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Methylation quantitative trait loci are largely consistent across disease states in Crohn’s disease

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Abstract
Recently, we identified 1,189 CpG sites whose DNA methylation level in blood associated with Crohn’s disease. Here, we examined associations between DNA methylation and genetic variants to identify methylation quantitative trait loci across disease states in (1) 402 blood samples from 164 newly diagnosed pediatric Crohn’s disease patients taken at 2 time points (diagnosis and follow-up), and 74 non-inflammatory bowel disease controls, (2) 780 blood samples from a non-Crohn’s disease adult population, and (3) 40 ileal biopsies (17 Crohn’s disease cases and 23 non-inflammatory bowel disease controls) from group (1). Genome-wide DNAm profiling and genotyping were performed using the Illumina MethylationEPIC and Illumina Multi-Ethnic arrays. SNP-CpG associations were identified via linear models adjusted for age, sex, disease status, disease subtype, estimated cell proportions, and genotype-based principal components. In total, we observed 535,448 SNP-CpG associations between 287,881 SNPs and 12,843 CpG sites (P < 8.21 × 10^{-16}). Associations were highly consistent across different ages, races, disease states, and tissue types, suggesting that the majority of these methylation quantitative trait loci participate in common gene regulation. However, genes near CpGs associated with inflammatory bowel disease SNPs were enriched for 18 KEGG pathways relevant to inflammatory bowel disease-linked immune function and inflammatory responses. We observed suggestive evidence for a small number of tissue-specific associations and disease-specific associations in ileum, though larger studies will be needed to confirm these results. Our study concludes that the vast majority of blood-derived methylation quantitative trait loci are common across individuals, though a subset may be involved in processes related to Crohn’s disease. Independent cohort studies will be required to validate these findings.

Keywords: methylation quantitative trait loci (mQTL); inflammatory bowel disease (IBD); Crohn’s disease (CD); DNA methylation (DNAm)

Introduction
A majority of inflammatory bowel disease (IBD) trait-associated genomic variants identified in genome-wide association studies (GWAS) reside in the genome’s noncoding regions (Farh et al. 2015). In the past decade, studies have focused on demonstrating the potential of these variants to influence specific traits or diseases through transcriptional regulation (Rockman and Kruglyak 2006; Farber and Lusis 2008; Ward and Kellis 2012). Previous expression quantitative trait loci (eQTL) studies in IBD proposed the genetic influences on gene expression are tissue-specific (Venkateswaran et al. 2018), disease-specific (Mo et al. 2018), and inflammation-specific (Thalayasingam et al. 2018; Venkateswaran et al. 2018). Meanwhile, recent advances in high-throughput array technologies have enabled genome-wide DNA methylation (DNAm) profiling for >850,000 CpGs. DNAm is one of the primary epigenetic mechanisms involved in gene expression regulation. Investigation of DNAm has the potential to advance our understanding of complex disease pathogenesis (Handy et al. 2011; Moore et al. 2013). In an epigenome-wide association study (EWAS), we recently identified 1189 CpGs showing differential DNAm in Crohn’s disease (CD) patients at diagnosis, compared to non-IBD controls (Somineni et al. 2019). A Mendelian randomization (MR) approach revealed that most of the DNAm...
changes observed in CD patients are consequences of the disease rather than causal factors influencing disease risk (Somineni et al. 2019). Since DNA methylation (DNAm) can be influenced by both environmental and genetic factors, in this study, we will focus on identification of methylation quantitative trait loci (mQTLs), or SNPs that associate with DNAm at specific CpG sites. We will compare mQTLs found within blood across disease state and progression, as well as age and race. Finally, we will compare patterns found in blood to those found in ileal (disease) tissue. In general, we are looking for mQTL patterns that distinguish CD cases from CD controls, and mQTLs that are context-specific.

Identifying SNP-CpG associations and comparing them between disease groups will provide epigenetic annotation of IBDM variants, facilitating their functional characterization. The comparative mQTL study has the potential to reveal differences in interactions between genetic and epigenetic mechanisms in IBDM patients vs controls. Further, the identification of mQTLs can aid MR approaches through identification of potential instruments to distinguish the causal vs consequential role of DNAm in complex diseases (Somineni et al. 2019), and can shed light on causal relationships between gene expression and DNAm in complex diseases (Hannon et al. 2018; Wu et al. 2018).

Investigating whether SNP-CpG associations are stable or dynamic over the course of the diseases can help inform whether the association might be a useful biomarker for disease, a useful marker for treatment, a potential cause of disease, or without obvious utility. A recent longitudinal analysis at 5 different stages showed that blood mQTLs are generally stable across the life span (Gaunt et al. 2016). However, among the 1,189 differentially methylated CpG sites identified in our previous study from blood derived DNA, we observed that during the CD disease course (at 1–3 years of follow-up), DNAm patterns in cases reverted back to levels observed in healthy controls. (Somineni et al. 2019). We also noted high correlations between DNAm at these 1,189 sites and levels of the inflammatory marker C-reactive protein (CRP) (Somineni et al. 2019). Investigation of mQTLs in CD patients pre- and post-treatment will reveal whether the genetic influence on DNAm at these sites varies over the disease course.

CD commonly affects the terminal ileum. Thus, in contrast to DNAm patterns in blood, methylation patterns in the ileum might be considered patterns seen at the “site of disease.” Consequently, ileal DNAm is more likely to be causative, compared to patterns observed in other tissues. Thus, we examine ileum-specific mQTL associations in CD patients.

Here, we used blood samples obtained from a total of 238 pediatric subjects; 164 patients with CD and 74 healthy controls sampled from the Risk Stratification and Identification of Immunogenetics and Microbial Markers of Rapid Disease Progression in Children with Crohn’s Disease (RISK) study (Kugathasan et al. 2017). We replicated our analysis in an independent cohort of 780 blood samples obtained from prospectively recruited adults in the Grady Trauma Project (GTP). Enrichment analysis showed that the previously identified IBD GWAS SNPs are significantly enriched for mQTLs in the CD cohort, but not in the adult cohort. To assess whether the mQTLs are specific to disease, disease course, or tissue type, we performed separate mQTL analyses in 164 CD cases at diagnosis, in cases at follow-up (1–3 years from the day of diagnosis), in 74 healthy controls and in 40 ileal biopsies (obtained from a subset of subjects included in the blood mQTL analysis; 23 controls and 17 patients with CD at diagnosis).

Methods
Study population and design of discovery cohort
The discovery cohort was a subset of pediatric subjects recruited under the Risk Stratification and Identification of Immunogenetic and Microbial Markers of Rapid Disease Progression in Children with Crohn’s Disease (RISK) study (Kugathasan et al. 2017) for whom DNA methylation data had been collected. A detailed description of the dataset is provided in our previous study (Somineni et al. 2019). In total, 164 newly diagnosed, pediatric patients with Crohn’s disease (cases) at 2 time-points (diagnosis and 3-year follow-up) and 74 non-IBD controls had DNA methylation measured for this study. Patients with no bowel pathology upon endoscopy, negative gut inflammation, and continued medication as asymptomatic for IBD during follow-up were considered as non-IBD controls.

Blood genotype data for CD cohort
Peripheral blood DNA genotypes were obtained for 238 subjects using Infinium Multi-Ethnic Global-8 Kit (Illumina, San Diego, CA, USA) and processed with GenomeStudio software. Detailed quality control (QC) steps for both samples and variants are provided in our earlier study (Somineni et al. 2019). Briefly, sample QC was performed by checking that (1) genotype call rates >95%, (2) inferred gender is consistent with clinical records, and (3) subjects are unrelated according to a pairwise identity-by-descent test. Similarly, SNP QC was performed by identifying and removing SNPs with low call rate (<95%), (1) Hardy-Weinberg disequilibrium (P < 1.0 × 10⁻⁶), or (2) minor allele frequency (MAF) < 5%, as well as (3) nonautosomal variants and (4) variants mapping to multiple locations. This resulted in a discovery data set consisting of 636,006 high-quality variants. These variants for all 238 subjects were then used to impute genotypes from the 1000 Genomes Project Phase3 autosomal reference panel, using IMPUTE2 software (Howie et al. 2012). Imputed SNPs with MAF < 1% and imputation quality scores <80% were removed. We also removed imputed variants with no dbSNP annotation, INDELs/CNV, and SNPs with missing genotype information in >5% of the samples. Last, SNPs with <5 individuals in each group [homologous reference (AA), heterozygous (AB), and homologous variant (BB)] were excluded. In total, 3,109,863 high-quality SNPs for all 238 subjects were retained across the entire genome.

Blood and Ileal DNA methylation data for CD cohort
DNA methylation data was profiled for 402 samples (164 CD cases at diagnosis and 3-years follow-up and 74 healthy controls at diagnosis) as described in our previous study (Somineni et al. 2019). In total, our discovery dataset consists of 77.7% Caucasian, 16.8% African American, and 5.5% other ancestries (Supplementary Table 1). In this study, we have added DNA methylation data for 40 ileal biopsies, performed in 17 CD cases and 23 non-IBD controls from the discovery cohort. Genome-wide DNA methylation for these samples was profiled at single-base resolution using MethylationEPIC BeadChips. QC for the blood DNA methylation is described in detail in Somineni et al. (2019). We applied the same QC protocol on the ileal biopsies and retained all 40 ileal biopsies. Data were normalized using the beta-mixture quantile dilation (BMQ) method (Teschendorff et al. 2013). Estimated cell counts for CD4+ T cells, CD8+ T cells, NK cells, B cells, monocytes, and granulocytes were calculated using Houseman’s approach (Houseman et al. 2012). After exclusion of CpGs with SNPs in their probe sequences, DNA
methylation of 609,192 CpG sites in 402 samples from 238 subjects was used in this study.

**Blood genotypes for GTP cohort**

Study design and genotyping of the replication cohort was previously described in recent studies (Almli et al. 2018; Nieuvergelt et al. 2019). Briefly, GTP individuals were prospectively recruited from waiting rooms at Grady Memorial Hospital in Atlanta, GA between 2005 and 2008 for a study of stressful life events and symptoms of post-traumatic stress disorder (PTSD). Seven hundred and ninety-one subjects from GTP were genotyped using Illumina HumanOmni1-Quad, HumanOmniExpress or Multi-Ethnic Global arrays. Genotypes were called using Illumina’s GenomeStudio software. QC analyses on the genetic data from the GTP cohort results, and calculated FDR on the actual test P-values using the Benjamini-Hochberg method, implemented in the p.adjust function in R. For these follow-up analyses in ileum, we restricted analysis to SNPs that had at least 2 individuals in each genotype category (AA/AB/BB) to ensure sufficient genetic variation. A statistical significance threshold of  \( P < 0.05 \) was used for replication analyses of the disease-, longitudinal-, and tissue-associated mQTLs. To formally test for disease-specific differences in CpG-SNP associations in ileum, we performed interaction tests by refitting the original model with the addition of a term for SNP \( \times \) disease status. We used the clump command in PLINK (Purcell et al. 2007) to identify the number of independent associations for each genomic variant with more than 1 significant SNP-CpG association with default parameters (distance threshold = 250 kb and \( r^2 \) threshold = 0.50).

**Annotation of SNPs and CpG sites**

We used the annovar package (Wang et al. 2010) to annotate SNPs, and the MethylationEPIC annotation file to annotate CpG sites.

**KEGG pathway enrichment analysis**

We used missMethyl (Phipson et al. 2016), a R/Bioconductor package, to identify KEGG database pathways that are more likely to occur in the associated CpGs than would be expected by chance. Genes with more CpG probes on the MethylationEPIC array are more likely to have differentially methylated CpGs, which could introduce potential bias when performing pathway enrichment analysis. The \( \gamma \)-m function implemented in missMethyl considers the varying number of CpGs per gene by computing prior probability for each gene based on the gene length and the number of CpGs probed per gene on the array.

**Results**

**Blood mQTL discovery**

To identify blood mQTLs, we tested the association between 3,109,863 SNPs and 609,192 CpG sites from 238 blood samples taken from 164 pediatric patients with CD at diagnosis and 74 matched non-IBD controls (Supplementary Table 1). We identified 535,448 associated SNP-CpG pairs involving 303,986 (9.8%) unique SNPs and 13,823 (2.3%) unique CpGs, at a Bonferroni adjusted genome-wide significance of \( P < 8.21 \times 10^{-14} \) (Fig. 1a). On average, each CpG site was associated with \( \sim 38 \) SNPs [interquartile range (IQR): 4–42], which likely reflects linkage disequilibrium (LD) between proximal SNPs, while each mQTL variant associated with 1.73 CpGs (IQR: 1–2). Using the clump command in PLINK (Purcell et al. 2007), we identified 16,313 LD pruned proximal SNPs that were associated with 13,202 CpG sites, which made a total of 24,523 associations.

 Ninety-two percent (\( n = 22,566 \)) of the SNP-CpG pairs involved associations between the LD-pruned genetic variants (\( n = 14,979 \)) and CpG sites (\( n = 12,260 \)) within 1 MB (\( \pm 500 \) kb; Fig. 1b) and were considered cis-acting. The remaining 8% appeared to be trans-acting (\( n = 1,966 \)). Overall, the full set of cis-acting mQTLs (regardless of LD) tended to have larger effect size (change in DNA methylation) with a mean absolute value of 0.087 [IQR: 0.053–0.108], compared to trans-acting mQTLs with a mean absolute value of 0.069 [IQR: 0.028–0.094] (Mann–Whitney–Wilcoxon \( P < 2.2 \times 10^{-16} \)) (Supplementary Fig. 1). A list of all cis- and trans- SNP-CpG pairs identified from 238 blood samples is provided in Supplementary Table 2. Enrichment analysis of cis-mQTL associated SNPs

**mQTL study**

All SNPs passing QC were tested for association with \( \sim 600K \) CpG sites using a linear mixed model implemented in matrixeQTL (Shabalin 2012). The model was adjusted for age, gender, estimated cell proportions, and 3 genotype-based principal components (PCs) to adjust for population sub-structure, and treated CpG-specific methylation as the outcome and SNP allele count as an explanatory variable. First, we tested each CpG site against all SNPs, and the MethylationEPIC annotation file to annotate CpG sites. A stringent Bonferroni threshold of \( P < 8.21 \times 10^{-14} \) was used to identify mQTLs in the CD (discovery) cohort. To replicate our findings, we further tested all significant associations in the GTP cohort using the same model as in the discovery cohort, adjusting for age, gender, PTSD status, first 3 PCs from genotype data, and estimated cell proportions from DNA methylation data. To identify whether these associations are specifically observed in the disease- and longitudinal datasets, we tested and compared them in the 74 healthy controls, 164 CD cases at diagnosis and at 3-year follow-up, separately. We also tested the significant blood mQTLs in a cohort of ileal samples (\( n = 40 \), which is a subset of individuals from the discovery cohort) to assess tissue-specific associations. To detect all the possible mQTLs for known IBD SNPs, we selected the mQTLs for those SNPs from the discovery cohort results, and calculated FDR on the actual test P-values using the Benjamini-Hochberg method, implemented in the p.adjust function in R. For these follow-up analyses in ileum, we restricted analysis to SNPs that had at least 2 individuals in each genotype category (AA/AB/BB) to ensure sufficient genetic variation. A statistical significance threshold of \( P < 0.05 \) was used for replication analyses of the disease-, longitudinal-, and tissue-associated mQTLs. To formally test for disease-specific differences in CpG-SNP associations in ileum, we performed interaction tests by refitting the original model with the addition of a term for SNP \( \times \) disease status. We used the clump command in PLINK (Purcell et al. 2007) to identify the number of independent associations for each genomic variant with more than 1 significant SNP-CpG association with default parameters (distance threshold = 250 kb and \( r^2 \) threshold = 0.50).

**DNA**

DNA was extracted from whole blood and interrogated using the MethylationEPIC BeadChip according to manufacturer’s instructions. Raw methylation beta values from the Infinium MethylationEPIC BeadChip were determined via the Illumina GenomeStudio program. Samples with probe detection call rates <90% and those with an average intensity value of either <50% of the experiment-wide sample mean or <2,000 arbitrary units (AU) were removed using the R package CpGassoc (Barfield et al. 2012). Data points with detection P-values > 0.01 were set to missing. CpG sites that cross-hybridize between autosomes and sex chromosomes were removed. BMIQ was used to normalize the distribution of types I and II probes (Teschendorff et al. 2013). In total, methylation of 608,245 CpG sites was examined in 780 blood samples of the replication cohort.

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Fig. 1. Genetic associations with DNAm genome-wide in discovery cohort. a) In total 555,448 SNP-CpG pairs are plotted, including both cis- and trans-associations. The x-axis indicates genomic variant position, whereas the y-axis indicates CpG site position. The color of each point represents absolute differences in DNAm per allele relative to the reference allele. Point size represents the significance of the association. A clear positive diagonal line indicates that most of them are cis-associations. b) In total, 14,797 cis-associations are plotted by position of CpG relative to SNP. The strongest associations are seen for proximal pairs of SNPs and CpG sites.

Blood mQTLs are highly replicable in an independent dataset regardless of age and race

To evaluate whether our blood mQTLs are unique to IBD, or more common to the human condition, we tested all cis associations in an independent cohort of 780 blood samples (GTP cohort) collected previously (Almli et al. 2018). More details about demographic information of this dataset are available in Almli et al. (2018) as well as in our methods section. Notably, while our discovery dataset consists of pediatric patients with predominantly Caucasian ancestry (78%; Supplementary Table 1), the GTP dataset is composed of prospectively recruited adult individuals with predominantly African American ancestry (93%), with a mean age of 42.26 (SD: 12.31) years (Supplementary Table 1). After QC, 5,971,966 genomic variants and 608,245 CpG sites were retained for 780 blood samples. To test for the replicability of blood mQTLs from the discovery cohort, we focused only on the SNPs and CpG sites identified in the discovery cohort. In total, 253,000 out of 287,881 SNPs (87.9%) and 12,808 out of 12,843 CpG sites (99.7%) were available to be tested in the replication cohort, respectively. Among the few mQTLs showing associations in the opposite direction (n = 393; 358 unique SNPs and 119 CpG sites) between the CD and GTP cohorts (i.e. an SNP associated with increased methylation in 1 cohort, and decreased methylation of the same CpG in another cohort), we did not observe significant enrichment for any KEGG pathways, including inflammation or immune-related pathways (data not shown).

CD-specific mQTLs in blood samples

To assess whether mQTLs vary with respect to disease status, we retested each of the 498,261 significant SNP-CpG pairs separately within controls (n = 74), within CD cases at baseline (n = 164), and within CD cases during the course of the disease (n = 164; Supplementary Table 7). We observed a strong positive correlation (r = 0.98) between effect sizes estimated from controls vs cases both at baseline and at disease course (Fig. 3, a and c). Collectively, our data establish that while methylation levels may vary with CD at specific sites (CD-associated CpG sites) (Somineni et al. 2019), the influence of SNPs on CpG methylation levels at CpG sites remains consistent in the presence or absence of disease.

Longitudinal profiling of mQTLs

In our previous report, we demonstrated that with treatment, DNAm changes associated with CD at diagnosis revert to the levels seen in healthy controls (Somineni et al. 2019). To examine the longitudinal trajectory of SNP-CpG associations and their dynamics during the course of the disease, we took advantage of our longitudinal DNA methylation data generated from same individuals from the discovery cohort (164 CD cases at diagnosis vs follow-up). We found that mQTL effect sizes were similar between diagnosis and at 3-year follow-up in CD patients, showing a strong positive correlation in magnitude and direction of effect (r = 0.99, Fig. 3b). The 2 outlier clusters in Fig. 3b represent 2 CpGs (cg22715629 and cg00924943) that are associated with 136 SNPs in LD. All discovery SNP-CpG associations (n = 497,689) were nominally significant at baseline (P < 0.05), and 99.5% (n = 497,443) were nominally significant during the course of the disease (P < 0.05). Therefore, our results suggest that these genetic associations are independent of disease status or treatment.

Tissue-specific associations between blood and ileum

To further evaluate how the mQTLs identified in our blood-based discovery dataset act in tissues more directly relevant to disease, we estimated SNP-CpG effect sizes using DNA methylation data from ileal biopsies (n = 40) and compared them to effects observed in blood. With the standard replication threshold of P < 0.05, we replicated 50.4% (n = 238,400) blood SNP-CpG associations in ileal biopsies. Overall, we noted an extremely strong correlation in effect sizes between ileal and blood samples, with 91.4% (n = 473,382) of mQTLs showing directional consistency (r = 0.75; Fig. 3d). A list of all ileal biopsy mQTL results are provided in Supplementary Table 8. We further investigated CpGs (n = 2,188) from SNP-CpG associations (n = 24,879) with directionally inconsistent effects...
between blood and biopsy. Although some of the directionally inconsistent effects may reflect the smaller sample size used in the ileal analysis, we did a KEGG pathway analysis to investigate enrichment for potential tissue-specific biological functions, and observed that these sites were more likely to localize to genes involved in metabolic pathways ($P = 3.34 	imes 10^{-8}$; Supplementary Table 9). Using summary statistics from 2 different studies, Qi et al. (2018) recently showed that mQTLs are highly correlated among the blood and brain tissues. Similarly, using blood and 4 regions of postmortem brain samples, we previously showed a
strong mQTL correlation among the tissues, especially within the 4 brain regions (Smith et al. 2014). Our current results show a similar pattern for blood vs ileum taken from the same patients, where the mQTL effects are highly correlated between blood and ileal biopsies.

**Enrichment of previously identified IBD susceptible SNPs in blood mQTLs**

To date, large-scale GWAS meta-analysis have identified 241 susceptibility loci for IBD (Liu et al. 2015; de Lange et al. 2017). A majority of these (>90%) localize to noncoding regions, presenting a key challenge to identify the functional variants and the relevant genes they act upon. Of these 241 previously identified IBD SNPs, 104 were genotyped and 64 were successfully imputed in our discovery dataset. We found that 37 (22.2%) of these 168 SNPs associated with 69 CpG sites in our discovery set of mQTL associations, which is greater than the 9.8% expected by chance (OR = 2.6, Fisher’s exact test \( P = 3e-6 \); Supplementary Fig. 2a; Supplementary Table 10). All but one of the associations were cis-mQTL associations: 90% \((n = 62)\) of these CpGs were located within 100 kb from the associated GWAS variant. Of these 62 CpGs, 13% are located within 1.5 kb of a transcriptional start site (TSS200 or TSS1500), and 47.8% are between the start and stop codon. These results provide an additional 31 genes potentially associated CpGs 7901 4 11

| Table 1. Summary of blood mQTLs identified in discovery and replication cohorts. |
|-----------------|-----------------|-----------------|
| **Cohorts**     | **Number of mQTL associations in total** | **IBD genomic variants from de Lange et al. (2017); \( n = 241 \)** | **CD-associated CpGs from Sommienen et al. (2019); \( n = 1,189 \)** |
| Discovery cohort \(( n = 238 \)** | Cis-associations | 498,261 | 69 | 565 |
|                 | SNP-CpG associations | 287,881 | 37 | 565 |
|                 | Unique SNPs | 12,843 | 69 | 22 |
|                 | Trans-associations | 37,187 | 1 | 0 |
|                 | SNP-CpG associations | 27,664 | 1 | 0 |
|                 | Unique SNPs | 1,118 | 1 | 0 |
|                 | Unique CpGs | | | |
| Replication cohort \(( n = 780 \)** | Cis-associations | 3,140,805 | 253 | 9,587 |
|                 | SNP-CpG associations | 1,429,468 | 87 | 8,848 |
|                 | Unique SNPs | 62,456 | 252 | 261 |
|                 | Trans-associations | 175,588 | 4 | 184 |
|                 | SNP-CpG associations | 106,900 | 4 | 85 |
|                 | Unique SNPs | 7901 | 4 | 11 |

Pathway enrichment of CpG sites that are under the influence of IBD-associated SNPs

To investigate the utility of DNAm as a tool to identify functional variants and underlying regulatory mechanisms that may contribute to our fundamental understanding of CD susceptibility and pathogenesis, we examined whether CpG sites associated with IBD-associated SNPs localize near genes associated with specific pathways. We first evaluated all 69 CpGs whose blood DNAm associated with IBD SNPs in the discovery analysis \((P < 8.21 \times 10^{-14})\) to identify the novel pathways that are relevant to CD. Our pathway enrichment analysis identified 18 KEGG pathways that were more likely to occur in the IBD SNP-associated CpGs than would be expected by chance \((FDR < 0.05; \text{Supplementary Table 11})\). Among these, 5 were relevant to immune function and other pathways, including human cytomegalovirus infection, insulin resistance, vascular smooth muscle contraction, hepatitis B, and chemokine signaling. Pathway enrichment analysis on an expanded set of CpG sites \((n = 454)\) that associated with IBD SNPs according to a looser significance threshold \((FDR < 0.05)\) showed enrichment for 7 KEGG pathways \((FDR < 0.05)\), including a natural killer cell mediated toxicity pathway. We also observed nominally significant \((P < 0.05)\) enrichment for immune and inflammatory related pathways such as MAPK signaling pathway, cytokine-cytokine receptor interaction, IBD, JAK-STAT signaling pathway, and Th17 cell differentiation (Supplementary Table 12).

Influence of previously identified IBD SNPs on DNAm in ileum mQTLs

We next investigated the 37 SNPs that were both IBD-associated (de Lange et al. 2017) and associated with CpGs in our discovery cohort \((P < 8.21 \times 10^{-14})\) to identify whether they were also mQTLs in ileum (Supplementary Table 13). Seventeen of 37 SNPs
were nominally significant mQTLs ($P < 0.05$) in ileum as well, and effect sizes showed a strong positive correlation between ileum and blood ($r = 0.789$, $P < 7.87 \times 10^{-10}$; Supplementary Fig. 2c).

We also followed up on the 108 FDR-significant IBD-associated SNPs in ileum. We tested 370 of the 454 blood mQTL pairs for association in ileum. These mQTL pairs included 85 of the 108 IBD-associated SNPs; the remaining 23 SNPs were not tested due to insufficient genetic variation in the smaller number of ileum samples. At a nominal threshold of $P < 0.05$, we detected associations for 51 of 85 IBD-associated SNPs. In total, 91 (24.6%) of 370 blood mQTLs in ileum were nominally significant, and the effect sizes were consistent in direction for 89 of these, indicating replication (Fig. 4c; Supplementary Table 14). The 2 IBD SNPs showing association in the opposite direction in ileum (rs35320439, and rs516246) were independently associated with CpGs cg07689252 and cg23216724 (Fig. 4c). Neither of these CpGs were associated with CD in [10]. The strong positive correlation and directionally consistent effects ($r = 0.68$, Fig. 4c) suggest that the IBD-associated SNPs influence the DNAm levels similarly across tissues for most CpGs. Performing the ileal mQTL analysis separately in CD cases ($n = 17$) and controls ($n = 23$) showed little correlation between effect sizes observed for cases vs controls ($r = 0.18$, Fig. 4d). In an interaction analysis of all ileal samples, the interaction between case-control status and SNP genotype was nominally significant ($P < 0.05$) for 17 SNP-CpG pairs involving 14 of the SNPs (Fig. 4d; Supplementary Table 14). These results suggest there may be disease-specific SNP-CpG associations in disease-relevant tissues such as ileum for CD. However, given our smaller sample size ($n = 40$) and the nominal significance level, further confirmation is needed in larger studies of disease-relevant tissues such as ileum.

### CD-associated differentially methylated CpGs in blood mQTLs

Recently, we identified 1,189 CD-associated CpG sites whose methylation level in blood distinguished CD cases from non-IBD controls (Somineni et al. 2019). After exclusion of 212 CpGs with one or more SNPs in their probe sequence, we tested 977 of our previously identified CpG sites for associations with SNPs within 1MB. In the discovery cohort, 22 (1.8%) of these 977 CpGs associated with 565 unique SNPs for a total of 565 SNP-CpG associations ($P < 2.81 \times 10^{-14}$; Supplementary Fig. 2b; Supplementary Table 15). Although we did not observe an enrichment of CD-associated CpG sites compared to the replication cohort (Table 1), 1 CD-associated CpG site cg20406979 had a strong association ($P = 7.60 \times 10^{-10}$) with SNP rs1819333, previously reported to associate with CD (Somineni et al. 2019). This SNP and CpG site were located 314 nucleotides apart, 3 kb upstream of the TSS for the gene RNASET2.

We further investigated the 977 CD-associated differentially methylated CpGs using an FDR-based significance threshold (FDR $< 0.05$) and observed a total of 7,819 cis-associations. 29% ($n = 285$) of 977 CD-associated CpGs were associated with 7,120 nearby genomic variants (Supplementary Table 15), with 59.9% of these CpGs associating with more than 5 SNPs (Fig. 5a). Further, separate analysis on CD cases ($n = 164$) and controls ($n = 74$) indicates that the mQTLs associated with CD-associated DNAm sites in the blood are extremely consistent across the disease types ($r = 0.91$, Fig. 5b).

Next, we investigated whether the CD-associated CpGs associated with 108 previously identified IBD risk SNPs within 1MB. We observed 2 IBD SNPs (rs1819333, rs3853824) associated with 4 CD-associated CpGs (cg20406979, cg23216724, cg15706657, and cg15815084) for a total of 4 cis associations. Of those, 3 CpGs cg20406979, cg23216724, and cg15706657 had stronger association with 1 IBD-associated SNP rs1819333 ($P = 7.60 \times 10^{-10}$, $P = 1.2 \times 10^{-11}$, and $P = 9.3 \times 10^{-07}$, respectively; Supplementary Table 15).

### Influence of SNPs on CD-associated CpGs in ileum mQTLs

Next, we investigated 22 of the 1,189 CpGs previously associated with CD in blood (Somineni et al. 2019) that were also blood mQTLs ($P < 8.21 \times 10^{-14}$) to see if they are also mQTLs in ileum.
In blood, these CpGs were involved in 565 significant SNP-CpG associations. We replicated 173 (30.6%) of these associations in ileal biopsies using a nominal threshold of \( P < 0.05 \), and observed effect sizes are consistent in magnitude and direction in ileum vs blood (\( r = 0.82, \ P < 2.2 \times 10^{-16} \); Supplementary Fig. 2d; Supplementary Table 16).

We performed similar comparisons for the 285 CD-associated CpGs that were significant at FDR < 0.05 in blood, which were involved in 7,819 significant SNP-CpG associations in blood. We followed up on 6,227 of these SNP-CpG pairs in ileum; the remainder were not tested due to insufficient genetic variation among the 40 ileal samples. At a nominal threshold of \( P < 0.05 \), we detected 47 of 285 significant CD-associated CpGs and 1,002 (16.1%) out of 6,227 blood SNP-CpG pairs in ileum (\( P < 0.05 \)). Among these 1002, 65.4% (\( n = 655 \)) showed directionally consistent associations (\( r = 0.15; \ P < 2.2 \times 10^{-16}; \) Fig. 5c). The 34.6% (\( n = 347 \)) of CpG-SNP associations that showed association in the opposite direction in blood vs ileum (Supplementary Table 16) comprised 12 CD-associated CpGs that were associated with 341 SNPs. Boxplots for the top SNP associated with the 12 CD-associated CpGs are compared between blood and ileum (Supplementary Table 16). None of the IBD SNPs showed the opposite mQTL effects between blood and ileum. Overall, only 1 IBD SNP (rs1819333) was nominally associated with a CD-associated CpG (cg15706657) in ileum (\( P = 0.040 \)), and the association was directionally consistent with blood results in our discovery cohort. Our analysis stratified by CD suggested possibly distinct effects for cases (\( n = 17 \)) vs controls (\( n = 23 \)), with nominally significant (\( P < 0.05 \)) interactions between SNP and disease status for 177 mQTLs (177 SNPs and 32 CD-associated CpGs) (Fig. 5d, Supplementary Table 17). However, given the nominal significance levels, this is merely suggestive, and a much larger sample would be required to test this formally.

**Discussion**

The primary focus of this study was to identify and characterize mQTLs in blood from 238 pediatric samples of largely (78%) European ancestry. Our findings establish that blood mQTLs are robust and reproducible, supporting their generalizability across age groups, ancestries, disease status, and DNA sample source, and offer a valuable source for future studies examining blood mQTLs. Recent studies have identified different sets of differential DNAm patterns in various tissue types (Zhang et al. 2013; Lokk et al. 2014), and previous epigenomic and transcriptomic studies in IBD suggest that disease-relevant tissue samples are needed to develop precise biomarkers for the disease subtypes (Sonawane et al. 2017; Barbeira et al. 2018; Venkateswaran et al. 2018; Yang et al. 2018). Our findings show that among genome-wide-significant mQTLs, genetic influences on DNAm are generally consistent across blood and ileum, even among IBD SNPs and CD-associated CpG sites. However, we observed lower cross-tissue reproducibility using a relaxed FDR-based significance criterion. Using a threshold of FDR < 0.05 in blood and nominal association [\( P < 0.05 \)] in ileum, we identified 2 IBD SNPs (Fig. 4c) and 12 CD-associated CpGs (Fig. 5c; Supplementary Fig. 3) with associations in the opposite direction between blood and ileum. This could suggest that a small portion of mQTLs act in a tissue-specific manner, but could also be due to the low power and relaxed significance criteria in the ileal analysis. Studies with large numbers of samples from both blood and ileal biopsies from the same cohort are needed to formally test for tissue-specific associations for the previously identified IBD SNPs or CD-associated CpGs.

Blood mQTLs were also consistent in comparisons of CD patients pre- vs post-treatment and in CD patients compared to healthy matched controls or to an external epidemiologic sample of predominantly African American adults. This trend was
Data availability
The blood DNA methylation data for all the 238 subjects (402 samples) included in this study have been deposited in the Gene Expression Omnibus (GEO) and are accessible through GEO series accession GSE112611. DNA methylation data for the 40 ileal biopsies are also deposited in GEO and can be retrieved through the accession number GSE135905. All the supplementary files related to this manuscript are placed in GSA figshare: https://doi.org/10.25387/g3.17430605.

Author Contributions
SV, KNC, DJC, AKS, and SK conceived of and designed the study. JP, DTO, and SK, processed samples for methylation profiling. VS, HKS, and VK performed the QC on the data. VS performed the analysis with input from DJC and KNC. AKS, SV, HKS, LAD, JSH, DJC, KNC, GG, AKS, and SK interpreted the results and wrote the manuscript. All authors reviewed and approved the manuscript prior to submission.

Ethics approval and consent to participate
Protocols including signed consent of all participants and/or consent of parents or legal guardians in the case of minors were approved by the IRBs of Emory University for CD RISK study supported by a research initiative grant from the Crohn’s and Colitis Foundation. The GTP study was approved by the Institutional Review Board of Emory University School of Medicine and the Grady Health Systems Research Oversight Committee. All participants provided written informed consent.

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Conflicts of interest
None declared.

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