Application of comparative transcriptional genomics to identify molecular targets for pediatric IBD

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Experimental models of colitis in mice have been used extensively for analyzing the molecular events that occur during inflammatory bowel disease (IBD) development. However, it is uncertain to what extent the experimental models reproduce features of human IBD. This is largely due to the lack of precise methods for direct and comprehensive comparison of mouse and human inflamed colon tissue at the molecular level. Here, we use global gene expression patterns of two sets of pediatric IBD and two mouse models of colitis to obtain a direct comparison of the genome signatures of mouse and human IBD. By comparing the two sets of pediatric IBD microarray data, we found 83 genes were differentially expressed in a similar manner between pediatric Crohn's disease and ulcerative colitis. Up-regulation of the chemokine (C–C motif) ligand 2 (CCL2) gene that maps to 17q12, a confirmed IBD susceptibility loci, indicates that our comparison study can reveal known genetic associations with IBD. In comparing pediatric IBD and experimental colitis microarray data, we found common signatures amongst them including: (1) up-regulation of CXCL9 and S100A8; (2) cytokine–cytokine receptor pathway dysregulation; and (3) over-represented IRF1 and IRF2 transcription binding sites in the promoter region of up-regulated genes, and HNF1A and Lhx3 binding sites were over-represented in the promoter region of the down-regulated genes. In summary, this study provides a comprehensive view of transcriptome changes between different pediatric IBD populations in comparison with different colitis models. These findings reveal several new molecular targets for further study in the regulation of colitis.

Keywords: interferon regulatory factor, chemokines, chitinase 3-like 1, transcription, bioinformatics

INTRODUCTION

Ulcerative colitis (UC) and Crohn’s disease (CD) are the two major forms of inflammatory bowel disease (IBD). The incidence rate of pediatric CD in the US is 43 per 100,000 and 28 per 100,000 for pediatric UC (1). As recently reported, the incidence and prevalence of pediatric IBD is rising in both developed and developing countries (2). Growth retardation poses a significant threat to the quality of life of 15–40% of children and adolescents with IBD (3). Although environmental factors, microbes in the gastroenterological tract, genetic susceptibility, and immune system dysfunction have been implicated, the etiology of pediatric IBD remains incompletely understood.

During the development of IBD, the colon tissue changes its genome transcription in response to pathological conditions, which is a result of dysregulated interaction between the immune system and enteric bacteria. The common feature of UC and CD inflamed tissue genome transcription provides new clues for pediatric IBD treatment. Although the microarray assay has been performed on pediatric IBD, there is no comprehensive genome transcription analysis for pediatric UC or CD. Here, we performed transcriptome analysis using two sets of pediatric IBD microarray data (4, 5), T-cell transfer colitis model microarray data (6), and dextran sodium sulfate (DSS)-induced colitis microarray data (7) generated from our laboratory and deposited in the National Center for Biotechnology Information Gene Expression Omnibus (NCBI GEO) database. Network and promoter analysis was performed to identify differentially expressed genes in the inflamed colon tissue from pediatric IBD patients versus experimental animal models. Comparison between pediatric IBD and experimental colitis microarray data revealed the similarly expressed genes and over-represented transcription factor binding sites (TFBS) in the promoter regions of the dysregulated genes.

MATERIALS AND METHODS

PEDIATRIC IBD MICROARRAY DATASETS

To get a comprehensive view of the pediatric IBD genome transcription profile, two sets of pediatric IBD microarray data were selected from NCBI. Those two sets of microarray data were obtained by using Affymetrix GeneChip Human Genome HG-U133 plus 2.0 arrays that provide the most comprehensive coverage of transcribed human genome and contain probes for approximately 22,634 genes. The microarray data were generated from
pediatric colon in healthy controls, colon only CD, and colon only UC. The dataset GSE10616 contained data from 11 control samples, 14 CD samples, and 10 UC samples (4); the dataset GSE9686 contained data from 8 control samples, 11 CD samples, and 5 UC samples (5). Colon RNA was isolated from biopsies obtained from patients and healthy controls at diagnosis. The pediatric Crohn’s Disease Activity Index (PCDAI) and Pediatric Ulcerative Colitis Clinical Activity Index (PUCAI) were used to assess the clinical severity of the IBD sample.

GENESIFTER ANALYSIS
Two sets of pediatric IBD microarray data were uploaded to GeneSifter software1 and normalized for comparison by Robust Multichip Average (RMA) method. The gene expression difference threshold was set to 2 with no upper limit. Data were analyzed with a Student’s t-test followed by a Benjamini and Hochberg post test to limit false discovery rates, as we previously reported (7).

INGENIETY PATHWAY ANALYSIS
To see the relationship between differentially expressed genes, the selected genes identified as dysregulated in pediatric CD and pediatric UC microarray data were then imported to IPA2 for network analysis. Genes that were related to each other in biological functions and/or diseases were organized into networks according to the Ingenuity Pathways Knowledge Base (IPKB). IPKB is a database derived from the data mining of the expression of and functional relationships between molecules; this information was extracted from published papers found in NCBI PubMed, Medline, and several other databases.

CIS-REGULATORY ELEMENTS ANALYSIS
To identify common properties of promoter regions of differentially expressed genes, the Affymetrix gene ID of identified genes in pediatric UC and CD were uploaded to the cREMaG system3 (8). The sequence upstream the transcription start site (TSS) is 5000 base pairs, and the downstream sequence of TSS is 1000 base pairs. Promoter sequences were scanned with TFBS matrices obtained from the JASPAR database and the public release of the TRANSFAC database using the TFBS BioPerl module (9, 10). The top 10 of the most over-represented binding sites were selected for comparison analysis.

COMPARISON BETWEEN PEDIATRIC IBD AND EXPERIMENTAL COLITIS MICROARRAY DATA
By directly comparing differential gene expression between human and mouse inflamed colon tissue, we assessed the similarity between human and mouse colitis. The dysregulated gene in DSS-colitis (GEO data base accession number GSE22307) and T-cell transfer colitis model (accession number GSE27302) were divided into eight classes according to the genes expression trends. In the DSS-colitis model, there were 1609 genes that were significantly altered during the colitis development, with 501 progressively up-regulated genes and 173 progressively down-regulated genes (7). In the T-cell transfer colitis model, there were 1775 gene expressions that were significantly changed, with 341 progressively up-regulated genes and 361 progressively down-regulated genes (6). The two sets of microarray data were obtained by using the same platform, Mouse Genome 430 2.0 Array (Affymetrix), which provided the most comprehensive annotated coverage of the mouse genome, composing of over 34,000 well-characterized mouse genes. The genes whose expression progressively changed were correlated with inflammation development and were selected for promoter binding sites analysis. The over-presented promoter binding sites were further compared with the over-presented binding sites obtained from pediatric IBD array data.

RESULTS
GENESIFTER ANALYSIS PEDIATRIC IBD MICROARRAY DATA
Analysis of GSE9686 pediatric CD microarray data showed that 242 genes were differentially expressed, 173 genes had up-regulated expression, while 69 genes had down-regulated expression. Analysis of the GSE10616 pediatric CD microarray data showed that there were 298 genes differentially expressed (the expression of 209 genes were up-regulated and 89 genes were down-regulated). After comparing two sets of pediatric CD microarray data, we found the expression of 167 genes was similarly changed. Among those 167 genes, 117 genes were up-regulated (Table S1 in Supplementary Material), and 50 gene expressions were down-regulated (Table S2 in Supplementary Material).

In GSE9686 pediatric UC microarray data, there were 3860 genes differentially expressed (1717 genes were up-regulated, and 2143 genes were down-regulated). While in GSE10616 pediatric UC, there were 1826 genes differentially expressed (1122 genes were up-regulated and 704 genes were down-regulated). After comparing the two sets of pediatric UC data, we found that 1071 genes were similarly up-regulated (Table S3 in Supplementary Material), and 736 genes were down-regulated (Table S4 in Supplementary Material).

After comparing the data in Tables S1 and S3 in Supplementary Material, we found that there were 65 genes up-regulated in pediatric CD and pediatric UC, as shown in Table 1 and Figure 1A. By comparing Tables S2 and S4 in Supplementary Material, we found that there were 18 genes down-regulated in pediatric CD and pediatric UC, as shown in Table 2 and Figure 1B.

Of the up-regulated genes, seven were from the CXC chemokine family: CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL9, and CXCL11, which are the key components of the cytokine–cytokine receptor interaction pathway. CXCL1 is expressed by epithelial cells, macrophages, and neutrophils (11, 12) and has neutrophil chemoattractant activity (13). CXCL2 is secreted by macrophages and monocytes and is a chemoattractant for polymorphonuclear cells, leukocytes, and hematopoietic stem cells (11, 14, 15). CXCL5 is expressed in eosinophils and stimulates the chemotaxis of neutrophils (16). CXCL6 is a chemoattractant for neutrophils (17). CXCL9 is an interferon (IFN)-dependent CXC chemokine, which plays a pro-inflammatory role and has been found to be expressed at high levels in UC tissue (18). CXCL11 is a chemoattractant for activated T cells (19).

Of the down-regulated genes, four were solute carrier genes: SLC16A9, SLC17A4, SLC23A3, and SLC3A1. The functions of

1 http://www.genesiffter.com
2 http://www.ingenuity.com
3 http://www.cremag.org
Table 1 | Similarly up-regulated genes in pediatric IBD.

| Gene ID | Gene identifier | GSE9686 CD | GSE10616 CD | GSE9686UC | GSE10616UC |
|---------|----------------|------------|--------------|------------|------------|
| ACSL4   | NM_022977      | 2.52       | 2.38         | 5.46       | 4.19       |
| ADM     | NM_001124      | 2.19       | 2.25         | 3.70       | 3.17       |
| ALDH1A2 | AB015228       | 2.22       | 2.93         | 11.29      | 8.36       |
| APCDD1  | N48299         | 2.3        | 2.28         | 3.45       | 2.94       |
| C4BPA   | NM_000715      | 2.01       | 2.50         | 10.31      | 5.94       |
| CCL2    | S69738         | 2.37       | 2.40         | 3.16       | 2.73       |
| CCR1    | NM_016557      | 2.04       | 2.13         | 5.18       | 3.13       |
| CDH11   | D21254         | 2.52       | 2.34         | 6.21       | 4.08       |
| CFB     | NM_001710      | 2.25       | 2.27         | 3.44       | 3.19       |
| CFI     | BC020718       | 2.04       | 2.10         | 6.36       | 3.38       |
| CH25H   | NM_003956      | 2.01       | 2.02         | 3.66       | 2.99       |
| CH3L1   | M80927         | 6.15       | 5.72         | 33.46      | 21.81      |
| CLDN1   | AF101051       | 2.17       | 2.54         | 8.57       | 4.59       |
| COL1A2  | NM_000089      | 2.02       | 2.51         | 5.01       | 4.08       |
| COL4A1  | NM_001845      | 2.03       | 2.09         | 4.27       | 3.65       |
| COL6A3  | NM_004369      | 2.07       | 2.35         | 4.24       | 3.97       |
| CXCL1   | NM_001511      | 5.60       | 4.66         | 18.35      | 13.43      |
| CXCL2   | AF002988       | 6.82       | 5.61         | 14.19      | 12.26      |
| CXCL3   | M57731         | 3.35       | 3.39         | 10.29      | 9.44       |
| CXCL5   | AK026546       | 6.04       | 6.49         | 44.64      | 22.12      |
| CXCL6   | NM_002993      | 4.84       | 5.97         | 30.42      | 18.36      |
| CXCL9   | NM_002416      | 4.70       | 3.23         | 4.13       | 3.95       |
| CYP27B1 | NM_000785      | 2.03       | 2.19         | 2.35       | 2.22       |
| CYR61   | NM_001554      | 3.11       | 2.98         | 3.69       | 3.94       |
| DERL3   | AI655697       | 2.25       | 2.11         | 5.50       | 3.96       |
| DUOX2   | NM_014080      | 11.06      | 9.73         | 29.14      | 25.35      |
| DUOX2A  | AI821606       | 2.24       | 3.79         | 14.00      | 13.6       |
| EMR2    | NM_013447      | 2.55       | 2.54         | 5.01       | 4.20       |
| FCLR1A  | X14355         | 2.82       | 2.39         | 2.88       | 2.61       |
| FCRIL5  | AF343662       | 2.47       | 2.53         | 6.18       | 4.60       |
| FN1     | X02761         | 2.08       | 2.07         | 2.15       | 2.32       |
| HSOD1B1 | NM_005525      | 2.68       | 2.57         | 4.12       | 3.84       |
| IGK1D-13| AW408194       | 3.19       | 3.25         | 6.96       | 4.87       |
| IGLV1-44| U96394         | 3.83       | 3.26         | 15.57      | 9.51       |
| IVD     | AF043583       | 3.00       | 3.08         | 11.48      | 7.58       |
| KDELR3  | NM_006855      | 2.38       | 2.50         | 5.09       | 4.13       |
| LCN2    | NM_005564      | 3.11       | 3.27         | 7.12       | 6.18       |
| LOXL2   | NM_002318      | 2.23       | 2.31         | 4.77       | 3.78       |
| LPL     | BF672975       | 2.19       | 2.73         | 6.44       | 4.22       |
| MMP1    | NM_002421      | 5.44       | 6.69         | 41.57      | 22.03      |
| MMP10   | NM_002425      | 3.71       | 4.86         | 44.39      | 34.11      |
| MMP3    | NM_002422      | 11.65      | 10.85        | 66.73      | 49.68      |
| MS4A2   | NM_000139      | 2.33       | 2.12         | 3.73       | 2.96       |
| NEBL    | NM_006300      | 2.24       | 2.29         | 3.94       | 3.24       |
| NIACR2  | NM_006018      | 4.90       | 4.04         | 7.94       | 8.03       |
| NID1    | BF940043       | 2.39       | 2.44         | 5.00       | 3.61       |
| NOS2    | L24553         | 2.12       | 2.02         | 4.07       | 3.54       |
| NTH3    | AF103529       | 3.64       | 2.91         | 9.44       | 6.10       |
| PCDH7   | NM_002589      | 2.07       | 2.16         | 7.21       | 4.27       |
| PCSK1   | NM_000439      | 3.25       | 4.54         | 11.66      | 8.33       |
| PHLDA1  | AAS76961       | 2.89       | 2.91         | 9.53       | 6.48       |
(Continued)
Table 1 | Continued

| Gene ID | Gene identifier | GSE9686 CD | GSE10616 CD | GSE9686UC | GSE10616UC |
|---------|----------------|------------|-------------|-----------|------------|
| PSAT1   | BC004863       | 3.49       | 2.49        | 9.33      | 5.50       |
| S100A8  | NM_002964      | 6.09       | 5.66        | 21.7      | 17.55      |
| SEC24D  | NM_014822      | 2.35       | 2.19        | 4.85      | 3.49       |
| SGMS1   | A1377497       | 2.07       | 2.17        | 5.15      | 3.56       |
| SLC6A14 | NM_007231      | 7.11       | 10.32       | 56.81     | 51.22      |
| S100A8  | NM_002964      | 6.09       | 5.66        | 21.7      | 17.55      |
| SEC24D  | NM_014822      | 2.35       | 2.19        | 4.85      | 3.49       |
| SGMS1   | A1377497       | 2.07       | 2.17        | 5.15      | 3.56       |
| SLC6A14 | NM_007231      | 7.11       | 10.32       | 56.81     | 51.22      |
| S100A8  | NM_002964      | 6.09       | 5.66        | 21.7      | 17.55      |
| SEC24D  | NM_014822      | 2.35       | 2.19        | 4.85      | 3.49       |
| SGMS1   | A1377497       | 2.07       | 2.17        | 5.15      | 3.56       |
| SLC6A14 | NM_007231      | 7.11       | 10.32       | 56.81     | 51.22      |

The two sets of pediatric microarray data are downloaded from NCBI Gene Expression Omnibus (NCBI GEO) database. Values are mean fold change normalized to control.

FIGURE 1 | Venn diagram illustration of gene expression similarity between pediatric CD and UC patient sample microarray data. (A) One-hundred seventeen genes were up-regulated from pediatric CD compared with 1071 up-regulated genes from pediatric UC patients, with 65 genes being common between the two groups. (B) Fifty genes were down-regulated from pediatric CD compared from 736 down-regulated gene from pediatric UC patients, with 18 genes being common between the two groups.

those down-regulated genes related to the transport of amino acids (20), monocarboxylate (21), glutamate (22), sodium-phosphate (23), and ascorbic acid (24).

INGENUITY ANALYSIS OF PEDIATRIC IBD MICROARRAY DATA

Those genes differentially expressed in pediatric CD and UC were uploaded to Ingenuity software for network analysis. Those differentially expressed genes in pediatric CD inflamed colon tissue were organized into eight networks. The molecules in each network and their top functions are listed in Table S5 in Supplementary Material. Those differentially expressed genes in pediatric UC inflamed colon tissue were organized into 25 networks, and the molecules in each network and their top functions are listed in Table S6 in Supplementary Material.

Figure 2 shows the first network of pediatric CD inflamed colon tissue differentially expressed genes with their cell-to-cell signaling functions and their interactions, as they relate to gastrointestinal and hepatic system disease. As shown in Figure 2, the transcription of nine chemokine genes was up-regulated, and those genes indirectly react with the NF-κB complex. Figure 3 shows the network 2 differentially expressed genes in pediatric CD inflamed colon tissue, which is composed of 15 up-regulated genes with functions related to connective tissue and genetic disorders. MMP-1 and MMP-3 are located in the center of pediatric CD network 2. ChI3L1 is also implicated in this network through its indirect reaction with IGFBP5.

Figure 4 shows the first network of pediatric UC with functions related to cellular movement and signaling. Pediatric UC network 1 is mainly composed of eleven G-protein-coupled receptors, which were all up-regulated. Transcription of eight members of the collage family was up-regulated as shown in Figure 5, with functions related to connective tissue. Interestingly, ChI3L1 was in the center of the pediatric UC network 2 (Figure 5), and ChI3L1 indirectly interacts with COL16A2 and TNC.

PROMOTER ANALYSIS OF PEDIATRIC IBD MICROARRAY DATA

Using the CREMaG system, we indentified over-presented TFBS in the differentially expressed genes. The over-presented TFBS of differentially regulated genes in pediatric CD is shown in Tables S7–S10 in Supplementary Material. TFBS over-presented in pediatric UC differentially expressed genes are shown in Table S11 in Supplementary Material (for up-regulated genes) and in Table S12 in Supplementary Material (for down-regulated genes). The fold-difference in TFBS frequency was computed by dividing the observed TFBS number by the background number.

By comparison, we found that there were six promoter sequences (RELA, NF-κB, IRF2, Evi1, and IRF1) that were over-presented in genes that were up-regulated in pediatric IBD. There were six TFBS (Lhx3, MEF2A, HNF1A, Nobox, NR2F1, and Foxa2) that were over-presented in the genes that were down-regulated in pediatric IBD-inflamed colon tissue.

In the pediatric CD microarray data analysis, the NF-κB binding sequence was over-presented in the inflammatory-related...
### Table 2 | Similarly down-regulated genes in pediatric IBD

| Gene ID | Gene identifier | GSE9686 CD | GSE10616 CD | GSE9686UC | GSE10616UC |
|---------|----------------|------------|-------------|------------|------------|
| ABCB1   | AF016535       | −2.13      | −2.02       | −4.98      | −4.04      |
| ABCG2   | AF098951       | −4.93      | −3.09       | −8.52      | −9.93      |
| APOBEC3B| NM_004900      | −2.09      | −2.05       | −2.78      | −2.80      |
| AQP8    | NM_001169      | −4.57      | −3.85       | −28.58     | −36.55     |
| KRT12   | NM_000223      | −2.53      | −2.22       | −4.34      | −3.69      |
| LOC389023| AI499651      | −2.07      | −2.81       | −3.56      | −3.60      |
| LOC643008| BF478120      | −2.02      | −2.00       | −4.50      | −2.58      |
| MEP1B   | NM_005925      | −5.08      | −3.82       | −6.37      | −8.09      |
| PCDH21  | AI825832       | −2.15      | −2.24       | −6.15      | −3.70      |
| PHLPPL  | AB023148       | −2.13      | −2.01       | −4.29      | −3.99      |
| PLA2G12B| BF939574       | −2.29      | −2.36       | −2.80      | −3.73      |
| PRAP1   | AA502331       | −2.93      | −2.33       | −3.99      | −4.65      |
| SGK2    | AI631895       | −2.6       | −2.92       | −4.76      | −4.35      |
| SLC16A9 | BG401568       | −4.05      | −3.08       | −6.67      | −4.65      |
| SLC17A4 | NM_005495      | −2.64      | −2.58       | −8.08      | −6.66      |
| SLC23A3 | AI263078       | −2.61      | −2.27       | −5.24      | −3.98      |
| SLC3A1  | M95548         | −3.11      | −2.43       | −3.25      | −4.76      |
| WSCD1   | AB011096       | −2.17      | −2.05       | −2.62      | −2.73      |

The two set of pediatric microarray data are downloaded from NCBI Gene Expression Omnibus (NCBI GEO) database. Values are mean fold change normalized to control.

![Network 1 of pediatric CD](image)

**FIGURE 2** | Network 1 of pediatric CD has biological functions associated with cell-to-cell signaling and interaction, gastrointestinal disease, hepatic system disease. Red shading indicates up-regulation, whereas green shading shows down-regulation.
FIGURE 3 | Network 2 of pediatric CD has biological functions associated with connective tissue disorders, genetic disorder, and cardiovascular disease. Red shading indicates up-regulation, whereas green shading shows down-regulation.

Comparison of differentially expressed genes in pediatric IBD and progressively up-regulated or down-regulated genes in experimental colitis is shown in Table 3 and Figures 6 and 7. The comparison of the over-presented promoters in the differentially expressed genes is shown in Table 4. Among them, the cytokine–cytokine receptor pathway was dysregulated in all the four sets of microarray data. CXCL9 and S100A8 were up-regulated in all the four sets of microarray data. The expression of S100A8 was also found to be up-regulated in the trinitrobenzene sulfonic acid (TNBS)-induced colitis rat model and T-cell-mediated colitis in SCID mice (25, 26).

Promoter analysis provided the possible common regulatory mechanism of the expression of the dysregulated genes. As shown in Table 4, the IRF1 and IRF2 binding sites were over-represented in the up-regulated genes in pediatric IBD and experimental colitis. The HNF1A and Lhx3 binding sites were over-presented in the down-regulated genes in the four sets of microarray data. HNF1A is a transcription factor that regulates the expression of cytokine-driven C-reactive protein, which is a clinical marker of inflammation (27). Lhx3 is a transcription factor that is required for pituitary and motor neuron development (28).
FIGURE 4 | Network 1 of pediatric UC has biological functions associated with cellular movement, cell signaling, and nucleic acid metabolism. Red shading indicates up-regulation, whereas green shading shows down-regulation.

As shown in Table 3, cytokine–cytokine receptor interaction pathway is dysregulated in pediatric UC, CD, T-cell transfer, and DSS-colitis model. While chemokine signaling pathway is dysregulated in pediatric UC, CD, and T-cell transfer colitis model, but not in DSS-colitis model. Additionally, NF-κB binding site is over-presented in the promoter region of up-regulated genes in pediatric CD, UC, and T-cell transfer colitis, but not in DSS-colitis model (Table 4). Thus, the comparison of dysregulated KEGG pathway (Table 3) and the over-represented TFBS (Table 4) showed that the T-cell transfer colitis model was better than the DSS-induced colitis model at simulating pediatric IBD; however, the DSS-colitis model was more similar to pediatric UC than pediatric CD, as the DSS model has more common dysregulated pathways and molecules (Table 3) and over-represented TFBS in the dysregulated genes with pediatric UC than CD (Table 4).

**DISCUSSION**

Our microarray analysis revealed that chitinase 3-like 1 (cartilage glycoprotein-39, CHI3L1) was up-regulated in pediatric IBD samples. CHI3L1 has the ability to enhance the adhesion and internalization of bacteria in epithelial cells (29). In vivo, neutralizing CHI3L1 with an antibody suppresses DSS-induced colitis, and this neutralization dramatically decreases bacteria adhesion and invasion of epithelial cells. It has been demonstrated that CHI3L1 expression is up-regulated in epithelial cells under inflammatory conditions. CHI3L1 also activates Akt signaling in epithelial cells through its chitin binding motif, and increases secretion of IL-8 and TNF-α in a dose-dependent manner (30). Fecal CHI3L1 levels are positively correlated with pathology score (31). Serum concentration of CHI3L1 is also elevated in IBD patients (32). Thus, CHI3L1 might be selected as both a target and a marker of pediatric IBD.

Cysteine-rich, angiogenic inducer 61 (CYR61 or CCN1) was up-regulated in pediatric IBD. It has been demonstrated that CCN1 up-regulates pro-inflammatory gene transcription, such as TNF-α, IL-1α, IL-1β, IL-6, and IL-12b in mice macrophages (33). This induction results from CCN1 direct activation of NF-κB and increased TNF-α synthesis. CCN1 supports macrophage
adhesion through integrin αMβ2 and syndecan-4. Because mice lacking CCN1 cannot develop in utero, involving vascular defects in the placenta, CCN1 is also related with vasculogenesis during embryogenesis (34). Moreover, CCN1 construct transfected mice showed increased angiogenesis in colon tissue (35). Together, these suggest that CCN1 might be a unique target to treat pediatric IBD through inhibition of pro-inflammatory gene expression and angiogenesis.

Chemokine (C–C motif) ligand 2 (CCL2), also known as monocyte chemotactic protein-1 or small inducible cytokine A2, was up-regulated in pediatric inflamed colon tissue. CCL2 attracts monocytes, memory T cells, and dendritic cells to sites of tissue injury, infection, and inflammation (36, 37). Interestingly, CCL2 is located in the confirmed CD and UC susceptibility loci 19q12 (38, 39). Increased expression of CCL2 (Table 1) in pediatric inflamed colon tissue supports the idea that CCL2 might be one of the causal genes of pediatric IBD. Interestingly, this has been demonstrated by nanomolar concentrations of CCL2 stimulating inflammatory responses of monocytes and effector T cells, whereas picomolar CCL2 exerts a global suppressive effect on T-cell trafficking into inflamed lymph nodes (40), as confirmed by picomolar levels of CCL2 ameliorating TNBS- and DSS-induced colitis (41). Thus, before targeting CCL2 for IBD therapy more information is needed regarding its dose effect on human colon tissue inflammation responses.

One major difference of genome transcription in adult IBD versus pediatric IBD colon tissue is that there is fewer common dysregulated genes found in adult IBD. Feng et al. found that 25 genes were up-regulated and 18 genes were down-regulated in adult IBD inflamed colon tissue (42). We found that 65 genes were up-regulated and 18 genes were down-regulated in pediatric IBD colon tissue. Compared with those two studies, CXCL2 and CXCL3 were both up-regulated in pediatric and adult IBD, and only ABCB1 was down-regulated in pediatric and adult IBD. This comparison suggests that there is a large difference between pediatric and adult IBD patients. Additionally, both studies show that there is a difference between CD and UC genome transcription patterns, which suggests that CD and UC have distinctive pathogenesis.

Promoter analysis provided potential targets at the transcription factor level. NF-κB transcription factors are comprised of
Table 3 | Common dysregulated pathways and genes.

| Dysregulated pathways                                                                 | Genes up-regulated                                                                 | Genes down-regulated |
|--------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|----------------------|
| T-cell model vs. pediatric CD                                                        | CXCL1, CXCL10, CXCL2, CXCL5, CXCL9, DUOXA2, GBP1, IL1B, MMP3, PSAT1, S100A8, TGM2 | ABCB1                |
| Cytokine–cytokine receptor interaction, chemokine signaling pathway, Leishmaniasis,  | ARL4C, CTSC, CD274, CDC6, CCR1, CXCL1, CXCL2, CXCL9, CSF2RB, C1R, C2, DUOXA2, EGR2, PSAT1, PYHIN1, RUNX2, S100A8, S100A9, S100A10, STAT1, SLAMF8, SLC7A11, SNX10, SOCS1, TM2, WARS, UBD, ZC3H12A | ABCB1, CHKA, DENND1B, FGFR3, HOXB5, HSD3B2, RAB26, SCIN, V5IG2 |
| Chagas disease, asthma, malaria, NOD-like receptor signaling pathway                 |                                                                                     |                      |
| T-cell model vs. pediatric UC                                                         | ARL4C, CTSC, CD274, CDC6, CCR1, CXCL1, CXCL2, CXCL9, CSF2RB, C1R, C2, DUOXA2, EGR2, PSAT1, PYHIN1, RUNX2, S100A8, S100A9, S100A10, STAT1, SLAMF8, SLC7A11, SNX10, SOCS1, TM2, WARS, UBD, ZC3H12A | ABCB1, CHKA, DENND1B, FGFR3, HOXB5, HSD3B2, RAB26, SCIN, V5IG2 |
| Cytokine–cytokine receptor interaction, chemokine signaling pathway, cell adhesion | ARL4C, CTSC, CD274, CDC6, CCR1, CXCL1, CXCL2, CXCL9, CSF2RB, C1R, C2, DUOXA2, EGR2, PSAT1, PYHIN1, RUNX2, S100A8, S100A9, S100A10, STAT1, SLAMF8, SLC7A11, SNX10, SOCS1, TM2, WARS, UBD, ZC3H12A | ABCB1, CHKA, DENND1B, FGFR3, HOXB5, HSD3B2, RAB26, SCIN, V5IG2 |
| molecules (CAMs), hematopoietic cell lineages, Leishmaniasis, malaria, Alzheimer’s    |                                                                                     |                      |
| DSS model vs. pediatric CD                                                            | ARL4C, CTSC, CD274, CDC6, CCR1, CXCL1, CXCL2, CXCL9, CSF2RB, C1R, C2, DUOXA2, EGR2, PSAT1, PYHIN1, RUNX2, S100A8, S100A9, S100A10, STAT1, SLAMF8, SLC7A11, SNX10, SOCS1, TM2, WARS, UBD, ZC3H12A | ABCB1, CHKA, DENND1B, FGFR3, HOXB5, HSD3B2, RAB26, SCIN, V5IG2 |
| Cytokine–cytokine receptor interaction, Toll-like receptor signaling pathway, ECM-receptor | ARL4C, CTSC, CD274, CDC6, CCR1, CXCL1, CXCL2, CXCL9, CSF2RB, C1R, C2, DUOXA2, EGR2, PSAT1, PYHIN1, RUNX2, S100A8, S100A9, S100A10, STAT1, SLAMF8, SLC7A11, SNX10, SOCS1, TM2, WARS, UBD, ZC3H12A | ABCB1, CHKA, DENND1B, FGFR3, HOXB5, HSD3B2, RAB26, SCIN, V5IG2 |
| interaction                                                                             |                                                                                     |                      |
| DSS model vs. pediatric UC                                                            | ARL4C, CTSC, CD274, CDC6, CCR1, CXCL1, CXCL2, CXCL9, CSF2RB, C1R, C2, DUOXA2, EGR2, PSAT1, PYHIN1, RUNX2, S100A8, S100A9, S100A10, STAT1, SLAMF8, SLC7A11, SNX10, SOCS1, TM2, WARS, UBD, ZC3H12A | ABCB1, CHKA, DENND1B, FGFR3, HOXB5, HSD3B2, RAB26, SCIN, V5IG2 |
| Cytokine–cytokine receptor interaction, complement and coagulation cascades, cell    | ARL4C, CTSC, CD274, CDC6, CCR1, CXCL1, CXCL2, CXCL9, CSF2RB, C1R, C2, DUOXA2, EGR2, PSAT1, PYHIN1, RUNX2, S100A8, S100A9, S100A10, STAT1, SLAMF8, SLC7A11, SNX10, SOCS1, TM2, WARS, UBD, ZC3H12A | ABCB1, CHKA, DENND1B, FGFR3, HOXB5, HSD3B2, RAB26, SCIN, V5IG2 |
| adhesion molecules (CAMs), Toll-like receptor signaling pathway, ECM-receptor interaction, | ARL4C, CTSC, CD274, CDC6, CCR1, CXCL1, CXCL2, CXCL9, CSF2RB, C1R, C2, DUOXA2, EGR2, PSAT1, PYHIN1, RUNX2, S100A8, S100A9, S100A10, STAT1, SLAMF8, SLC7A11, SNX10, SOCS1, TM2, WARS, UBD, ZC3H12A | ABCB1, CHKA, DENND1B, FGFR3, HOXB5, HSD3B2, RAB26, SCIN, V5IG2 |
| hematopoietic cell lineages                                                             |                                                                                     |                      |

FIGURE 6 | Venn diagram illustration of gene expression similarity between pediatric CD and experimental colitis model microarray data. (A) One-hundred seventeen genes were up-regulated from pediatric CD compared with 341 up-regulated genes from T-cell colitis model and 501 up-regulated genes from DSS-colitis model. (B) Fifty genes were down-regulated from pediatric CD compared from 361 down-regulated gene from T-cell colitis model and 173 down-regulated genes from DSS-colitis model.

FIGURE 7 | Venn diagram illustration of gene expression similarity between pediatric UC patient sample microarray data and experimental colitis model microarray data. (A) One-thousand seventy one genes were up-regulated from pediatric UC compared with 341 up-regulated genes from T-cell colitis model and 501 up-regulated genes from DSS-colitis model. (B) Seven-hundred thirty six genes were down-regulated from pediatric UC compared from 361 down-regulated gene from T-cell colitis model and 173 down-regulated genes from DSS-colitis model.
Table 4 | Comparison promoter between animal model and pediatric IBD.

| T-cell model vs. pediatric CD | Over-represented in up-regulated genes | Over-represented in down-regulated genes |
|------------------------------|----------------------------------------|------------------------------------------|
| IRF1                         | Lhx3                                   |                                          |
| IRF2                         | MEF2A                                  |                                          |
| NFκB                         | HNF1A                                  |                                          |
| RELA                         | NoBox                                  |                                          |
| ELF5                         |                                        |                                          |

| T-cell model vs. pediatric UC | Over-represented in up-regulated genes | Over-represented in down-regulated genes |
|------------------------------|----------------------------------------|------------------------------------------|
| IRF1                         | HNF1A                                  |                                          |
| IRF2                         | Lhx3                                   |                                          |
| NFκA                         | Pax4                                   |                                          |
| RELA                         | MEF2A                                  |                                          |
| FOXF2                        | IRF2                                   |                                          |
| NFκB                         | NoBox                                  |                                          |

| DSS model vs. pediatric CD   | Over-represented in up-regulated genes | Over-represented in down-regulated genes |
|------------------------------|----------------------------------------|------------------------------------------|
| IRF1                         | HNF1A                                  |                                          |
| IRF2                         | Lhx3                                   |                                          |
| FOXF2                        | IRF2                                   |                                          |
| Pax4                         | Lhx3                                   |                                          |

| DSS model vs. pediatric UC   | Over-represented in up-regulated genes | Over-represented in down-regulated genes |
|------------------------------|----------------------------------------|------------------------------------------|
| IRF2                         | HNF1A                                  |                                          |
| IRF1                         | Lhx3                                   |                                          |
| FOXF2                        | IRF2                                   |                                          |

In conclusion, we performed pediatric IBD transcriptome analysis and its cross-species comparison with experimental colitis models. Identification of common dysregulated gene expression profiles, over-represented transcription binding sites, and related transcription factors controlling dysregulated gene expression changes reveal several molecular targets that serve as novel pathways for further study and potential therapy for pediatric IBD.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://journal.frontiersin.org/article/10.3389/fimmu.2015.00165/abstract

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