Rectal microRNAs are perturbed in pediatric inflammatory bowel disease of the colon

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KEYWORDS
Inflammatory bowel disease; Crohn's disease; Ulcerative colitis; MicroRNA; Serum microRNA; Circulating microRNA

Abstract

**Background and aims:** Changes in intestinal microRNAs have been reported in adult patients with ulcerative colitis or Crohn's disease. The goal of this study was to identify changes in microRNA expression associated with colitis in children with inflammatory bowel disease.

**Methods:** Rectal mucosal biopsies (n = 50) and blood samples (n = 47) were collected from patients with known or suspected inflammatory bowel disease undergoing endoscopy. Rectal and serum microRNA levels were profiled using the nCounter® platform and the TaqMan® low-density array platform, respectively. Significantly altered microRNAs were validated in independent sample sets via quantitative RT-PCR. In vitro luciferase reporter assays were performed in the human colorectal Caco-2 cell line to determine the effect of miR-192 on NOD2 expression.

**Results:** Profiling of rectal RNA identified 21 microRNAs significantly altered between control, UC, and colonic CD sample groups. Nine of the ten microRNAs selected for validation were confirmed as significantly changed. Rectal miR-24 was increased 1.47-fold in UC compared to CD samples (p = 0.0052) and was the only microRNA altered between IBD subtypes. Three colitis-associated microRNAs were significantly altered in sera of disease patients and displayed diagnostic utility. However, no serum microRNAs were found to distinguish ulcerative colitis from Crohn's colitis. Finally, miR-192 inhibition did not affect luciferase reporter activity, suggesting that miR-192 does not regulate human NOD2.

**Conclusion:** This study has demonstrated that rectal and serum microRNAs are perturbed in pediatric inflammatory bowel disease. Future studies identifying targets of inflammatory bowel disease-associated microRNAs may lead to novel therapies.

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**Abbreviations:** IBD, inflammatory bowel disease; UC, ulcerative colitis; CD, Crohn's disease; L2 CD, Crohn's colitis; miRNA, microRNA; UTR, untranslated region; SAM, Significance Analysis of Microarrays; TuD, tough decoy; NOD2, nucleotide-binding oligomerization domain containing 2; ROC, receiver operating characteristic; AUC, area under the ROC curve.

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Rectal microRNA in pediatric IBD

1. Introduction

Crohn's disease (CD) and ulcerative colitis (UC) are the predominant forms of inflammatory bowel disease (IBD), a debilitating disorder of the gastrointestinal tract for which no cure has been developed. Approximately 30% of patients with IBD receive their diagnosis during childhood. Children with IBD often experience pediatric-specific complications including growth failure and pubertal delay. The diagnosis of IBD in children is commonly delayed, which can exacerbate these complications; the majority of children with IBD have growth failure at the time of diagnosis. Current therapeutic interventions are unable to permanently ameliorate symptoms and many are associated with potentially severe side effects. Elucidating the causal and perpetuating mechanisms of IBD in children will facilitate novel therapeutic approaches, enabling normal maturation and reducing morbidity.

MicroRNAs (miRNAs) are short, non-coding RNAs that repress protein translation by recruiting RNA-induced silencing complex (RISC) proteins to target mRNAs, thereby promoting deadenylation and inhibiting ribosomal machinery. The function of miRNAs is critical to the development and homeostasis of the intestine; mouse intestinal epithelia devoid of all mature miRNAs lack proper barrier integrity, are depleted of goblet cells, and develop inflammation reminiscent of IBD, suggesting that miRNAs are required for proper intestinal function. MiRNA expression is perturbed in numerous pathologies, including IBD. For example, Wu et al. characterized miRNA levels in sigmoid colon biopsies from adult patients with active and inactive UC, and controls. They identified 11 miRNAs that are significantly changed in active UC patients and show promising diagnostic characteristics. In this study, rectal miRNAs were profiled in children with UC or Crohn's colitis (Paris classification: L2 CD) and in control patients. Several of the most abundant rectal miRNAs were significantly altered in IBD biopsies. A panel of nine miRNAs was confirmed as altered by qRT-PCR in an independent set of samples. Several miRNAs altered in rectal IBD tissues were also significantly changed in the serum of IBD patients and show promising diagnostic characteristics.

2. Methods

2.1. Study subjects

All patient samples were obtained at the Center for Pediatric Inflammatory Bowel Disease at the Children's Hospital of Philadelphia as part of an institutional review board-approved protocol. All subjects or parents/guardians gave informed consent to participate in the study. Control samples were obtained from patients undergoing endoscopy for suspicion of IBD whose tissues were subsequently identified as histologically normal. All IBD patients had histological evidence of chronic rectal inflammation. The diagnoses of UC and CD were made by the treating physician and JRF based on standard endoscopic, histologic, and radiographic criteria. No significant difference in the endoscopic appearance of the rectum was found between IBD groups (Table 1). All CD patients included in the study had disease restricted to the colon (L2 CD, Paris classification), as these can be the most difficult for physicians to distinguish from UC. There was no significant difference in time from an IBD diagnosis to rectal biopsy between UC and L2 CD groups. Only one UC patient and one L2 CD patient were treatment naive at the time of biopsy. Patient demographic and clinical information is summarized in Table 1.

2.2. RNA isolation

Total RNA was prepared from tissue biopsies and sera using the mirVana™ miRNA Isolation Kit (Life Technologies, Grand Island, NY). Frozen tissue biopsies were pulverized on dry ice using a chilled pestle and immediately processed according to manufacturer’s instructions. For circulating RNA experiments, RNA was isolated from 60 μL of serum. Following denaturation, exogenous Caenorhabditis elegans miRNAs (6 μL containing cel-miR-54 at 22.5 pM and cel-miR-238 at 15.0 pM) were added for use as normalizers and 3.0 μg MS2 RNA (Roche, Basel, Switzerland) was added as a carrier to increase RNA yield.

2.3. MiRNA profiling and quantitative RT-PCR

Rectal miRNA levels were determined using the nCounter® Human v1 miRNA Expression Assay Kit (NanoString Technologies, Seattle, WA). Data was processed following the removal of all erroneous miRNAs measured by the Human v.1 kit but no longer recognized as miRNAs by miRBase (Supplemental Table 1). Serum miRNA profiling was performed using TaqMan® Low Density Array Human MicroRNA Panels (Life Technologies). Data was normalized using the median threshold cycle (Ct) of each array. Reverse transcription and real-time PCR amplification of miRNAs was performed using TaqMan® MicroRNA Assays (Life Technologies) according to manufacturer’s Instructions. For tissue and serum RNA experiments, 33 ng of RNA and 3.33 μL of eluted RNA preparations were reverse transcribed, respectively. MiRNA expression from tissue RNA was normalized using the average of RNU44 and RNU48 levels and from serum using the average level of the exogenous C. elegans miRNAs added during RNA isolation. Reverse transcription of miRNAs was performed with oligo(dT) and random hexamer priming and using SuperScript® II RTase (Life Technologies) with 1 μg RNA as template. Real-time PCR amplification was performed using Power SYBR® Green PCR Master Mix (Life Technologies).
2.4. Expression and reporter plasmids

Tough decoy (TuD) expression plasmids targeting miR-192/215 (TuD192) or containing a scrambled sequence binding site (TuDctrl) were generated by ligating double-stranded oligonucleotides (TuD192fwd/TuD192rev and TuDctrl-T/TuDctrl-B, respectively) into BfuAI cut pEN TuD parent plasmid. The pEN TuD parent was derived from pEN_hU6 miRc224 by replacing the 768 bp NdeI-XbaI fragment with a double stranded oligo (hU6TuDtop/hU6TuDbot) that introduced the forward and reverse ‘stem 1’ sequences of the TuD hairpin, separated by a linker containing two BfuAI sites. To create a human nucleotide-binding oligomerization domain containing 2 (NOD2)′ untranslated region (UTR) reporter plasmid, a 1292 bp fragment containing the 3′ UTR of NOD2 was amplified via nested PCR from HEK293 genomic DNA, digested with XhoI and NotI, and cloned into pMiRCheck2. Site-directed mutagenesis of predicted miR-192 binding sites was performed via overlap PCR. The miR-192 sensor reporter plasmid was generated by ligating a double-stranded oligonucleotide (192 sensor fwd/rev) into XhoI-NotI cut pMiRCheck2. Oligonucleotide sequences used in this study are listed in Supplemental Table 2.

2.5. Cell culture and luciferase assay

Caco-2 human colonic epithelial cells (ATCC, Manassas, VA) were maintained at 5% CO2 in DMEM supplemented with penicillin, streptomycin, and 20% fetal bovine serum. For reporter and expression experiments, 7.5 × 10⁴ cells were seeded in 24-well plates with 500 μL of culture media. After 24 h, cells were transfected with 900 ng of TuD plasmid and 10 ng of reporter plasmid in growth media lacking antibiotics using FuGENE® HD transfection reagent (Promega, Madison, WI). Renilla and firefly luciferase activities were determined 48 h after transfection using the Dual-Luciferase® Reporter Assay System (Promega). Renilla:firefly ratios were corrected for the effect of TuD expression on an empty pMiRCheck2 Table 1: Patient characteristics.

<table>
<thead>
<tr>
<th>Rectum</th>
<th>Control (n = 20)</th>
<th>UC (n = 18)</th>
<th>L2 CD (n = 12)</th>
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<tbody>
<tr>
<td>Age, y</td>
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<td>15.8 ± 3.6</td>
<td>15.4 ± 3.9</td>
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<tr>
<td>Sex, % male</td>
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<td>38.9</td>
<td>33.3</td>
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<tr>
<td>Race, % white</td>
<td>70.0</td>
<td>77.8</td>
<td>66.7</td>
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<tr>
<td>Left-side colitis, %</td>
<td>0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Years since diagnosis</td>
<td>N/A</td>
<td>3.2 ± 3.3</td>
<td>2.6 ± 2.1</td>
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<tr>
<td>Endoscopic appearance of rectum, n</td>
<td>N/A</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Normal</td>
<td>N/A</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Mild</td>
<td>N/A</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Moderate</td>
<td>N/A</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Severe</td>
<td>N/A</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Naive patients, n</td>
<td>N/A</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Treatment at time of bx, n</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Steroids</td>
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<td>11</td>
<td>3</td>
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<tr>
<td>Anti-TNF</td>
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<td>3</td>
</tr>
<tr>
<td>Methotrexate or 6-MP</td>
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<td>2</td>
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<td>Enteral therapy</td>
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<td>0</td>
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<td>Serum</td>
<td>Control (n = 18)</td>
<td>UC (n = 18)</td>
<td>L2 CD (n = 11)</td>
</tr>
<tr>
<td>Age, y</td>
<td>15.1 ± 2.2</td>
<td>16.1 ± 3.4</td>
<td>15.2 ± 4.1</td>
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<tr>
<td>Sex, % male</td>
<td>38.9</td>
<td>38.9</td>
<td>27.3</td>
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<tr>
<td>Race, % white</td>
<td>67.7</td>
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<td>72.7</td>
</tr>
<tr>
<td>Left-side colitis, %</td>
<td>0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Years since diagnosis</td>
<td>N/A</td>
<td>3.2 ± 3.2</td>
<td>2.5 ± 2.1</td>
</tr>
<tr>
<td>Endoscopic appearance of rectum, n</td>
<td>N/A</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Normal</td>
<td>N/A</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Mild</td>
<td>N/A</td>
<td>7</td>
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</tr>
<tr>
<td>Moderate</td>
<td>N/A</td>
<td>6</td>
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<tr>
<td>Severe</td>
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<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Naive patients, n</td>
<td>N/A</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Treatment at time of bx, n</td>
<td></td>
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</tr>
<tr>
<td>Steroids</td>
<td>0</td>
<td>10</td>
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<td>Anti-TNF</td>
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<td>Methotrexate or 6-MP</td>
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<tr>
<td>Enteral therapy</td>
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Continuous variables are presented as mean ± standard deviation. UC, ulcerative colitis; L2 CD, Crohn’s colitis; 6-MP, mercaptopurine; bx, biopsy. No significant difference between groups was observed for any continuous variable. No significant difference was observed for endoscopic appearance or any treatment modality between IBD groups.
plasmid and the Renilla:firefly for each reporter plasmid was scaled such that the mean Renilla:firefly of TuDctrl-treated cells equaled one.

2.6. Statistical analysis

Significance Analysis of Microarrays (SAM) software (Stanford University, Stanford, CA) was used to analyze nCounter and TaqMan low-density array data. Remaining statistical calculations were performed with Stata 11.0 (StataCorp, College Station, TX). Hierarchical cluster analysis using complete linkage with Euclidean distance was performed using expression data for all 96 miRNAs detected in each patient sample. Heat maps were generated using Matrix2png. Differences in miRNA and mRNA expression among validation groups were determined using the Kruskal–Wallis test with the Holm–Bonferroni post-hoc method. Differences in luciferase activity were determined using the Wilcoxon signed-rank test. Differences in nominal variables were determined using the chi-square test. Differences in categorical variables were determined using the Wilcoxon rank-sum test. Spearman's rank correlation coefficient was used to determine statistical dependence between variables.

3. Results

3.1. Profiling of rectal miRNA in pediatric inflammatory bowel disease

To determine if colitis in pediatric IBD patients is associated with changes in intestinal miRNA levels, rectal miRNAs were profiled in patients with UC or L2 CD and age- and sex-matched controls (n = 6, 5, 6) using the nCounter® Human v.1 miRNA Expression Assay Kit (NanoString Technologies). This platform detects 654 human miRNAs and provides direct miRNA counts without the need for amplification, thereby allowing assessments of relative miRNA abundance. L2 CD patients were selected because this is the specific population in which distinguishing CD from UC may be the most difficult; thus differential miRNA expression may have diagnostic utility in this population. In all three sample groups, miR-21 and miR-142-3p were the most abundant miRNAs, together accounting for over 20% of the total rectal miRNA (Fig. 1A, Supplemental Table 3). MiR-192 and miR-194, previously shown to be abundant in intestinal epithelia, were also highly expressed. Hierarchical clustering analysis revealed that rectal miRNAs distinguish UC rectal biopsies from those of L2 CD and control patients (Fig. 1B). SAM analysis was performed to identify miRNAs...
significantly altered among sample groups. Of the 96 miRNAs expressed above background in all 17 samples, 21 miRNAs were significantly altered at a false discovery rate of 3.1% (Fig. 1C).

3.2. Confirmation of IBD-associated rectal miRNA

To validate the IBD-associated rectal miRNAs, qRT-PCR was performed using RNA from the remaining rectal biopsies: a distinct set of control (n = 14), UC (n = 12), and L2 CD (n = 7) subjects. Of the 10 miRNAs selected for validation, all except miR-150 were significantly altered between sample groups. Four miRNAs that are enriched in epithelial cells (miR-192, miR-194, miR-200b, and miR-375) were significantly decreased by at least 50% in UC patients compared to controls (Fig. 2A). Likewise, four miRNAs that are highly expressed in inflammatory cells (miR-142-3p, miR-146a, miR-21, and let-7i) were increased in UC patients compared to controls (Fig. 2B). Only miR-375 and miR-21 were significantly altered in L2 CD patients relative to controls. Significant associations between rectal miRNAs and treatment groups were observed in UC patients receiving the immunomodulator 6-mercaptopurine or methotrexate. These patients showed a significant elevation of miR-375 and miR-192 compared to UC patients not receiving immunomodulators (Supplemental Fig. 1A). There was no significant correlation between rectal miRNA levels and the time since IBD diagnosis.

3.3. MiR-192/215 does not directly target NOD2

In an attempt to identify IBD-relevant mRNA targets of the colitis-associated miRNAs, Ingenuity Pathway Analysis software (Ingenuity Systems, Redwood City, CA) was used to identify potential miRNA binding sites in known IBD risk genes. Two predicted miR-192 binding sites are located in the 3′ UTR of human NOD2 (Fig. 3B). NOD2 encodes a pattern recognition receptor critical for bacterial sensing and the induction of autophagy.30,31 Previous studies have shown NOD2 is expressed in the intestinal epithelium and that polymorphisms in NOD2 confer increased risk of developing IBD.32–34 NOD2 mRNA levels were not significantly altered between UC, L2 CD, and control samples (p = 0.11) (data not shown). To determine if NOD2 is regulated by miR-192,
Caco-2 cells were transfected with tough decoys and luciferase reporter constructs. A tough decoy targeting miR-192/215 (TuD192) significantly increased the activity of Renilla luciferase with a 3′ UTR containing tandem artificial miR-192/215 binding sites (192 sensor) compared to a control tough decoy (TuDctrl) (Fig. 3A). Luciferase activity in cells transfected with the wild-type NOD2 3′ UTR luciferase construct was unchanged in cells co-transfected with TuD192 compared to a scrambled sequence TuD (TuDctrl) (Fig. 3C). The complete disruption of either miR-192/215 binding site had no effect on luciferase activity in TuD192-treated cells (Fig. 3C).

3.4. Serum miRNA are IBD biomarkers

Circulating miRNAs are potential biomarkers of pediatric CD. To determine if IBD-associated changes in rectal miRNAs are reflected in sera of IBD patients with colitis, qRT-PCR was performed using RNA isolated from sera of control (n = 18), UC (n = 18), and L2 CD (n = 11) subjects collected at the time of study enrollment. Because miRNA abundance is low in circulating fluids compared to tissue samples, MS2 phage RNA was added during RNA isolation to increase RNA yield. Several of the IBD-associated rectal miRNAs were elevated in the serum of IBD patients (Fig. 4A). MiR-192 and miR-21, two circulating miRNAs previously associated with pediatric CD, were elevated in both UC and L2 CD samples relative to controls. However, serum levels of the IBD-associated rectal miRNAs were not significantly different between L2 CD and UC. ROC analysis was performed to determine the utility of serum miRNAs in identifying pediatric IBD colitis (Fig. 4B). MiR-192, miR-142-3p, and miR-21 correctly classified 78.72%, 72.34%, and 72.34% of patients, respectively (Table 2). In patients from whom both serum and rectum miRNAs were measured (n = 33), serum miRNA levels did not significantly correlate with those of the rectum (Fig. 4C). In an effort to identify circulating miRNAs that can differentiate L2 CD and UC, serum miRNAs were profiled in a subset of the analyzed IBD serum samples (UC and L2 CD, n = 6 each) using a
microfluidic qRT-PCR low-density array platform. SAM analysis revealed that no miRNAs were significantly altered between groups (data not shown). Thus, serum miRNAs may not possess utility in distinguishing UC from L2 CD in children.

4. Discussion

Perturbations of intestinal miRNA expression have previously been reported in adults with IBD. The present study is the first to profile miRNAs in rectal biopsies of children with IBD. Implementation of the nCounter® platform facilitated the evaluation of absolute miRNA abundance in rectal mucosal biopsies; this is an advantage over other assay methods (such as hybridization- or TaqMan-based platforms). The most abundant miRNAs in each sample group were miR-21 and miR-142-3p. Although also expressed by immune cells, miR-21 expression was recently identified in intestinal epithelial cells of adult UC patients.\(^\text{17,36}\) MiR-142-3p is highly expressed in the T cell lineage\(^\text{37}\) and may represent the resident T cell population of the rectal mucosa. Interestingly, these miRNAs were not among the most highly expressed miRNAs in a previous survey of the adult intestinal tract.\(^\text{13}\) MiR-192 and miR-194 were found to be the third- and fifth-most abundant

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**Figure 4** Circulating miRNAs are IBD biomarkers. A) Box plots showing serum miRNA levels in control (n = 18), UC (n = 18), and CD (n = 11) samples. Box, 25th–75th percentiles; line, median; whiskers, adjacent values\(^\text{47}\); filled circles, values outside the adjacent values. For each miRNA shown, the expression significantly differs among groups (Kruskal–Wallis, p < 0.05). Holm–Bonferroni-corrected p-values following post-hoc testing are shown. B) Receiver operating characteristic (ROC) curves displaying the ability of three serum miRNAs to distinguish children with IBD colitis from control patients. C) Scatter plots displaying the correlation between rectum and serum miRNA levels in 33 patients. There was no significant correlation between rectum and serum levels observed for any miRNA. \(\rho\), Spearman’s rank correlation coefficient. Ctrl = control; UC = ulcerative colitis; CD = Crohn’s colitis. AUC = area under the ROC curve.
miRNAs in pediatric control samples, respectively. The miR-192/194/215 family is also highly expressed in intestinal mucosal tissue of adults and mice.\(^\text{10,13}\) Wu et al. previously demonstrated via in situ hybridization that miR-192 is expressed predominantly in epithelial cells of the human colon.\(^\text{12}\) Likewise, McKenna et al. found miR-194 to be highly expressed in the epithelial cells of mouse intestine.\(^\text{10}\) Here, miR-192 and miR-194 expression was positively correlated with both miR-200b and miR-375 levels in all sample groups, suggesting that miR-200b and miR-375 are also expressed in intestinal epithelial cells.

The present study has identified and validated a panel of nine miRNAs altered in rectal tissue of pediatric IBD patients. Of the miRNAs chosen for validation, four were significantly decreased (miR-192, miR-194, miR-200b, and miR-375) and four were significantly increased (miR-21, miR-142-3p, miR-146a, and let-7i) in pediatric UC compared to controls. There is considerable overlap between these results and previous studies examining miRNA changes in adult IBD. For example, decreased miR-192, miR-200b, and miR-375 expression has been reported in the sigmoid colon of adult UC patients.\(^\text{12,38}\) Adult UC is also associated with increases in miR-21, which appears to localize to intestinal epithelial cells.\(^\text{12,17,36,39,40}\) The inflammatory cell-associated miR-146a is also elevated in colonic mucosal biopsies of adult UC patients.\(^\text{41}\) Interestingly, miR-124 was demonstrated in situ hybridization that miR-192 is expressed in pediatric CD.\(^\text{35}\) However, serum levels of the colitis-associated rectal miRNAs were unable to distinguish UC from L2 CD. Likewise, large-scale profiling of serum miRNAs in UC and L2 CD was unable to identify any miRNAs significantly different among groups. Thus, while these results indicate that tissue miR-24 levels may help differentiate UC from L2 CD, circulating miRNAs may not be useful in this context.

There are a number of limitations to the study. It is possible that the study was underpowered to detect relatively small changes in tissue or serum miRNAs that may have been of diagnostic utility. Given the small amount of serum used, relevant miRNAs may have fallen below the limit of detection. The number of subjects was not sufficient for most subgroup analyses, such as those based on duration of disease or therapy, age groups, or individual medications. It will be important to conduct further studies validating our findings of tissue miRNA biomarkers of IBD, L2 CD versus UC.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.crohns.2014.02.012.

Conflicts of interest

AMZ, NJH, and JRF have submitted a U.S. patent application (no. 13/851,623) on the use of circulating miRNAs in the diagnosis of CD.

Acknowledgments

The authors are grateful to the patients who generously agreed to participate in IBD research. The authors also wish to thank Theresa Kerbowski, Kelly E. Kachelries, and Cynthia Escajadillo for their essential contributions. This project was supported by funding from the Center for Pediatric Inflammatory Bowel Disease at the Children’s Hospital of Philadelphia. AMZ is supported by a Ruth L. Kirschstein National Research Service Award for Individual Postdoctoral Fellows (F32) from the National Institute of Diabetes and Digestive and Kidney

### Table 2

<table>
<thead>
<tr>
<th>MiRNA</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
<th>Correctly classified, %</th>
<th>AUC</th>
<th>95% CI</th>
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<tr>
<td>miR-192</td>
<td>79.31</td>
<td>77.78</td>
<td>78.72</td>
<td>0.757</td>
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<td>miR-142-3p</td>
<td>75.86</td>
<td>66.67</td>
<td>72.34</td>
<td>0.723</td>
<td>0.573–0.873</td>
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<td>miR-21</td>
<td>75.86</td>
<td>66.67</td>
<td>72.34</td>
<td>0.718</td>
<td>0.568–0.869</td>
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</table>

IBD, inflammatory bowel disease; AUC, area under the curve; CI, confidence interval.
Diseases. AMZ conceived of, and designed, the study, carried out the experiments and data analyses and drafted the manuscript. NJH designed and generated TuD and reporter tools, and participated in the study design and manuscript revision. DMT carried out sample analyses and critically revised the manuscript. RNB participated in the study design and coordination and revised the manuscript. JRF conceived of, and designed, the study, participated in data analyses, and critically revised the manuscript. All authors read and approved the final manuscript.

References


