF) is a rare entity originally described in young male patients, commonly presenting with enlarged lymph nodes in the neck area, usually adjacent to the parotid or submandibular glands (Am J Clin Pathol 1982; 78:493-9). The entity is considered an extreme form of follicular hyperplasia, resolving 1-36 months from presentation. The pathophysiology remains unknown and there are no reports of molecular studies in primary cases. We report on clonality and long term follow-up in a series of 9 patients.

**Design:** Retrospective chart review of histologic, immunophenotypic and genetic findings data on 9 patients diagnosed with RFHGF was undertaken.

**Results:** The patients were all males with an age range of 7 to 19 years (median 15 years) who presented with enlarged lymph nodes (1-4.5 cm, average 3 cm) in the peri-parotid region (8/9) or groin (1/9). All cases had similar histologic findings with effacement of nodal architecture due to enormous follicles, some of which extended across the entire length of the lymph node. The follicles revealed a back-to-back localization and occasionally were serpiginous with an expansile and sometimes coalescing appearance. Mantle zones were frequently attenuated or there was follicle lysis. The germinal center cells were intermediate to large, associated with increased tingible body macrophages, and Bcl-2 negative. Four cases were tested for Epstein Barr virus mRNA by in situ hybridization (EBER); only 1 was positive.

There was evidence of B-cell clonality in 5/5 cases as evidenced by IgH gene rearrangements. One of these had a trisomy 15 clone (29%) by cytogenetics. Flow cytometry showed clonality in 2/4 cases. Irrespective of clonality, 6/9 cases have at least >8 years of follow-up with no recurrence or progression.

**Conclusions:** This case series of 9 patients with RFHGF confirms that the entity is most common in adolescent boys who usually present with solitary peri-parotid or cervical lymphadenopathy. We demonstrate that the process is clonal by IgH gene rearrangement analysis (5/5), by flow (2/4) and even by cytogenetic analysis. The trisomy 15 identified in the single cytogenetically abnormal case has been show to have 'doubtful significance' in hematologic malignancies (Am J Clin Path 2008; 129:478-85).

Despite clonality RFHGF is a benign process as it resolves without therapy and does not recur or transform to lymphoma with long follow-up. RFHGF shares many features with Bcl-2 negative "pediatric follicular lymphoma" (Blood 2002;99:1958-64) which has been described to occur more commonly in boys (M:F= 2.3:1) with head/neck/cervical nodes, stage I disease, with coalescing follicles of large cells, increased tingible body macrophages, and no BCL2 reactivity. That they are not the same entity is difficult to dispute. Despite clonality in RFHGF, we conclude it is best considered a "hyperplasia" rather than "pediatric follicular lymphoma".
6 Testicular Microlithiasis in Pediatric Population
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**Background:** Testicular microlithiasis is a condition of microcalcifications located within the spermatic tubules. These microcalcifications can be identified on ultrasound examination as punctate echogenic foci. The etiology of these calcifications is not definitely known. The clinical interest of this condition is primarily due to its association with testicular malignancy. The studies focused on testicular microlithiasis in pediatric populations are scarce.

**Design:** The data of this retrospectively study derived from pathology and radiology reports databases of a tertiary children's hospital with a busy urology clinic, including all patients younger than 18 years old at the time presentation who had a scrotal ultrasound study. The pathology reports and slides of all related were reviewed by a pediatric pathologist. The ultrasound imaging studies with microlithiasis were reviewed by a pediatric radiologist.

**Results:** 3,155 patients had a scrotal ultrasound from 8 year period of time. 59 (1.9%) patients with testicular microlithiasis were identified and included in the study group. 17 (28.8%) patients had followup ultrasound ranged from 1 to 14.5 years (mean 4.2 years). The age ranged from 7 months to 17.9 years (mean 10.8 years). 48 (80%) patients had bilateral microlithiasis and 11 (20%) patients had unilateral microlithiasis. 40 (68%) patients had classic microlithiasis and 18 (31%) patients had limited microlithiasis. One patient had classic microlithiasis in one testis and limited microlithiasis in the other. There was a statistically significant positive correlation between increasing age group and prevalence. Imaging appearance of microlithiasis was stable in 10 (71%), and increased in 3 (21%) of the patients. Only one patient the microlithiasis decreased on followup. 6 patients in our population with microlithiasis (6/59, 11.8%) had coexisting testicular neoplasm, 3 of which were benign or premalignant neoplasms (Sertoli cell tumor, nodular benign Leydig cell hyperplasia, intratubular germ cell neoplasm, one case each), and three of which were malignant (all mixed germ cell tumors). 4 patients in our population without microlithiasis (4/3096, 0.1%) had testicular neoplasms, 2 of which were benign (Sertoli cell tumor and mature teratoma) and 2 of which were malignant (both mixed germ cell tumor). Coexistence of microlithiasis and neoplasm (3/59) was much higher than the number of neoplasms without microlithiasis (4/3096). No patients in our study were found to develop a neoplasm within a testicle that had preexisting microlithiasis. The most common underlying scrotal abnormality in our population with microlithiasis was cryptorchidism (6/59), followed by Peutz-Jeghers syndrome (3/59). The pathological and radiological patterns of microlithiasis associated with specific entities were also studied.

**Conclusions:** Our retrospective institution's review of pediatric testicular microlithiasis is the largest of its kind to date. It answered many critical questions regarding to its prevalence, natural history and associated clinical and pathological conditions.
Histopathologic Delineation of the Transition Zone in Short-Segment Hirschsprung Disease

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**Background:** Failure to resect the transition zone (TZ) between aganglionic and neuroanatomically normal bowel is considered one reason for persistent post-operative obstructive symptoms in patients with Hirschsprung disease (HD). However, the existence of different, often subjective, histopathologic definitions for TZ compromise existing studies of the clinical significance of TZ pull-through. Comprehensive comparative analysis of putative TZ features in the same set of HD patients, in an effort to develop objective criteria, has not been reported.

**Design:** Full-circumference, transverse sections at 1 cm intervals from the rectums of 9 non-HD autopsy patients and resections from 15 infants (<6 mos) with short-segment HD were immunostained with Hu C/D (ganglion cell bodies) and GLUT1 (perineurium of extrinsic nerves). The following 5 putative features of TZ were examined morphometrically: (1) Partial myenteric or submucosal aganglionosis (≥1/8th of the circumference), (2) Myenteric or submucosal hypoganglionosis (<80% of the full-circumference ganglion cell density at the proximal margin), (3) Hypertrophic submucosal nerves (≥2 nerves ≥40µ-thick per high power field), (4) GLUT1+ submucosal innervation, and (5) Submucosal "neuronal dysplasia" (≥9 ganglion cells in 4% or more of ganglia).

**Results:** GLUT1+ submucosal innervation, hypertrophic nerves, and hypoganglionosis were absent or restricted to the distal 2 cm of the rectum in non-HD controls; neither partial circumferential aganglionosis nor submucosal neuronal dysplasia was observed in controls. One or more feature of TZ, typically all 5, was present proximal to the aganglionic segment of each HD resection. The length of the TZ ranged from 1-9 cm depending on which neuropathologic feature was considered in an individual case. No consistent spatial relationship was observed between the various features, but submucosal and myenteric hypoganglionosis generally correlated and submucosal neuronal dysplasia accounted for the longest TZ estimates. Excluding submucosal neuronal dysplasia, the TZ was generally ≤5 cm.

**Conclusions:** The length of the TZ in HD depends on which of its purported neuropathologic features is applied. Investigation of the clinical significance of TZ pull-through requires standardized objective histopathological criteria, like those characterized in this study. Many features of TZ cannot be excluded with a biopsy, as opposed to a full-circumference section. Some features of the TZ cannot be assessed with intraoperative frozen sections.
Zebrafish Cirhin Morphants Have p53-Mediated Developmental Biliary Defects, and Are a Model for North American Indian Childhood Cirrhosis

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Background: North American Indian Childhood Cirrhosis (NAIC) is a rare, autosomal recessive, progressive cholestatic disease of infancy described in the Cree-Ojibway First Nations of Quebec. All affected patients are homozygous for a missense mutation (R565W) in CIRHIN/UTP4, which encodes a conserved nucleolar protein found in the t-Utp subcomplex of the small subunit (SSU) processome, important in ribosomal RNA transcription and small subunit assembly. NAIC has thus been proposed as a "ribosomeopathy"; however, investigation of the pathophysiologic mechanism of this disease has been impaired by lack of an animal model.

Design: We have generated a zebrafish model of NAIC using a morpholino oligonucleotide (MO)-based loss-of-function strategy.

Results: Zebrafish cirhin shows substantial homology to the human protein, and is expressed in developing hepatocytes and biliary epithelial cells by 3 days post-fertilization (dpf). Injection of two independent MOs directed against cirhin at the one-cell stage causes defects in canalicular and biliary morphology in 5 dpf morphants. In addition, 5 dpf morphants have dose-dependent defects in biliary function, as assayed by gallbladder accumulation of an ingested fluorescent lipid substrate. Previous yeast and in vitro studies have proposed a role for the nucleolus as a cellular stress sensor, whereby defects in ribosome biogenesis cause stabilization and nuclear accumulation of p53, leading to p53-mediated cell cycle arrest and/or apoptosis. In accordance with this hypothesis, defects in biliary function seen in cirhin morphants are completely abrogated in p53-mutant larvae.

Conclusions: Our findings provide the first in vivo model for NAIC, and support the hypothesis that ribosome biogenesis defects may cause developmental disease through a p53-mediated cellular stress response.
Wnt and BMP Pathways Contribute to the Preferential Activation of mTOR Signaling in Pediatric Yolk Sac Tumors

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**Background:** Germ cell tumors (GCTs) are broadly classified into seminomas / germinomas (GERs) and non-seminomatous germ cell tumors (NSGCTs) such as yolk sac tumors (YSTs). Current cisplatin-based therapy fails to cure 15% of patients and is associated with significant long-term toxicities, such as kidney damage, hearing loss, early onset cardiovascular disease, and secondary malignancies, indicating a need for safer and more effective therapeutic alternatives. We have previously shown that Bone Morphogenetic Protein (BMP) signaling is preferentially active in YSTs and others have shown similar results for Wnt signaling, but these pathways are currently not direct therapeutic targets. Rapamycin-sensitive mTOR complex 1 (mTORC1) is a central regulator of cell growth, proliferation, and differentiation, and has emerged as a therapeutic target in many cancers but has not been investigated in GCTs.

**Design:** In a cohort of pediatric YSTs and GERs, we queried the mTORC1 and Wnt signaling pathways by immunohistochemistry for protein expression, and qRT-PCR using pathway-specific arrays for gene expression. In two GCT cell lines, NTERA-2 and NCCIT, we investigated the effect of Wnt3a and BMP2 on mTORC1 pathway activation. We also studied the effect of mTORC1 inhibitor Rapamycin on GCT cell proliferation and viability.

**Results:** YSTs showed higher immunoexpression of phosphorylated mTOR and its downstream targets phosphorylated S6 ribosomal protein, Cyclin D1, HIF1A, and GLUT1. By qRT-PCR for mTOR pathway, YSTs expressed higher levels of positive regulators of PI3K-mTOR (IRS1, INSR, AKT3, PLD2, MAPK3 and HRAS) and mTORC1 target genes (VEGF and HIF1A), while GERs expressed higher levels of mTOR inhibitor REDD2/DDIT4L. GERs also expressed higher levels of MTOR and RPS6KA5, likely a feedback response to the low levels of mTORC1 activity. By qRT-PCR for Wnt pathway, YSTs expressed higher levels of the ligand Wnt11, receptors (FZD2/4/5/7/8 proteins and LGR5), the transcriptional coactivator TCF7L1, target genes (AES, PITX2) and Wnt pathway regulators (FZDB, SFRP1, CTBP1, CXX4 and TLE2). Treatment of NTERA-2 and NCCIT cells with purified Wnt3a or BMP2 caused increased activity of mTORC1, as shown by increased phosphorylation of S6. Treatment of NTERA-2 and NCCIT cells with 10 µM Rapamycin blocked phosphorylation of S6 and inhibited cell proliferation. Higher doses of Rapamycin impaired the viability of both cell lines (EC50 17 µM for NTERA2 and 19 µM for NCCIT).

**Conclusions:** Our results show a preferential activation of mTORC1 and Wnt signaling in pediatric YSTs compared to GERs. We further show that mTORC1 activity in GCTs is driven at least in part by Wnt and BMP (we have previously shown BMP signaling to be preferentially active in YSTs). Importantly, we also demonstrate that mTORC1 inhibitor Rapamycin potently inhibits the proliferation and survival of GCT cells. Our results provide the rationale for exploring mTORC1 inhibition as an adjuvant or alternative therapy for GCTs.
CLOVES and Klippel-Trenaunay Syndromes Share Somatic Mosaic Activating Mutations in PIK3CA

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Background: Congenital Lipomatous Overgrowth with Vascular, Epidermal, and Skeletal anomalies (CLOVES) is a sporadically occurring, non-hereditary rare disorder characterized by asymmetric somatic hypertrophy and anomalies in multiple organs. We recently employed massively parallel sequencing of fresh and fixed archival tissue to identify somatic mosaic activating mutations in PIK3CA in individuals with CLOVES syndrome. Klippel-Trenaunay syndrome (KTS) exhibits similar features with CLOVES, i.e. vascular anomalies with overgrowth, but with a more limited distribution of involvement, suggesting KTS involves a common genetic pathway.

Design: We assessed for 3 common PIK3CA mutations (E542K, E545K, H1047R) in genomic DNA from archival lesional tissue of 10 KTS individuals using competitive allele-specific PCR or PCR amplification followed by subcloning and Sanger sequence analysis. Unaffected tissue (when available) and mutation positive and negative tissues were employed as controls.

Results: We identified somatic mosaic PIK3CA mutations in lesional tissue from 8 KTS individuals. All three mutations were identified in KTS, whereas the E545K mutation has not yet been identified in CLOVES tissue. When assessed, mutant allele frequencies were < 25% in affected tissue from multiple embryonic lineages, similar to findings in CLOVES tissue and supporting a mosaic hypothesis.

Conclusions: CLOVES and KTS are caused by post-zygotic activating mutations in PIK3CA. We are currently performing massively parallel PIK3CA sequence analysis in lesional tissue from 30 additional CLOVES and KTS individuals to determine if genotype-phenotype correlations exist, although the syndromes may also represent an allelic spectrum determined by timing and distribution of mutant cells during development.
The "Myotrophoblast": Smooth Muscle Differentiation in the Endovascular Trophoblast of the Rat

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**Background:** The conspicuous similarities of the rat to the human placenta, in particular hemochorial placentation which is associated with deep trophoblastic invasion, make rat models very attractive in the study of placental diseases. Studying the species differences in depth is required to project from animal models to humans. We have previously shown dysregulation of the NOS and endothelin systems in our maternal hyperinsulinemia IUGR rat model, similar to human disease.

**Design:** Placentas of normal pregnant dams were examined on gestational days 13 (onset of endovascular trophoblastic invasion), 16 and 21 (term), with special emphasis on the endovascular trophoblasts (EVT). Paraffin and frozen sections were immunostained for cytokeratin (CK), alpha-smooth muscle actin (alpha SMA), alpha heavy chain of smooth muscle myosin (SMM), non-muscle myosin (NMM), and Rho proteins. Co-staining of CK and alpha SMA was also performed on frozen and paraffin sections. We also studied the expression of endothelin receptors A and B (ETA and ETB). Transmission electron microscopic (TEM) study was performed on glutaraldehyde fixed tissue.

**Results:** Expression of alpha SMA in varying degrees was noted in most EVT in the central artery on day 21, co-localizing with CK. Small bundles of thin fibers with an average diameter of 6.607nm, consistent with actin fibers, were observed in these cells by TEM. Localization of alpha SMA at the periphery of the cells, in accordance with the cellular localization of the thin fibers by TEM was evident by confocal microscopy of double-immunofluorescent stained frozen sections. Expression of SMM, NMM and Rho proteins was also seen in EVT. Expression of both endothelin receptors, i.e. ETA and ETB, was evident as well. Expression of smooth muscle contractile proteins was observed as early as day 13 of gestation.

**Conclusions:** The expression of smooth muscle contractile proteins co-localizing with cytokeratin in EVT, along with the expression of endothelin receptors, raise the possibility that some control of central artery contraction is retained in the rat placenta despite degeneration of vascular smooth muscle cells. This may play a role in situations of dysregulation of the vasoactive systems in the rat, such as in models of pre-eclampsia or IUGR.
Diagnosis of Congenital CMV Using PCR Performed on Formalin-fixed, Paraffin-Embedded Placental Tissue

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Background: Congenital cytomegalovirus (CMV) infection may be asymptomatic until hearing loss or developmental delay is detected in childhood. The diagnosis of congenital CMV requires viral detection within an infant's first 21 days of life; urine shell vial culture is the gold-standard. The placenta provides a unique opportunity to identify congenital CMV exposure in individuals in whom CMV is not suspected until later in life.

Design: With Institutional Review Board approval, a database of all CMV shell vial cultures performed from July 2001 to March 2012 was used to identify infants under 100 days of age with urine CMV culture results. The pathology database was then queried to identify corresponding placentas with positive or negative CMV cultures, as well as placentas in which CMV immunohistochemistry (IHC) was performed. All placentas were reviewed for the presence of chronic villitis, the cellular composition of the villitis, and the CMV IHC result. Infant data, labs, and physical exam findings were recorded, as were maternal CMV serologies during pregnancy. DNA was extracted from each formalin-fixed paraffin embedded placental tissue block, and quantitative CMV PCR performed.

Results: Seventeen (17) placentas were identified belonging to fetuses/neonates under 100 days of age with positive CMV cultures. Of the 9 cases with positive cultures in the first 21 days of life, PCR confirmed CMV in 8 cases, while IHC was negative in 4 cases and inconsistently positive in two others. The neonate with a negative PCR had two negative urines, one at day 1 and another at day 4 of life, before a positive urine culture at day 17 of life, suggesting a post-natal infection. Of the 8 infants whose CMV cultures were positive only after day 21 of life, all had negative CMV IHC, but one placenta from an infant whose urine was not tested until day 25 of life was positive by CMV PCR. Placentas from 20 infants with negative CMV urine cultures were all CMV PCR negative; 12 of these infants also had mothers with positive CMV serologies. CMV PCR was also performed on 26 placentas with chronic villitis by histology (no urine cultures available), and one placenta was positive. In the 10 individuals with CMV PCR positive placentas (8 with positive urines within the first 21 days, 1 with positive urine at 25 days, 1 with no urine tested), CMV IHC was consistently positive in only 4 cases.

Conclusions: This study demonstrates the utility of CMV PCR testing of formalin-fixed, paraffin-embedded placental tissues for the diagnosis of congenital CMV. Confirmation of CMV exposure in utero by this method may inform treatment decisions and identify infants requiring close follow-up for hearing impairment and developmental delay.
13 Dual Mechanisms of Ethanol-Impaired Placentation: Experimental Model

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**Background:** One of the major adverse effects of maternal ethanol consumption is intrauterine growth restriction (IUGR). In an experimental rodent model, we demonstrated that ethanol-induced IUGR is mediated by impaired placentation. Ethanol exposure reduces trophoblast motility and invasiveness through inhibition of insulin/IGF signaling leading to impaired vascular transformation at the implantation site. Furthermore, ethanol reduces the number of secondary giant cells that mediate vascular invasion in rat placentas. We hypothesize that in addition to impairing trophoblastic motility and invasiveness, ethanol impairs placentation by inhibiting progenitor cell survival, proliferation, and differentiation.

**Design:** Pregnant Long Evans rats were fed isocaloric liquid diets containing 0% or 8% (v/v) ethanol. Diets were initiated at different embryonal days (E) corresponding with the temporal program of stem cell activation (E6), the appearance of secondary trophoblast giant cells (E10), prior to (E15) and after (E16) trophoblast invasion into the decidua and mesometrial triangle. The pups and placentas were harvested on E19. Fetal body weight and length were documented. Placental histopathological studies were used to determine the trophoblastic differentiation and vascular transformation at the implantation site. Since vascular transformation is mediated by invasive trophoblasts, we analyzed their distribution at the implantation site with cytokeratin immunostaining and unbiased 3-D stereology. Phenotypic analysis of trophoblastic cells was performed by qRT-PCR in control and ethanol-exposed placentas by measuring mRNA levels of genes encoding for specific trophoblastic cells and normalized to ribosomal 18S RNA measured in parallel reactions. Inter-group comparisons were made using one-way ANOVA with Tukey's multiple comparison test.

**Results:** Early gestational ethanol exposure (E6 and E10) significantly impaired fetal growth relative to control and late exposure groups. Maternal vascular transformation was inhibited in all ethanol groups relative to control, but the effects were greater when the ethanol diets were initiated on E6 and E10. Ethanol exposure significantly reduced number of cytokeratin-positive invasive trophoblastic cells at the implantation site and their precursor secondary giant cells. The degree of reduction correlated with earlier ethanol exposures. The mRNA expression levels of genes encoding for stem cell (Cdx2), progenitor cell (Tpbpa), trophoblast giant cell (Prl3d1), glycogen cell (Gjb3) and invasive trophoblast (Prl5a1, Prl7b1) were significantly reduced by ethanol exposure regardless of its timing.

**Conclusions:** Gestational ethanol exposure has dual inhibitory effects on placentation mediated by reduced progenitor cells and impaired trophoblastic motility.
14  Intestinal Metaplasia of the Gastric Antrum in the Pediatric Population, Is It Clinically Significant?
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Background: Intestinal metaplasia found in the gastric antrum of pediatric patients is an incidental finding that can cause confusion in the clinical setting. Pediatric intestinal metaplasia has been described in a number of clinical situations. Most commonly, it has been identified in association with atrophic gastritis that results from infection with Helicobacter pylori. It has also been seen in the setting of chronic reflux, the presence of anti-gastric antibodies, and surgical manipulation of the aerodigestive tract. There is some dispute as to whether this finding is truly a pathological one. It has been suggested that intestinal epithelium can be a component of ectopic tissues found in pancreatic rests that are commonly seen in pediatric antral biopsies. Another explanation is that the finding of intestinal epithelium merely represents inadvertent sampling of normal small bowel as the division between gastric antrum and duodenum is not as well demonstrated in children. Though accepted as a premalignant lesion in adults, the extreme rarity of gastric adenocarcinoma in the pediatric population speaks to the inappropriateness of qualifying the lesion similarly in this population.

Design: This was a retrospective review of biopsy series from 401 upper GI endoscopy procedures in patients up to the age of 21 in a community hospital setting. Cases displaying intestinal metaplasia were confirmed with a PAS/Alcian blue stain.

Results: Intestinal metaplasia was identified and confirmed in six gastric antrum biopsies, an incidence of 1.49% in our patient population. Four patients presented with nonspecific symptoms and had no factors that might predispose them to developing intestinal metaplasia in their histories. One patient had been diagnosed with Crohn's disease. The final patient had undergone surgical repair of a tracheoesophageal fistula. No patient had a history of H.pylori infection. All the examples of intestinal metaplasia we identified were of the complete type. None were identified in the proximity of small bowel epithelium and none were in association with a pancreatic rest.

Conclusions: Four out of six examples were identified without an associated H.pylori infection and without other predisposing factors. This lead us to conclude that the clinical settings in which this lesion are found are likely more varied than earlier described. Furthermore, the role of H.pylori is uncertain. Our examples also occurred only in the context of gastric epithelium. As a result, we also concluded that our examples were a truly metaplastic process as opposed to inadvertent sampling of the small bowel or ectopic tissue present in a pancreatic rest.
Background: Non-iatrogenic neonatal gastric perforation (NGP) is a rare and life-threatening condition whose etiology is often unclear. Previous animal and human studies conducted abroad have shown a decrease in interstitial cells of Cajal (ICC) in cases of "idiopathic" gastric perforation. Interstitial cells of Cajal act as gastrointestinal pacemaker cells and express the proto-oncogene c-Kit. In the first study of its kind in the United States, the authors propose that a lack of ICC in the stomach musculature may be implicated in the development of non-iatrogenic gastric perforation in neonates. Moreover, the authors suggest a change in the often confusing labeling of this condition, changing the prevalent idiopathic/non-idiopathic terminology to a more comprehensive and clear "iatrogenic/non-iatrogenic."

Design: 6 cases of non-iatrogenic NGP were identified at our institution over a period of 17 years. They presented with no mechanical, pharmacologic, or otherwise medical-related intervention previous to rupture. Patient gastric biopsies/subtotal resections and matched controls from autopsy material were examined using H&E and immunohistochemical stains for c-Kit (CD117). The number of c-Kit positive ICC in non-necrotic muscularis propria from five random HPF (400X) per specimen was compared using light microscopy. Statistical significance was determined using Student's T-test.

Results: The number of ICC was significantly lower in the specimens from cases of non-iatrogenic NGP compared to controls (p = 0.008). Five of the six patients survived. One case died of fulminating fungal peritonitis secondary to gastric perforation, and two cases received medical treatment for MRSA peritonitis.

Conclusions: A reduced number of gastric interstitial cells of Cajal predisposes neonates to gastric perforation. Further large-scale studies, including molecular and genetic analysis, may be helpful in understanding this phenomenon.
16 Congenital Lower Lip Pits (Van Der Woude Syndrome): What Pediatric Pathologists Need to Know?

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**Background:** The specimens from lower lip pits come to the attention of pediatric pathologists from time to time, many of them representing a congenital malformation known as Van der Woude syndrome. This syndrome was named after Van de Woude who in 1954 first gave a detailed description of this condition. It is an autosomal dominant condition with penetrance close to 100%, and estimated to affect 1:75000 to 100,000 individuals, being the most common syndromic condition for cleft lip and palate. Some significant publications on this subject are available but thitherto it has never been a focus in any pathology journal, which led to inadequate acquaintance of this condition for pathologists, even for those who specialize in head and neck pathology and those who work in the children's hospital settings.

**Design:** 19 specimens from 17 patients were collected from our institutional surgical pathology files in the span of 25 years. The histopathology and relevant clinical information were studied.

**Results:** The locations without exception were vermilion of the lower lip on both sides of the midline, and symmetrical. Both Caucasian and black patients were represented as well as one white non-identical (or dizygotic) twin patients. There is male predominance (14:3). The classic histology spectrum of the pits ranged from mere depression to deep draining sinus tracts to fistulas, which may or may not communicate the subjacent minor salivary glands; they were lined by acanthotic non-keratinizing stratified squamous mucosa in continuity with the labial epithelium. Most of the times two pits specimen were submitted simultaneously but occasionally only one pit was repaired at the time. The most salient clinical feature is association of cleft lip and palate, mostly bilateral, but variable expression including unilateral and submucosal cleft cases were represented. Significant number of cases was noted to have otitis media and associated hearing loss. The primary differential diagnosis considerations include epidermal inclusion cyst, mucocele. Most cases were signed out descriptively without realizing the syndromic implications. Some other common pathology can happen in this location (nevus, hemangioma) but typically does not constitute any diagnostic challenges.

**Conclusions:** Located in chromosome 1q32-q41, the mutated gene responsible for this condition is IRF6 (Interferon Regulatory Factor 6), which is also responsible for popliteal pterygium syndrome. Besides these two conditions, lip pits can also be observed, though very rarely in type 1 orofaciodigital syndrome and Kabuki makeup syndrome. The management is primarily surgical, and genetic counseling is indicated. Adequate knowledge of this condition is necessary for all pediatric pathologists and head and neck pathologists as indicated by its relatively high frequency.
Distinct Histologic Features of Secondary Pulmonary Alveolar Proteinosis in Children Compared to Surfactant Deficiencies

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Background: In the last few years there has been great progress made in understanding the etiology, genetic basis and pathophysiology of alveolar proteinosis. This has led to more specific definitions of pediatric and adult diseases that were often confused in the past. Surfactant deficiency disorders are mostly genetic defects in surfactant proteins while primary pulmonary alveolar proteinosis is due to defects in the GM-CSF receptor. In contrast, secondary or acquired alveolar proteinosis is related to macrophage dysfunction.

Design: We reviewed 480 pediatric lung biopsies performed at the University of Chicago Medical Center over a period of 17 years (1994-2012) in order to define histologic characteristics of macrophage dysfunction diseases (secondary alveolar proteinosis). We identified four pediatric cases of pulmonary alveolar proteinosis and compared their findings to surfactant deficiency disorders.

Results: There were two male and two female patients ranging in age from 4 to 14 years of age. Three patients had acute myeloid leukemias and one had juvenile rheumatoid arthritis. Two of the patients with leukemia also had fungal pneumonia. Away from the areas of pneumonia the findings in all 4 cases were similar, with alveoli filled with PAS positive material. There were minimal or no interstitial changes with little inflammatory reaction to the material and only focal type II pneumocyte hyperplasia. These findings are similar to those found in patients with mutations in the GM-CSF receptor and contrast with those in patients with confirmed surfactant deficiency who have variable proteinosis, interstitial inflammation or fibrosis, desquamated cells and type II pneumocyte hyperplasia. Electron microscopy was performed on 2 specimens and showed cellular debris and no abnormalities of lamellar bodies.

Conclusions: Clinical, genetic and pathologic findings are distinct in secondary pulmonary alveolar proteinosis as compared to pediatric surfactant deficiency disorders but are histologically similar to primary pulmonary alveolar proteinosis.
Pulmonary Neuroendocrine Cell Expression in Second Trimester Trisomy 21 Fetuses
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**Background:** The pulmonary neuroendocrine cell system (PNEC) is widely distributed throughout the respiratory mucosa. It responds to both mechanical and hypoxic stimuli. It consists of solitary neuroendocrine cells (NEC) and neuroepithelial bodies (NEB), representing clusters of 4-100 NEC. PNEC is highly represented in fetal and neonatal lungs and it declines afterwards. Both elements produce a variety of neuropeptides, including bombesin, potentially involved in lung growth and differentiation. Pulmonary structural anomalies, such as enlarged alveolar ducts and alveoli and reduced alveolar number, may be found in Down's syndrome. These changes are thought to be late gestation in development. The possible involvement of PNEC in Down's alveolar dysmorphology was studied in fetal lungs.

**Design:** The study group consisted of 11 fetuses with Down's syndrome ranging from 17 to 22 weeks gestational age and subdivided in two small groups, 17 to 19 weeks, and 20 to 22 weeks, according to the different stage of pulmonary development. The control group consisted of 9 normal fetuses, at the same gestational age with no chromosomal or congenital abnormalities. Cases with potential PNEC alteration were excluded, such as severe chorioamnionitis (where bombesin is overexpressed) and oligohydramnios with pulmonary hypoplasia. Immunohistochemistry for synaptophysin and bombesin was performed in both lungs in each fetus and evaluated according to different levels of the respiratory tree. For each lung, single NEC and NEB were counted only in the mucosal lining, analyzing 2 sections of segmental bronchi, 3 terminal bronchioles and 3 respiratory units (respiratory bronchioles, alveolar ducts and immature alveoli). Morphometrical analysis was applied to measure the perimeter of the structures and the PNEC cells were expressed as a ratio per length. NEC and NEB cells were distinguished according to synaptophysin and bombesin expression. Fisher's test was applied to evaluate the PNEC expression in the two different fetal populations.

**Results:** Mean lung synaptophysin and bombesin NEB and NEC cell distribution did not differ between normal and Down's syndrome fetuses, according to age. No statistical significant difference ($p > 0.05$) was found between the expression of synaptophysin and bombesin in NEC and NEB in normal and Down's syndrome fetuses.

**Conclusions:** According to these results, at least in early stages of lung maturation, even comparing a small fetal population, PNEC cell expression does not appear to be significantly different in normal and Down's syndrome fetuses.
Background: Transient abnormal myelopoiesis (TAM) and Myeloid leukemia associated with Down syndrome (AML of DS) are two morphologically indistinguishable disorders. Though TAM is usually congenital and regresses within the first three months of life, some cases of TAM have persistence of blast elevation and marrow dysplasia beyond 90 days and are termed AML of DS. In their 2004 paper, McElwaine et al. employed microarray transcript profiling and identified PRAME as a marker that is increased in AML of DS but not in TAM. The gene PRAME (preferentially expressed antigen of melanoma) expresses a tumor antigen recognized by cytotoxic T cells, and expressed in a variety of tumors including melanomas, lung non-small cell carcinomas, renal carcinomas, head and neck squamous cell carcinomas, sarcomas, and acute leukemias. An immunohistochemical antibody to PRAME has been developed, but its utility in distinguishing TAM that will regress from that which will persist beyond 90 days and give rise to AML of DS has not been studied. The purpose of this current study is to use the PRAME antibody to determine if it can differentiate between TAM and AML of DS.

Design: With IRB approval, the pathology database was searched for bone marrow biopsy or placenta cases of transient abnormal myelopoiesis and myeloid leukemia associated with Down syndrome. From the reports, the patient's age at the time of the biopsy and percent blasts identified within the marrow were recorded. Slides from each biopsy and placenta were stained with anti-PRAME antibody (Abcam, ab89097, mouse polyclonal) and evaluated for expression.

Results: Biopsies with interpretable PRAME staining were available for four cases of TAM, including two placentas, and fourteen cases of AML of DS. Positive PRAME staining in megakaryoblasts usually consisted of a single large dot in the cytoplasm. Of the four cases of TAM, two cases were positive for PRAME, including one with follow-up demonstrating AML of DS. The other individual with positive PRAME staining of TAM is disease free at 32 months of age. Of the fourteen cases of AML of DS, ten had at least a subset of cells with positive PRAME staining, while four were negative for PRAME. Of note, PRAME also stained dysplastic megakaryocytes and fibroblasts, which were prominent in multiple cases of AML of DS.

Conclusions: This study is one of the first reports to evaluate protein expression of PRAME in transient abnormal myelopoiesis (TAM) and Myeloid leukemia of Down syndrome (AML of DS) by immunohistochemistry. Consistent with prior reports, PRAME localized to the cytoplasm. Based upon our results, we suggest that the gene expression array findings of increased PRAME mRNA in AML of DS (McElwaine et al, 2004) could be in part due to expression in fibroblasts and dysplastic megakaryocytes, as staining was not restricted to blasts.
Mucosal Overexpression of T-Bet Promotes Mixed Cellular Inflammatory Infiltrate During Intestine Transplant Rejection

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Background: Recurrent acute cellular rejection (ACR) limits graft survival after intestinal transplantation (ITx). In our recent genome-wide gene expression study of peripheral blood, the gene T-bet, a classical type I inflammatory transcription factor, and the cytotoxic gene, granulysin, were found to be upregulated before ITx, and after ITx rejection, but not during ACR among rejection-prone recipients in a discovery cohort of 20 recipients. We hypothesized that the T-bet and granulysin proteins would be upregulated in inflamed allograft mucosa during ITx rejection, explaining decreased expression of corresponding gene in peripheral blood.

Design: The study was done after IRB approval. 1. Single color immunohistochemistry (IHC) was performed in residual formalin-fixed-paraffin-embedded tissue obtained at surveillance biopsies from 12 pediatric ITx recipients. Appropriate stain controls were performed. All children received rabbit anti-human thymocyte globulin induction, and tacrolimus with steroids. Rejectors (n=8 of 12) experienced biopsy-proven ACR within 60 days after ITx. Counts/high power field (hpf) were evaluated in the lamina propria of biopsies for each subject within 4 hours after graft reperfusion, at days 15-18 after ITx or at the time of first ACR, and at months 6-12 after ITx. The differentially expressed T-bet protein was further evaluated for expression in CD14+monocytes, CD8+T-cytotoxic cells, and CD16+NK cells in random blood samples from 5 additional ITx recipients, using flow cytometry, and 3. in corresponding leukocyte subsets in the lamina propria of selected biopsies in the IHC cohort using confocal microscopy.

Results: On IHC staining, T-bet was encountered in the nuclei of lamina propria mononuclear cells. Compared with non-rejectors, rejectors demonstrated higher (mean+SEM) T-bet+cell counts/hpf at reperfusion (13.1±3.9 vs 40.3±11.2, p=0.06) and during rejection in early post-ITx biopsies (14.2±4.1 vs 61.1±15.7, p=0.023). No significant differences were noted late after ITx (18.1±5.2 vs 28.4±8.1, p=NS). Granulysin-stained cell counts/hpf were not different between outcome groups (reperfusion: 3.1±0.9 vs 5.1±2.8, p=NS; early after ITx 1.4±0.2 vs 2.2±0.7, p=NS; late post-ITx: 2.6±1 vs 1.9±0.5, p=NS). In random blood samples, rejectors (n=3) demonstrated higher frequencies of CD14+ (96.3±3.1 vs 6.6±3.4, p:0.0003), and CD8-cytotoxic cells (88.9±5.5 vs 8.1±0.9, p: 0.04) which expressed T-bet, compared with non-rejectors (n=2). In confocal staining of a biopsy from a rejector, T-bet nuclear staining co-localized with CD14+monocytes with which it was strongly correlated (Spearman r=0.691, p=0.019), and with T-cytotoxic and CD16+NK cells.

Conclusions: Intestine transplant rejection is characterized by overexpression of nuclear T-bet, an inflammatory transcription factor, which likely promotes a mixed cellular inflammatory infiltrate comprising inflammatory monocytes and cytotoxic T- and NK cells.
Background: Atypical spitz nevi (AS) can morphologically resemble melanoma and hence cause diagnostic confusion.

Design: We retrospectively reviewed the charts and slides of patients (pts) diagnosed with atypical spitz tumor and melanoma at Children's Mercy Hospital during the period 2002-2011. Data pertaining to age, sex, diagnosis, recurrence, metastasis, last follow up and histology were collected. For the diagnosis of AS, the criteria selected were extension into deep dermis, mitoses in deep dermis and size >0.5 cm. All melanoma diagnoses were confirmed by external consultants and at least 3 pathologists.

Results: There were 15 melanoma (7 female, 8 male) pts with a median age of 11 years (range 1-17 y). There were 10 AS (3 females, 7 males) pts with a median age of 6 years (range 1-16 y). The melanoma pts had a median follow up of 47 months (2-96 months); 6 of them had nodal metastasis and 2 had local recurrence. The AS pts had a median follow up of 32 months (1-55 months); 1 of them had nodal dissection (no metastasis) and 1 had local recurrence. Both melanoma and AS pts showed the histological parameters of pagetoid spread (60% versus 30%), epidermal ulceration (27% versus 30%), mitoses in deep dermis (60% versus 20%). While not all cases had HMB45 immunostaining performed, the ones that were available showed no difference in expression between melanoma and AS. Only 3 of 15 melanoma pts exhibited vascular, neural or glandular invasion (1 each). Metastasis outside of lymph nodes was not seen in melanoma pts. All of the 25 pts are alive.

Conclusions: There is a significant overlap of histological features between AS and melanoma. Only frank invasion of nerves or vessels; or nodal metastasis can be a reliable feature to diagnose melanoma on histological grounds. Follow up has revealed no spread of melanoma beyond the lymph nodes. Thus, the outcomes of both AS and melanoma in children are favorable.
The Angiotensinogen Thr235 Variant is Associated with Preterm Labor in Japanese American Women

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Background: The angiotensinogen A(-6)G promoter variant tightly linked to the Met235Thr polymorphism (SNP rs699) has been shown to be associated with severe preeclampsia in Japanese women and Utah Caucasian women. It has also been associated with intrauterine growth restriction (IUGR) and placental abruption. The placentas from pregnancies complicated by severe preeclampsia, IUGR, and many cases of preterm labor (PTL) show histologic features of relative uteroplacental insufficiency. If idiopathic PTL is a disease of relative uteroplacental insufficiency similar to preeclampsia and IUGR, it may also be associated with rs699. In turn, the frequency of this allele should be significantly increased in idiopathic PTL compared to normal term pregnancy controls.

Design: We identified all pregnancies from Japanese American women in the Pacific Research Center for Human Early Development's (PRCED) pregnancy phenotyping database at the John A. Burns School of Medicine in Hawaii. We sorted them into negative controls (normal term deliveries) and idiopathic PTL leading to preterm birth. Cases were excluded if the patient reported less than 75% Japanese heritage within three generations, which yielded a total of 97 negative controls and 42 PTL cases for SNP rs699 genotyping. Maternal and fetal metrics, including gender were available for analysis. Data were analyzed by Chi-square.

Results: The angiotensinogen Thr235 allele frequency was 0.70 in the negative control cohort similar to previous reports of Japanese women (0.71). Interestingly, the allele frequency in negative controls was significantly different depending on newborn gender (males q=0.65, females q=0.77). Overall, the allele frequency was significantly increased in PTL (q=0.83) compared with negative controls (p<0.05). The association was especially strengthened if the baby was a boy (q=0.88; p-value <0.01), but not if the baby was a girl (q=0.80).

Conclusions: Although the sample size is limited, our results suggest fetal gender coupled with maternal angiotensinogen genotype may affect the risk of relative uteroplacental insufficiency leading to preterm labor.
Perinatal Deaths Due to Umbilical Cord Compromise in a Community Hospital
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Background: There is wide variation in the reported incidence of umbilical cord accident as a cause of perinatal death. In recent years gross and microscopic criteria have been suggested for diagnosing cord compression. In a community hospital, cases with a prenatal diagnosis of chromosomal or genetic anomaly, or major malformation are usually referred to a University center for management. Thus, perinatal deaths in community hospitals are usually not associated with known fetal disease. A relatively high incidence of umbilical cord accidents as a cause of perinatal death might be expected at community hospitals.

Design: All perinatal deaths at or after 20 weeks of gestation at Rochester General Hospital from 2007-2010 were reviewed, 42 cases in total. Gross photographs and slides were available in all cases. Complete autopsies were done on 32 fetuses and external examinations were done on 6 more. Photographs and microscopic slides were reviewed. The cases were placed in 4 categories: "Death due to cord compression" group 1; "Cord compression present, possibly contributing to death", group 2; "Cord compression present, probably not contributing to death", group 3; "No cord compression", group 4. Diagnoses were reached by consensus of two pediatric pathologists.

Results: Group 1 had 12 cases, group 2 had 6, group 3 had 9 and group 4 had 15. Cord compression caused death in 28.6% of the cases, and was present to some degree (groups 1-3) in 64.3%. There was a difference in gestational age between group 4 and the others. In cases from group 4, 13/15 were at 30 weeks gestation or less. Cases from group 4, 13/15 were at 30 weeks gestation or less. Cases from groups 1-3 had 22/27 over 30 weeks gestation. 22/24 (91.7%) deaths over 30 weeks gestation had some degree of cord compression with 10/24 (41.7%) having death due to cord compression. Umbilical cord features associated with cord compression include: increased twists, furcate insertion, tight true knot, and congestion/edema. Placental lesions associated with cord compression include vascular ectasia, thrombi, avascular villi, and "hemorrhagic endovasculopathy". Vascular ectasia is the least specific of these. Fetal features associated with cord compression include cardiac left ventricular dilation, right atrial dilation and multiple effusions (pleural, pericardial, and ascites). Placentas with diffusely swollen congested umbilical cords generally permitted confident prediction that effusions would be found, with an enlarged heart with dilated right atrium and left ventricle. Various other fetal and/or placental signs of hypoxia were generally present. At this institution, this constellation of associated findings is referred to as Cord Compression Syndrome.

Conclusions: In a community hospital, unexpected perinatal deaths after 30 weeks gestation have a high likelihood of cord compression and almost half of them will have cord compression as the cause of death. A diffusely swollen congested umbilical cord on placental exam is highly predictive of effusions and a dilated heart.
Correlation Between Neonatal C-reactive Protein and Histologic Placental Inflammation
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Background: Despite the large body of literature examining C-reactive protein (CRP) as a marker of neonatal sepsis, there is no universally accepted standard of practice for the use of CRP in the assessment of neonatal sepsis. Infants asymptomatic at birth and those with little or no risk factors for infection are challenging to diagnose and treat. Our goal was to examine the utility of serial serum CRP in newborn babies being evaluated for sepsis and correlate these with histologic diagnosis of chorioamnionitis.

Design: An enterprise data warehouse search for neonates with at least two CRP values in the first two days of life was performed from 1/1/2009-12/31/2010. Retrospective review of newborn charts and associated maternal placenta surgical pathology reports was used to determine eligibility for inclusion. 268 singleton neonates with at least 2 CRP values and available placental pathology were included. Placental inflammation was categorized and staged as maternal acute inflammation (stage 1-3), fetal acute inflammation (stage 1-3), or chronic inflammation. Statistical analyses were performed using SPSS.

Results: At least one abnormal CRP (>0.5 mg/dL) was present in 39% of the infants. There were robust associations with abnormal 12-hour and 24-hour CRP levels, and any abnormal CRP at <72 hours with both maternal and fetal acute placental inflammation (P<0.001). These associations remained significant after adjustment for gestational age, birth weight, gender, and race/ethnicity, and for further adjustment for other potential confounders such as prolonged rupture of membranes, maternal steroids, and infant antibiotic therapy. Only 7 (3%) neonates had positive blood culture. Positive blood culture correlated with any fetal acute inflammation (P<0.05), and with high stage maternal acute inflammation (amnionitis) (P=0.006), but not with abnormal CRP.

Conclusions: Abnormal/elevated 12 and 24 hour neonatal serum CRP values correlate strongly with acute maternal and fetal placental inflammation, and therefore, may reflect intrauterine inflammation. The presence of any fetal acute inflammation and amnionitis correlate the most strongly with positive blood cultures in the neonate. Since only a small number of neonates typically have a positive blood culture, placental inflammation and serial CRP can be used to guide diagnosis and treatment.
**25 Placental Involvement of Congenital Multisystem Langerhans Cell Histiocytosis**

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**Background:** Congenital presentations of multisystem Langerhans cell histiocytosis (MS-LCH) are uncommon, present diagnostic challenges, and result in significant morbidity and mortality. The placenta is likely the first fetal tissue examined at birth and may provide the initial histologic evidence of disease. Such early identification may enable prompt therapeutic intervention for this aggressive neoplasm. Interestingly, no apparent cases of placental involvement by LCH have been reported in the literature. Herein, we present two such cases.

**Design:** Two cases of congenital MS-LCH with placental involvement were retrieved from the archives of Children's Hospital Boston and Saint Francis Hospital with appropriate IRB approval. Routine and immunohistochemical stains were performed using standard clinical protocols and reagents. Positive and negative controls were included for evaluation and showed appropriate patterns of reactivity.

**Results:** The first case involved premature delivery of a 33 week male fetus with a placenta that was large for gestational age, exceeding the 90th percentile for dates. The second case involved the intrauterine fetal demise of a 34 week fetus with a placental weight below the 10th percentile for gestational age. In each case, the skin and mucous membranes contained multiple dark hemorrhagic-appearing ("blueberry muffin-like") lesions with associated multiorgan involvement and/or multisystem failure. Microscopic examination of both placentas revealed multiple foci of chronic villitis characterized at low power by histiocytic elements, scant eosinophils, and rare lymphocytes. Higher magnification revealed grooved nuclei and indistinct cell membranes within a subset of the histiocytic cells. This population coexpressed CD1a, Langerin and S100. Skin biopsies from each case exhibited similar histologic and immunophenotypic features, establishing the diagnosis of LCH.

**Conclusions:** MS-LCH can involve placenta, which may explain phenomena such as transmission in familial (twin-twin) disease. The two cases presented here highlight the value of placental microscopic examination in disease detection and expand the differential diagnosis of chronic villitis.
Effects Of Transcutaneous Electrical Nerve Stimulation (TENS) On Fetal Development And Expression Of Hypoxia Related Markers In An Experimental Model Of Placental Insufficiency

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Background: Despite studies in humans reporting TENS as a treatment for placental insufficiency, experimental models demonstrated negative effects of this current on placental vascularization. The purpose of this study was to evaluate the effect of TENS on fetal development.

Design: On day 15 of gestation, 8 Wistar rats were submitted to the right uterine artery ligation. The contralateral horn was used as control. TENS was applied in 4 rats (frequency: 80 Hz; pulse duration: 200 microseconds; intensity: 4-6 mA) from the immediate postoperative period until the euthanasia (on day 19). For classification of intra-uterine growth restriction, fetal external measures as crown-rump length (CRL), fronto-occipital (FOD), thoracic ventral-dorsal (TVDD) and abdominal ventral-dorsal (AVDD) distances were analyzed, as well as the area occupied by fetal internal organs such as brain (BA), lung (LUA) and liver (LVA). After immunohistochemistry, the expression of glucose transporter 1 (GLUT-1) was analysed in fetal brain, lung and liver. Placental expression of Hypoxia Inducible Factor alpha (HIF-1), Interferon gamma (IFN), Inducible Nitric Oxide Synthase (iNOS) and p53 protein was analyzed by real time Polymerase Chain Reaction (qPCR).

Results: In the ligated horn, there was significant reduction of CRL, FOD, TVDD, AVDD, BA, LUA and LVA (p ≤ 0.001). In cases stimulated, in addition to significant reduction of FOD, TVDD, AVDD (p ≤ 0.001) and LVA (p = 0.030), there was significantly increased expression of GLUT-1 in fetal brain, lung and liver (p ≤ 0.001), and IFN in the placenta (p = 0.004). Evaluating the interaction between the restriction of blood flow and electrical stimulation, in the group with ligation and stimulated (LS) there was a significantly decrease in CRL, FOD, TVDD, AVDD, BA, LUA and LVA (p ≤ 0.001) and increased expression of GLUT-1 in brain, lung and liver (p ≤ 0.001). The groups that had higher placental expression of IFN were the contralateral to ligation with the stimulus (CS) and LS, respectively. Placental expression of HIF-1 was higher in the ligated horn, without differences in cases stimulated.

Conclusions: Our results confirm the negative effects of TENS on uteroplacental circulation, and also demonstrate its negative influence on fetal development, advising against its use during pregnancy.
A Stereological Study of Chronic Uteroplacental Insufficiency Associated with Normal Birth Weight – A Distinct Entity

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Background: Chronic uteroplacental insufficiency (CUPI) causes accelerated villous maturation (AVM). In 810 low risk pregnancies, 83 had AVM, in whom pre-eclampsia (PET) and thrombophilia were excluded. Of these 63 were associated with normal infant birth weights (CUPI-NBW), and 20 with SGA (CUPI-SGA).

Design: Ten placentas were assessed from each of the following groups: normal pregnancy and birth weight (NBW), CUPI-NBW, CUPI-SGA and PET with IUGR (PET-IUGR). The placental disc volume was measured, followed by uniform random sampling of 10 full thickness biopsies. 5 fields were examined from coded H&E stained sections. Stereology comprised star volume and surface area measurements of terminal villi and capillaries. Two-dimension counts of syncytial knots were also performed.

Results: The CUPI-NBW had significantly reduced capillary star volume and surface area, but had a normal villous surface area compared to NBW. This contrasted with CUPI-SGA in which all the parameters, including surface area, were reduced similar to PET-IUGR. The PET-IUGR capillary star volume however was partially reduced compared to NBW although capillary surface area measurement was significantly reduced. (Possibly related to maternal antihypertensive therapy). Fetal distress requiring caesarean section/instrumental delivery in 41% of CUPI-NBW compared to 20% in CUPI-SGA and 18% in NBW controls.

Conclusions: The normal total villous surface area in CUPI-NBW compared to NBW and reduced terminal villous vascular volume similar to PET-IUGR provides an understanding of previously unexplained intrapartum hypoxia.
Variables Impacting QNS Sweat Chloride Collection

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Background: The Cystic Fibrosis Foundation recommends that the qns (quantity not sufficient) rate for sweat collection be ≤10% for infants <3 months old. The <3 month age group is particularly susceptible to obtaining a qns collection, with literature indicating a qns rate up to 40%. Several demographic factors impact the ability to obtain sweat, and because of this there are collection centers where patients may be deferred for collection at a later time. Deferral policies are not universal, resulting in the potential for variable qns rates among organizations due to demographic factors alone. The purpose of our study was to prospectively collect data at a single children's healthcare system to determine if history of prematurity, age, race, or weight at collection, either individually or in combination, is associated with an increased likelihood of qns collection.

Design: 217 sweat chloride tests were performed on 199 patients; 17 patients were tested twice and one three times using the Macroduct sweat collection system at Children's Healthcare of Atlanta. Data consisting of patient age, weight, history of prematurity, and race were prospectively collected by the phlebotomist at the time of service. Characteristics of patients with and without qns results were then compared and analyzed using chi-square tests, two sample t-tests, multivariate logistic regression, and regression trees.

Results: A significant association exists between the qns rate and patient weight ≤3 kg (p < 0.001) or history of prematurity (p < 0.001). Of the 44 patients ≤3kg, the qns rate was 32%, compared to 7% for those infants weighing >3 kg. Infants with a history of prematurity had a qns rate of 46%, compared to infants without a history of prematurity, where the qns rate was 7%. Infants ≤54 days of age or less at the time of sweat collection had lower qns rates (10%) compared to infants older than 54 days (24%). No association was found between African American race and qns rates. Multivariate logistic models revealed an interaction between the age of the infant at collection, history of prematurity, and weight ≤3kg (p = 0.047). As a result, the infant most likely to have a high qns rate was an infant with a history of prematurity, ≤3kg, and older than 54 days. Of the 7 infants with a combination of history of prematurity, ≤3kg, and >54 days old at sweat collection, 1 (14%) successfully completed the test compared to 6 out of 7 (86%) who had qns results. There was no difference in qns rates between older and younger patients who did not have a history of prematurity or were >3 kg (both 96%, respectively).

Conclusions: There is a higher likelihood of having a qns result for sweat collection on infants <3 months old if the infant is ≤3kg, has a history of prematurity, or is >54 days old at the time of collection. This information can be used to determine if deferral of sweat testing should be considered for such patients, and if the reporting of qns rates should be case adjusted depending on demographics of the infants undergoing sweat collection at a given institution.
A Follow Through of DNA Based Testing to Understand Utilization
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Background: DNA based testing for the diagnosis of genetic disorders is an ever-growing field with new tests available almost daily. The cost of this testing is substantial. With the changes in healthcare, hospitals are continually tasked with decreasing costs and increasing the efficient utilization of resources. At Children's Hospitals and Clinics of MN our reference lab budget was approximately $5 million dollars in 2011. An analysis of customer ordering patterns of DNA based testing was performed during the first two months of 2012.

Design: All DNA based testing was tracked including routine karyotyping. Each test type, ordering provider, inpatient vs. outpatient status, reference laboratory, and campus of origination was logged. The pathologist added the ordering physician group or subspecialty as well as the inheritance pattern of the disorder if testing was for a known syndrome. The data was then compiled and analyzed.

Results: During the two month period a total of 517 DNA based tests were ordered. Of these 22% were ordered on inpatients. Fifty-four percent of the total tests were ordered by genetics. Of the inpatient population the services with the greatest number of orders were neonatology, hematology/oncology, and genetics. In the outpatient arena genetics ordered the majority of testing with neurology and developmental pediatrics trailing a good distance behind. CGH and karyotyping were the most frequent test in the inpatient and outpatient setting with a potpourri of defined syndromic testing making up the remaining testing. In the outpatient setting the situation was similar except for a greater number of orders for Fragile X.

Conclusions: A detailed analysis of DNA based ordering patterns is invaluable in making decisions for the laboratory and hospital. In Minnesota there is a movement by some payers to only reimburse for testing previously approved by a geneticist or genetic counselor. With the increasing costs of healthcare one can imagine that this trend will continue. Information from the follow-through study can be used to assess future staffing needs of the genetics department if all DNA based tests are to be ordered with their consultation. Finally, capitated inpatient billing results in decreased reimbursement for testing. Education of clinicians ordering DNA based testing should be targeted to ensure the correct test is ordered in the appropriate setting in order to more efficiently use healthcare dollars.
Extramedullary Myxopapillary Ependymoma as a Neoplasm of Children and Adolescents

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Background: Myxopapillary ependymoma (MEPN) is a slow growing tumor of the central nervous system (WHO grade I) that occurs in the intradural space at the conus medullaris-filum terminale. Extramedullary presentation is rare, usually documented in case reports; these cases are thought to represent an anomaly of the posterior neuropore. The incidence of extramedullary MEPNs in the pediatric population is not well-established. To better understand the clinical and pathologic aspects of MEPNs in children and adolescents, we reviewed all available cases from our institutional files.

Design: The surgical pathology database was searched for all available cases of MEPN, spanning the years 1990 to 2012. Cases were tabulated based on age, gender and site of clinical presentation. Only primary extramedullary cases, not involving direct extension by an intradural spinal cord MEPN, were considered as true extramedullary examples. Groups were then compared using the two-tailed Fisher's exact test or student's t-test.

Results: A total of 49 cases of MEPN were identified in individuals between the ages of 3 weeks (wk) and 68 years (yr). Of these, 11 (22%) were diagnosed in individuals less than 20 years old (age range: 3 wk–18 yr; mean 10.3 yr) and 38 cases (78%) were adults (age range: 21-68 yr; mean age 41 yr). Primary extramedullary sites were present in 9/49 (18%) cases, of which 7/9 (78%) were diagnosed before 20 years old. The percentage of extramedullary MEPNs was statistically significant between adult (2/38=5%) and pediatric (7/11=64%) cases (P value <0.0001). The extramedullary tumors occurred at a significantly younger age (3 wk-17 yr; mean age: 7.4 yr) when compared to the intradural/spinal MEPNs in the first two decades (age range 12-18 yr; mean age: 15.3 yr) (P value= 0.047). There was a male preponderance in the same age group among the spinal MEPNs (M:F= 3:1) which was not evident in the extramedullary cases (M:F = 0.8:1). Extramedullary sites in pediatric cases were confined to the skin and/or soft tissues of the sacrococcygeal region and pelvis. Other than the location of extramedullary MEPNs, the pathologic features of papillary profiles around a central vascular and myxoid stroma were indistinguishable from the spinal tumors.

Conclusions: MEPN is an uncommon neoplasm regardless of age which presents in the distal portion of spinal cord, but unlike most other CNS neoplasm it is known to present rarely outside of the neural axis. In our series of 49 cases, 18% were extramedullary and 7 of 9 cases (78%) occurred in children with a mean age of 7.4 yr. Only 2 (5%) of 40 spinal MEPNs were diagnosed before 20 yr of age. Though not documented in all cases, there was an associated spinal dysraphism among the extramedullary tumor. Although uncommon, our series suggests that MEPN should be considered in the differential diagnosis of children and adolescents with tumors of the skin and soft tissue of the pelvic and sacrococcygeal regions.
bFGF But Not Forskolin or Dibutyryl Cyclic AMP is Permissive to Schwannian Differentiation in BRDU Treated IMR-32 and SH-SY5Y Neuroblastoma Cell Lines

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Background: Neuroblastoma is a devastating malignant tumor that develops from sympathetic neuronal lineage cells and is the most common extracranial solid tumor found in children. The proportion of Schwannian stroma contained in these tumors is used as a prognostic marker for their pathological classification. The lineage of the Schwannian stroma is still not definitively characterized. Transformation of neuroblastic cells to Schwannian or mesenchymal lineages in cell lines provides one model system for studying the biological genesis and significance of Schwannian stroma.

Design: Two neuroblastoma cell cultures, IMR-32 and SH-SY5Y, were grown on 96 well plates for a total of twelve days. Throughout their growth period, 16 unique differentiation treatments using bromodeoxyuridine (BRDU), forskolin, dibutyryl cyclic AMP (db cAMP) and basic fibroblast growth factor (bFGF)- either singly or in combination- were administered to both cell lines. They were subsequently subjected to immunofluorescence staining for tyrosine hydroxylase (TH) as a neural marker, and S100 as a Schwannian marker.

Results: Untreated control cells showed negative staining for both TH and S100 antibodies. Schwannian S100 positivity was only found in cells that had undergone BRDU differentiation treatment alone and in the combination treatment of BRDU and bFGF. The other two combination treatments containing BRDU: db cAMP and BRDU, as well as forskolin and BRDU, showed negative S100 staining.

Conclusions: Treatment of IMR-32 and SH-SY5Y neuroblastoma cell lines with BRDU alone results in Schwannian differentiation and we hypothesize that this would generalize to any anti-proliferative treatment. The action of bFGF is permissive to the Schwannian differentiation. Conversely, the presence of forskolin or dbcAMP in conjunction with BRDU prevented the Schwannian differentiation. We hypothesize that this would generalize to other treatments that raise cyclic AMP levels. Further work is needed to fully elucidate the biochemical pathways involved in Schwannian transformation of neuroblastoma cells.
Role of Yes-Associated Protein (YAP) in Hepatoblastomas

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**Background:** The Hippo kinase signalling system has been implicated in carcinogenesis but their role in liver tumors is not well-recognized. Recent studies have reported development of hepatocellular carcinomas in rodents following deregulation of Hippo kinase pathway. The downstream target of the pathway is a transcriptional coactivator called yes-associated protein (YAP) which is known to initiate several proliferative genes such as Survivin, Glypican 3 and cyclin D1. We have shown these targets to be upregulated in HCC and cholangiocarcinoma and have showed overall increased expression of YAP in HB. The aim of this study was to determine the distribution and significance of expression of YAP by immunohistochemistry in various subtypes of HB. We hope to determine if YAP staining is a useful method to differentiate subtypes of HB.

**Design:** The study was retrospective and deemed exempt by Institutional IRB. 15 samples of HB from 13 patients were stained with YAP (Cell Signaling Technologies, Danvers, MA). Case selection was based on availability of tissue and presence of different histologic components on review. Appropriate positive and negative controls were run. The staining pattern was recorded as localization: cytoplasmic or nuclear; as well as intensity of staining 1-3, and histologic subtype of tumor staining.

**Results:** The patients ranged in age from 5 months to 8 years (Mean: 2.65 years) with a M:F ratio of 8:5. There were 5 biopsy cases, 2 of which had their posttherapy resection specimen available for testing. The remaining cases included 7 post-therapy cases and 1 case of tumor recurrence. YAP was expressed normally in the cytoplasm of adjacent liver tissue serving as internal controls. Well-differentiated fetal areas were seen in 2 cases, both showing mainly cytoplasmic staining with a rare nuclear stain. The fetal with mitoses (crowded fetal) areas showed cytoplasmic staining with nuclear staining in 8/10 cases with intensity of 1-2. Embryonal areas were seen in 12 cases of which 4 were negative and 8 cases showed nuclear staining (intensity 1-3). The small cell areas (SCU) showed strong staining in all 4 cases where present. One case with cholangiolar differentiation showed nuclear staining while another teratoid example also had strong staining. Mesenchymal component also showed strong staining in 4/5 cases.

**Conclusions:** YAP appears to be over-expressed in HB and is seen in crowded fetal and embryonal cases as well as in SCU areas. They are not significantly expressed in well-differentiated fetal areas suggesting their role in proliferation rather than differentiation. They may affect more than one target gene that may include glypican 3 and cyclin D1, both known to be upregulated in HB. This study suggests the role of the Hippo kinase pathway in pathogenesis of HB.
Utility of N-Terminal and C-Terminal Beta-Catenin Staining in Hepatoblastoma
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Background: The role of beta-catenin (BC) in hepatoblastoma (HB) has been slowly unraveled over the years. It is now known that beta-catenin mutations play an important role in the pathogenesis of hepatoblastomas and may be an important pathway in carcinogenesis by activating downstream targets such as cyclin D1 or c-myc. However, its exact role is still unclear and while mutations are detected in most HB, with differences in type of deletion/mutations in fetal and embryonal HB, the morphology of this tumor is variable. We have shown nuclear localization within HB cells to be a marker of BC mutation in HB. We have also seen a role for BC in proliferation and differentiation of hepatocytes during development and have demonstrated the role of calpain in truncation of the BC protein which leads to differentiation in mouse studies, with a possible similar result in HB. We hope to expand the same hypothesis to determine a role for BC N-terminal and C-terminal antibodies in differentiating fetal and embryonal HB.

Design: The studies were performed as part of an IRB exempt study and involved retrospective review of cases with selection of cases with adequate material for staining for IHC. Positive and negative controls were run. The antibodies used were beta-catenin (#14, Ventana Med) and N-terminal beta-catenin (AA1-18, Abcam). The staining pattern of cytoplasmic, membranous and nuclear was determined for both in all areas of HB. The staining intensity was graded as 1-3.

Results: A total of 42 cases from 38 patients were selected for staining based on availability of tissue. The age ranged from 6 months to 13 years with M:F = 11:8. Both biopsy and post-treatment resections were selected based on availability. A total of 33 cases showed fetal components with 4 cases showing well-differentiated fetal and 6 showing fetal with mitoses or pleomorphism. 19 of these cases also had embryonal areas. Two examples of teratoid HB were also identified with fetal and embryonal areas. The fetal areas of all cases showed membranous and some cytoplasmic BC while nuclear staining to any extent was seen in only 6 cases, all these were negative for nuclear N-terminal BC. The embryonal areas showed nuclear BC staining with both stains in all cases. In pleomorphic epithelial tumors the staining was variable with 3 showing nuclear staining for both markers and 2 not showing either. Staining in small cell areas was variable but primitive mesenchyme was positive.

Conclusions: In this study we find the N-terminal BC stain to be useful to identify those tumors with less-differentiated areas especially embryonal component which suggests presence of the entire BC protein as opposed to only the truncated form identified by the C-terminal probe only in fetal HB areas. This is a beta-catenin mutation independent finding and appears to be a function of the non-mutational or non-deletional allele, raising questions about the oncogenic potential of beta-catenin in HB. Use of N-terminal BC stain may help differentiate embryonal HB from fetal HB in difficult cases.
Gene Expression Profiles of Alveolar and Embryonal Rhabdomyosarcoma in Formalin-Fixed Paraffin Embedded Tissue
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**Background:** Rhabdomyosarcoma (RMS) is divided into two major histologic subtypes: alveolar (ARMS) and embryonal (ERMS). ARMS is associated with poor prognosis and places the patient in a high-risk category. ARMS is associated with the characteristic translocations t(2;13) and t(1;13) resulting in the fusion genes PAX3/FOXO1 and PAX7/FOXO1, respectively. However, there are cases of fusion-negative ARMS (nARMS). Recently, nARMS and ERMS were shown to have indistinguishable gene expression profiles and similar event-free survival while fusion-positive ARMS (pARMS) was genetically distinct. The fusion status and genetic profile regardless of histology was relevant to patient outcome. These molecular studies were performed on fresh tissue. In this study we show gene expression profiles reflective of fusion status can be determined from formalin-fixed paraffin embedded tissue (FFPE).

**Design:** The molecular characteristics of archival FFPE tissue of 19 untreated RMS cases (9 ERMS, 4 pARMS, 1 nARMS and 5 fusion-unknown ARMS) were determined. RT-PCR was performed to determine fusion status of the unknown ARMS cases. Gene expression of two previously determined metagene sets (21 genes associated with pARMS and 23 genes associated with ERMS and nARMS) for the 19 RMS samples was performed using the nCounter Gene Expression Assay (NanoString, Seattle, WA). In this method, each gene is associated with two probes. One is a short sequence complimentary to a singular metagene mRNA coupled to an affinity tag, and the second is a complimentary sequence coupled to a unique color-coded tag that is the detection signal. These 88 unique probes are hybridized together in a single case, and the level of expression for each gene is measured by counting the unique signal for each mRNA.

**Results:** RT-PCR determined 1 of the 5 unknown ARMS cases was fusion positive. The remaining cases gave unsatisfactory results due to RNA degradation. However, Expression of 11/21 fusion-positive genes in the metagene set was elevated in the majority of the unknown/pARMS but not ERMS cases. Expression of 8/23 fusion-negative genes in the metagene set was elevated in the majority of the nARMS/ERMS cases but not pARMS cases.

**Conclusions:** Our study suggests FFPE archival material can be used to determine gene expression profiles of RMS samples. Although RNA from these samples could not determine fusion status, gene expression from fusion-positive and fusion-negative metagene sets aided in distinguishing pARMS from nARMS and ERMS. The ability to distinguish these entities in FFPE could prove beneficial for diagnostically challenging cases where fresh tissue is not available.