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


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 VDRameliorated 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). The increase in 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 (Table 1) 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. The ENS appears to improve over time, which may be attributed to the plasticity of the ENS.

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

<table>
<thead>
<tr>
<th>Component</th>
<th>Total Sample (n=198)</th>
<th>Tertile 1 (n=66)</th>
<th>Tertile 2 (n=66)</th>
<th>Tertile 3 (n=66)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitochondrial function</td>
<td>-2.341 (24.611)</td>
<td>-7.278 (29.6)</td>
<td>-2.987 (24.670)</td>
<td>3.241 (17.152)</td>
<td>0.0037</td>
</tr>
<tr>
<td>Adenosine - epithelial proliferation</td>
<td>1.622 (7.569)</td>
<td>3.024 (7.311)</td>
<td>2.423 (6.380)</td>
<td>0.589 (6.438)</td>
<td>0.0058</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Component</th>
<th>Total Sample (n=206)</th>
<th>Tertile 1 (n=69)</th>
<th>Tertile 2 (n=69)</th>
<th>Tertile 3 (n=68)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intimate immune response</td>
<td>2.956 (11.907)</td>
<td>2.887 (12.529)</td>
<td>4.035 (12.204)</td>
<td>1.946 (11.023)</td>
<td>0.065</td>
</tr>
<tr>
<td>Adaptive lymphocyte immune response</td>
<td>2.741 (12.515)</td>
<td>2.29 (13.058)</td>
<td>4.110 (13.074)</td>
<td>1.753 (11.401)</td>
<td>0.006</td>
</tr>
</tbody>
</table>

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.

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 SSRIs 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 P60 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 (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 the female offspring (7.6% vs 7.6% p=0.001; n=6-9) 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.

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 cardiac, vascular, digestive, urogenital, and musculoskeletal abnormalities, or VACTERL (vertebral, anorectal, cardiac, tracheoesophageal, renal and limb anomalies) association. Recent discovery of the VACTERL association in FOXF1 and ZIC3, but the molecular etiology for most VACTERL cases remains unknown. Chromosome abnormalities, including aneuploidies and microdeletions, accounts for 6-10% of 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. Aims: 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 saliva samples were obtained and DNA was extracted from peripheral blood lymphocytes. Next Generation Sequencing (NGS) of DNA was performed using a custom VACTERL gene panel of 137 genes previously associated with EA/TEF and 83 genes identified through whole-exome sequencing of patients with EA/TEF. Results: Four novel CNVs were identified and classified as de novo variants. Three patients had CNVs that segregated with the disease phenotype in a Mendelian fashion, while one patient had a CNV that did not segregate with the disease phenotype. The most significant CNV identified was a 54.3 kb deletion in patient 3, identified at the breakpoint. Conclusion: The identified CNVs were shown to be de novo and are likely causative of the condition. These findings provide additional insights into the complex and heterogeneous nature of EA/TEF and may have implications for the future diagnosis and management of EA/TEF patients.
Twenty individuals (7mo-30 years) 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 tros. Eight out of 9 patients (5 males) with complex phenotype had mutations in any of the genes implicated in vesicle trafficking (PCDH1, APC2, PKD2G2), vesicle trafficking (RAB4B, AP2G1, and AP2E1) (TGFPR1). 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 study could be used to test the feasibility of genome 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.

MULTICHANNEL INTRALUMINAL IMPEDANCE WITH PH TESTING REDUCES PROTON PUMP INHIBITOR USAGE IN CHILDREN WITH NONEROSE REFLUX
Lisa B. Mahoney, Rachel Rosen

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 impact of pH-MII on 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 8h of 24h pH-MII trial prior to diagnostic studies, though all studies were performed on PPI therapy. Children with cosinophilic 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 esophagitis phenotypes: those with normal 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 medical 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 between 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% increased continued PPI, 17% increased 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, 35% 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.

QUALITY OF LIFE IN CHILDREN WITH TYPICAL AND ATYPICAL SYMPTOMS OF GASTROESOPHAGEAL REFLUX
Lisa B. Mahoney, Rachel Rosen

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 extracorporeal reflux 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 subcales for symptom burden as well as school and social impact. Results at follow-up on the pH-MII were compared with those on the PGSQ. Results: There were no significant differences in PGSQ total and sub-scales in patients reporting typical symptoms and those reporting atypical symptoms (p > 0.08). 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 = 0.05), social (p = 0.03) and total scores (p = 0.03) compared to patients with pain. Patients reporting nausea had higher symptom (p = 0.09), total (p = 0.03) and social scores (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 symptom. Conclusion: 29% of patients had an abnormal pH-MII study and 19% of patients had microscopic esophagitis present on histology. Neither refluxacid 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.