Sa2009

BIOAVAILABLE SERUM VITAMIN D AND RECTAL VITAMIN D RECEPTOR EXPRESSION AT DIAGNOSIS IN PEDIATRIC ULCERATIVE COLITIS: ASSOCIATIONS WITH DISEASE SEVERITY, CLINICAL OUTCOMES, AND RECTAL PATTERNS OF GENE EXPRESSION

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Background: Serum vitamin D (VD) deficiency and vitamin D receptor (VDR) polymorphisms have been associated with the development and severity of inflammatory bowel disease (IBD). Deletion of intestinal epithelial VDR results in more severe murine colitis, and transgenic over-expression of intestinal epithelial VDR ameliorated both spontaneous and chemical models of colitis. **Aims:** To associate disease severity and short term outcomes with serum VD fractions and rectal VDR expression in pediatric Ulcerative Colitis (UC), and to explore key biologic pathways mediated by VDR. **Methods:** 431 newly diagnosed pediatric UC patients from 29 centers were enrolled in the PROTECT study. Clinical severity was determined using the Pediatric Ulcerative Colitis Activity Index (PUCAI), and endoscopic severity was determined using the Mayo endoscopic sub-score. In 206 UC and 20 controls, the global pattern of rectal gene expression was determined using RNA sequencing. Free and total serum VD fractions were measured and bioavailable VD was calculated. Mucosal healing at week 4 was defined as fecal calprotectin (FCP) < 250 mcg/g. **Results:** Adjusted and unadjusted models revealed that while higher bioavailable VD and rectal VDR expression were associated with lower clinical severity at diagnosis (p<0.001), only higher rectal VDR expression was associated with lower endoscopic severity (p=0.0016). Consistent with this, higher levels of baseline rectal VDR expression were associated with higher rates of week 4 clinical remission (PUCAI<10, p=0.03) and mucosal healing (38% in the highest tertile vs. 14% in the lowest tertile, p=0.02). Higher baseline rectal VDR expression remained significantly associated (p=0.003) with week 4 mucosal healing in a multivariable model which included serum albumin and pan-colitis. Gene set enrichment analysis was applied to 5296 genes differentially expressed between UC patients and controls (1.5 fold-change and FDR of 0.001), and core biologic pathways were defined. Associations between plasma VD fractions, rectal VDR expression, and the first principle component (PC1) for each biologic pathway gene signature were tested. We detected associations between tertiles of serum bioavailable VD (Table1) or rectal VDR expression (Table 2) and pathways involved in epithelial cell function, but not in pathways defining innate or adaptive immune cell function. Immunohistochemistry confirmed reduction of VDR abundance on rectal epithelial cells in UC cases compared to controls. **Conclusions:** Rectal VDR expression is inversely associated with clinical and endoscopic severity at diagnosis and with initial treatment responses in pediatric UC. Gene expression analysis implicates VD-associated pathways regulating intestinal epithelial cell functions including energy production and cellular proliferation.

Table 1: Associations between Plasma Bioavailable Vitamin D and Epithelial and Immune Gene Expression Signatures.

		Tertile of Plasma Bioavailable Vitamin D			
	Total Sample (n=198)	Tertile 1 (n=66)	Tertile 2 (n=66)	Tertile 3 (n=66)	P-value
		<1.05 ng/mL	1.06-1.755 ng/mL	>1.755 ng/mL	
Component:					
Epithelial Functions					
Mitochondrial function	-2.341 (24.611)	-7.278 (29.6)	-2.987 (24.670)	3.241 (17.152)	0.0137
Adenocarcinoma-epithelial proliferation	1.622 (7.549)	3.024 (7.313)	2.423 (8.380)	0.580 (6.438)	0.00581
Immune Functions					
Innate immune response	2.956 (11.907)	2.887 (12.529)	4.035 (12.204)	1.946 (11.023)	0.6651
Adaptive lymphocyte immune response	2.741 (12.515)	2.29 (13.058)	4.180 (13.074)	1.753 (11.401	0.806

Data are shown as the mean(SD) of the first principle component of gene expression for the indicated pathways. P-values for analysis of variance.

Table 2: Associations between Rectal VDR Expression and Epithelial and Immune Gene Expression Signatures.

		Tertile of Rectal VDR Expression			
	Total sample (n=206)	Tertile 1 (n=69)	Tertile 2 (n=69)	Tertile 3 (n=68)	
		≤15.772 TPM	>15.773-22.290 TPM	>22.291-62.13 TPM	P-value
Component					
Epithelial functions					
Mitochondrial function	-2.432 (24.839)	-26.76(24.74)	4.377 (12.674)	15.340 (11.21)	<0.0001
Adenocarcinoma - epithelial proliferation	1.641 (7.522)	9.399 (4.782)	0.946 (3.753)	-5.526 (4.582)	<0.0001
Immune functions					
Innate immune response	2.844 (12.126)	1.131 (15.118)	6.590 (9.690)	0.782 (10.043)	0.877
Adaptive lymphocyte immune response	2.603 (12.665)	0.429 (15.845)	6.33 (10.018)	1.027 (10.601)	0.773

Data are shown as the mean(SD) of the first principle component of gene expression for the indicated pathways. P-values for analysis of variance.

Sa2010

INFLUENCE OF MATERNAL SSRI EXPOSURE ON THE DEVELOPMENT OF THE ENTERIC NERVOUS SYSTEM AND ENTEROCHROMAFFIN CELLS

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Background: Antidepressants, including selective serotonin reuptake inhibitors (SSRIs) are commonly used during pregnancy. Approximately 7% of women in North America require SSRI in the perinatal period. Perinatal exposure to SSRIs has been shown to disrupt the development of serotonergic signaling pathways in the brain and enteric nervous system (ENS). Serotonin (5-HT) signaling in the intestine is critical for intestinal function and dysregulation of this pathway is associated with intestinal disease. The gastrointestinal (GI) tract contains 95% of the body's 5-HT. **Aims:** To test the hypothesis that perinatal exposure to SSRIs can influence the development of the ENS and enterochromaffin (EC) cells. **Methods:** Female Wistar rats were given fluoxetine (10mg/kg/d) or vehicle (cookie dough) from 2 weeks prior to mating until weaning (postnatal day [P] 21). Offspring were collected from SSRI-treated and control rats on P1, P21 and P6 months. Enteric neurons in the myenteric plexus were visualized in whole mount preparations of jejunum, ileum and colon. EC cells were visualized in the colon. Total number (#) of enteric neurons (EN), serotonergic neurons (SN) and EC cells were visualized with immunostaining using antibodies to HuC/D and 5-HT, respectively. Percentage of SSRI-expressing enteric neurons were calculated as a percentage of total neurons and EC cells were expressed per 10 crypt/villus. using image analysis software (Velocity). Female (F) and male (M) offspring were analyzed separately. **Results:** On P1, a significant decrease between the total # of EN and SN was found in the SSRI-exposed F offspring colon (13.6% vs 9.3%; p=0.04; n=6-9) in the fluoxetine exposed group compared to controls. On P21, significant differences were found in the percentage of SN in both F (1.4% vs 7.6%; p=0.009); n=5-6) and M colons (1.7% vs 6.9%; p=0.002; n=5-8). The number of EC cells at P1 and P21 was not statistically significant. At 6 months of age, there was no significant difference in the percentage of total # of EN, SN nor in EC between SSRI-exposed and control offspring. **Conclusions:** Our results suggest that SSRI exposure in utero and perinatal period play a role in serotonergic signaling pathways involved in the development of the ENS of the developing offspring with most notable effects at P21 which correlates to maximal exposure of SSRI. However, there was no statistically significant differences in EC cells. The ENS appears to improve over time, which may be attributed to the plasticity of the ENS.

Sa2011

NOVEL CANDIDATE GENES IN ESOPHAGEAL ATRESIA/ TRACHEOESOPHAGEAL FISTULA IDENTIFIED BY WHOLE EXOME SEQUENCING

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Introduction: Esophageal atresia/tracheoesophageal fistula (EA/TEF) is a congenital anomaly with an estimated incidence of 1 in 2500 to 4000 live births. Approximately half of affected patients have associated birth defects and are classified as syndromic or complex. Associated malformations include cardiovascular, digestive, urogenital, and musculoskeletal malformations, or VACTERL (**v**ertebral, **a**norectal, **c**ardiac, **t**racheo**e**sophageal, **r**enal and **l**imb anomalies) association. Reports exist linking the VACTERL association to mutations in *FOXF1* and *ZIC3*, but the molecular etiology for most VACTERL cases remains unknown. Chromosome anomalies, including aneuploidies and microdeletions, account for 6-10% of syndromic EA/TEF. In an effort to identify novel, *de novo* genetic variants and genes associated with EA/TEF that would enhance diagnostic capability and improve prognostic ability, we performed exome sequencing on 20 individuals with EA/TEF and their biological parents. **Aim:** To delineate the genomic architecture of EA/TEF and refine the clinical characterization of malformations associated with EA/TEF. **Methods**: Patients with isolated and non-isolated EA/TEF were recruited from two medical centers (Columbia University Medical Center in New York, USA and Cairo University General Hospital in Cairo, Egypt). Blood and/or saliva

samples were obtained from the probands and both biological parents. Exome sequencing was performed at an average of 85X read depth, and *de novo* variants were identified. **Results:** Twenty individuals (7mo-30years) with EA/TEF were studied. Seven probands had isolated EA/TEF, 13 probands had neurocognitive delay and/or at least one additional congenital defect and were classified as complex. We identified 30 *de novo* variants; 11 novel *de novo* predicted deleterious variants were identified in 9 of 20 trios. Eight out of 9 patients (5 males) had complex phenotypes. Three cellular pathways were implicated: cell adhesion/migration (*PCDH1*, *APC2*, *PIK3C2G*), vesicle trafficking (*RAB3GAP2*, *AP1G2*), and autophagy (*TECPR1*). **Conclusions**: In our pilot study, we identified 11 novel *de novo*, predicted deleterious variants in genes not previously implicated in EA/TEF. Information from this pilot can be used to test the feasibility of genomic characterization to provide more accurate clinical prognostic information and combine the same type of analysis with other congenital malformations to provide a unified understanding of how the human body develops.

Sa2012

MULTICHANNEL INTRALUMINAL IMPEDANCE WITH PH TESTING REDUCES PROTON PUMP INHIBITOR USAGE IN CHILDREN WITH NONEROSIVE REFLUX

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Introduction: Proton pump inhibitors (PPIs) are widely used in the diagnosis and management of pediatric gastroesophageal reflux (GERD). However, recent data in adults and children suggests that these medications can be associated with significant adverse effects and should be used judiciously. The current gold standard for diagnosing GERD remains multichannel intraluminal impedance with pH testing (pH-MII). However, little is known about whether pH-MII results change management or predict clinical outcomes. The aim of this study was to determine the prevalence of PPI therapy for different reflux phenotypes at follow-up after pH-MII. **Methods:** Children ≥ 5y who underwent upper endoscopy and pH-MII for evaluation of typical reflux symptoms (pain, heartburn, chest pain, reflux or regurgitation) between 2004 and 2016 were included. All children underwent a minimum of an 8-week PPI trial prior to diagnostic studies, though all studies were performed off PPI therapy. Children with eosinophilic or erosive esophagitis, a history of thoracic or abdominal surgery and those who did not report symptoms during pH-MII were excluded. The results of pH-MII were used to categorize patients into nonerosive esophageal phenotypes: those with abnormal esophageal acid exposure (pH < 4 for > 6% of the study) were classified as having nonerosive reflux disease (NERD), those with normal acid exposure but a positive symptom index (SI) were classified as reflux hypersensitivity (RH) and those with normal acid exposure and negative SI were classified as functional heartburn (FH). PPI use at follow-up after pH-MII was determined from review of the medial record. **Results**: A total of 45 children met inclusion criteria: 29% were classified as NERD, 29% had RH and 42% had FH. Follow-up medication data was available in 39 children as 6 children were lost to follow-up after pH-MII (2 NERD, 1 RH and 3 FH). Patients remaining on PPI at the time of the first follow-up visit after pH-MII included 79% of all patients, 91% of NERD patients, 92% of those with RH and 63% with FH. Based on pH-MII results in patients with NERD, 46% continued PPI at the same dose, 27% increased dose, 18% changed brands and 9% discontinued PPI. In patients with RH, 42% continued PPI, 17% increased dose, 25% decreased dose, 8% changed brands and 8% discontinued PPI. In patients with FH, 31% continued PPI, 44% discontinued PPI, 13% changed brands, 6% increased dose and 6% decreased dose. In total, 33% of children either discontinued PPIs or decreased the total dose based on pH-MII results. **Conclusions**: The majority of children remain on PPI therapy after pH-MII testing. However, pH-MII results led to decreasing the PPI dose or discontinuing the medication in one third of patients. Children with FH were most likely to have reduced PPI usage.

Sa2013

QUALITY OF LIFE IN CHILDREN WITH TYPICAL AND ATYPICAL SYMPTOMS OF GASTROESOPHAGEAL REFLUX

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Introduction: Symptoms of gastroesophageal reflux disease (GERD) can have a significant impact on quality of life (QOL) in children. Adult studies suggest that patients with typical esophageal symptoms have worse QOL scores compared to patients with atypical or extraesophageal manifestations. However, little is known about the impact of reflux symptom type on QOL in children. **Methods:** We performed a prospective study of 47 children ≥ 5y undergoing multichannel intraluminal impedance with pH (pH-MII) testing and upper endoscopy for the evaluation of suspected GERD. The pH-MII study was considered abnormal if there was > 73 reflux episodes or if the pH < 4 for ≥ 6% of the study. Patient reported QOL scores were assessed using the validated Pediatric Gastroesophageal Symptom and Quality of Life Questionnaire (PGSQ), which provides subscales for symptom burden as well as daily, school and total impact. Higher scores indicate more significant impact on daily life. Previously reported mean scores for healthy control patients are < 0.2. Symptoms reported during pH-MII were categorized into typical reflux symptoms (heartburn, chest pain, epigastric abdominal pain and regurgitation) and atypical reflux symptoms (cough, dysphagia, throat clearing, gagging, nausea, vomiting, spitting, hoarseness, gulping and dyspnea). **Results**: The mean PGSQ score was 0.96 ± 0.66 for symptom sub-scale, 1.45 ± 3.42 for daily life sub-scale, 0.56 ± 0.92 for school sub-scale and 1.03 ± 0.72 for total score. There were no significant differences in PGSQ total and sub-scores in patients reporting typical symptoms and those reporting atypical symptoms (p > 0.06). When examining individual symptoms reported during pH-MII testing, patients reporting heartburn had a worse QOL with higher symptom (p = 0.04), school (p = 0.04) and total scores (p = 0.03) compared to patients without heartburn. Patients reporting pain had higher symptom (p = 0.01) and total (p = 0.03) scores. Report of nausea was associated with higher symptom (p = 0.008), school (p < 0.0001) and total (p = 0.03) scores. Patients reporting vomiting had a higher total score (p = 0.02). There were no differences in QOL scores for any other symptoms. 28% of patients had an abnormal pH-MII study and 19% of patients had microscopic esophagitis present on histology. Neither reflux/acid burden on pH-MII nor the presence of microscopic esophagitis had any significant impact on QOL scores (p > 0.17). **Conclusions**: There are no significant differences in QOL scores in children with typical versus atypical reflux symptoms. Specific reports of heartburn, pain, nausea and vomiting during pH-MII testing are associated with worse QOL scores. Abnormal reflux or acid burden on pH-MII and the presence of microscopic esophagitis is not associated with worse QOL scores.

Sa2014

GASTROESOPHAGEAL REFLUX AND FUNDOPLICATION STATUS DO NOT PREDICT MEDIUM-TERM LUNG ALLOGRAFT OUTCOMES IN CHILDREN

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Background: Gastroesophageal reflux disease (GERD) is common in patients post-lung transplantation. Aspiration of gastric contents is considered a potential cause for the development of chronic allograft failure. However, the exact mechanisms are not well understood and evidence in children remains unclear. Our aim was to characterize GERD in pediatric lung transplant recipients and to evaluate the impact of GERD and fundoplication status on survival and development of obstructive chronic lung allograft dysfunction (CLAD). **Methods:** Patients who underwent lung transplantation at Texas Children's Hospital between September 2011 and February 2017, survived at least 6 months and had post-transplant combined pH and multichannel intraluminal impedance (pH-MII) studies were retrospectively reviewed. Demographic data, bronchoalveolar lavage fluid analysis, transbronchial biopsy results, pulmonary function testing, and fundoplication status were also assessed. **Results:** Fifty-nine patients met inclusion criteria, with median age at time of lung transplant 10.8 years (range 0.2-18.8 years). pH-MII studies were performed at median of 47 days post-transplant with median total follow-up time of 23.8 months. Eighteen of 59 patients (30.5%) had abnormal reflux by pH monitoring; 3 (5%) had abnormal reflux by impedance, and 5 (8.4%) had abnormal pH and MII. Overall reflux profiles are shown in Table 1. GERD parameters by pH-MII testing (study time pH<4, number of reflux episodes (acid, non-acid and total), proximal reflux burden) were not associated with development of CLAD, graft failure or mortality. Patients who subsequently underwent fundoplication (n=21) had significantly higher reflux burden compared to patients without fundoplication (n=38): (mean %time pH<4: 14.7% versus 4.7%, respectively, p=.03; mean total number of reflux episodes: 55.9 versus 31.2, respectively, p=.04). However, patients with and without fundoplication did not have significantly different rates of CLAD, graft failure or mortality. **Conclusions:** Although GERD is common in children post-lung transplantation, reflux parameters measured by pH-MII testing and fundoplication status do not appear to predict medium-term lung allograft outcomes. Further studies are needed to understand the role of GERD in development of CLAD and optimal management.

Reflux Parameters by pH-MII Monitoring in Children Post-Lung Transplantation (n=59)

Reflux Parameter	Mean	Standard Error
Study %Time with pH<4	8.24%	1.8%
Total reflux episodes	40.0	4.9
Total acid reflux episodes	19.7	2.9
Total nonacid reflux episodes	19.8	2.9
Total full column reflux episodes	10.4	1.9
Full column %	22.8%	2.7%

Sa2015

THE TIMED BARIUM ESOPHAGOGRAM, A USEFUL ADJUNCT IN EVALUATING PEDIATRIC ESOPHAGEAL MOTOR DISORDERS

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Background: The timed barium esophagogram (TBE) is a further development of the barium swallow, introducing functional and dynamic dimensions. As it provides functional data on esophageal emptying, it has been valuable in achalasia and other disorders in adults. It is also helpful in studying the response to treatment such as dilation or surgery. It is however, a test that currently is not common in children. *Aim:* The purpose was to assess the usefulness of TBE in children with symptoms suggestive of esophageal emptying disorders. To do so, we focused on the impact-on-treatment made by the information provided by TBE, compared with that provided by high-resolution esophageal manometry (ESMO). *Approach:* We studied 5 children with symptoms suggestive of an esophageal emptying disorder. After fasting, patients drank100-250 mL of low-density barium sulfate suspension. Radiographs of the esophagus were exposed at 1, 2 and 5 min after barium ingestion. The heights and widths of the barium column and changes in these parameters over time (esophageal emptying) were assessed. *Results:* Patients were 14-18 year old and 3 were female. Healthy individuals empty their esophagus effectively and promptly within 2 min. In patients diagnosed with achalasia (3) all TBE variables differed profoundly compared to the controls, and at 5 minutes the column of barium had not decreased from the 1 and 2 min measurements. In one patient there were 2 problems detected: One was a mid-esophageal body stenosis associated with a prior TEF repair which was known, but the TBE made apparent a second problem: poor emptying of the distal esophagus that was causing progressive worsening of dysphagia. In all cases with abnormal TBE, the study led to clinically successful esophageal dilatation. The ESMO results would have led to the same treatment in 2/3. In one patient, however, where 2 previous dilatations had already been done, it did not show an increased LES pressure, only poor body motility and thus the result was not indicative of a need to repeat dilation. The TBE however demonstrated the persistent esophageal-emptying problem, as the barium column did not decrease at 5 min compared to 1 and 2 min films. This patient received third esophageal dilation and clinically improved. *Conclusions:* In this study we compared the value in regard to impact-on-treatment of two tests, TBE and ESMO in children with symptoms suggestive of an esophageal emptying disorder. Our results show a clear difference in the TBE values in patients with achalasia and those in controls suggesting its diagnostic value. We also find that the information provided by the two tests is complementary