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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

ORIGINAL RESEARCH

Variant-to-Gene-Mapping Analyses Reveal a Role for the Hypothalamus in Genetic Susceptibility to Inflammatory Bowel Disease

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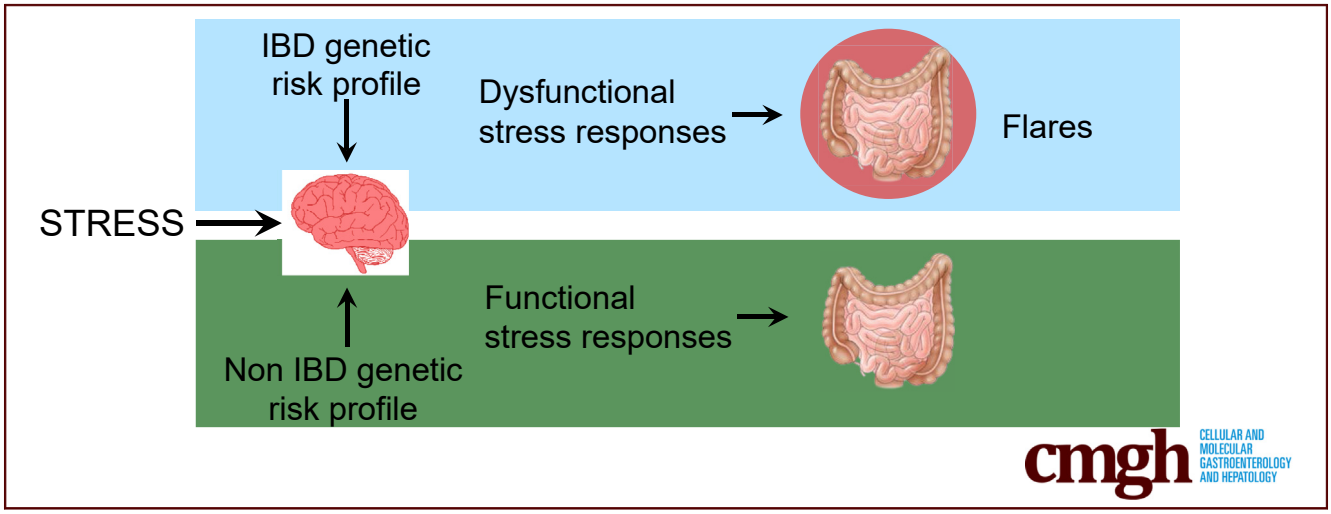

SUMMARY

Inflammatory bowel disease is associated with stress and depression. These 2 comorbidities are influenced by the hypothalamus. Integrating our 3-dimensional genomic data with publicly available genome-wide association study data, our results implicate a subset of inflammatory bowel disease loci conferring their effect via the hypothalamus. Our findings warrant further investigation.

BACKGROUND & AIMS: Inflammatory bowel disease (IBD) is a polygenic disorder characterized principally by dysregulated inflammation impacting the gastrointestinal tract. However, there also is increasing evidence for a clinical association with stress and depression. Given the role of the hypothalamus in stress responses and in the pathogenesis of depression, useful insights could be gleaned from understanding its genetic role in IBD.

METHODS: We conducted genetic correlation analyses on publicly available genome-wide association study summary statistics for depression and IBD traits to identify genetic commonalities. We used partitioned linkage disequilibrium score regression, leveraging our ATAC sequencing and promoter-focused Capture C data, to measure enrichment of IBD single-nucleotide polymorphisms within promoter-interacting open chromatin regions of human embryonic stem cell-derived hypothalamic-like neurons (HNs). Using the same data sets, we performed variant-to-gene mapping to implicate putative IBD effector genes in HNs. To contrast these results, we similarly analyzed 3-dimensional genomic data generated in epithelium-derived colonoids from rectal biopsy specimens from donors without pathologic disease noted at the time of colonoscopy. Finally, we conducted enrichment pathway analyses on the implicated genes to identify putative IBD dysfunctional pathways.

RESULTS: We found significant genetic correlations (rg) of 0.122 with an adjusted P (P_{adj}) = 1.4×10^{-4} for IBD: rg =

0.122; $P_{adj} = 2.5 \times 10^{-3}$ for ulcerative colitis and genetic correlation (rg) = 0.094; $P_{adj} = 2.5 \times 10^{-3}$ for Crohn's disease, and significant approximately 4-fold ($P = .005$) and approximately 7-fold ($P = .03$) enrichment of IBD single-nucleotide polymorphisms in HNs and colonoids, respectively. We implicated 25 associated genes in HNs, among which *CREM*, *CNTF*, and *RHOA* encode key regulators of stress. Seven genes also additionally were implicated in the colonoids. We observed an overall enrichment for immune and hormonal signaling pathways, and a colonoid-specific enrichment for microbiota-relevant terms.

CONCLUSIONS: Our results suggest that the hypothalamus warrants further study in the context of IBD pathogenesis. *(Cell Mol Gastroenterol Hepatol 2021;11:667–682; https://doi.org/10.1016/j.jcmgh.2020.10.004)*

Keywords: HPA; Stress; Colonoids; hESC.

Inflammatory bowel disease (IBD) is an immune-mediated trait, consisting principally of Crohn's disease (CD) and ulcerative colitis (UC), caused by inflammation of the gastrointestinal tract with the disease course ranging from chronically active to intermittent/rare flares. Multiple genetic and environmental factors are known to contribute to the pathogenesis of IBD. More than 230 independent genetic loci for IBD have been reported from genome-wide association studies (GWAS) to date, with many implicated in host-microbiome immunologic-mediated interactions.[1–5]

The environmental factors responsible for triggering the initial presentation and subsequent relapses, as well as their related mechanisms, are not clearly understood. Psychosocial stress is considered one such factor and the majority of IBD patients report a link between their illness and their stress levels[6]; indeed, clinicians have observed an exacerbation of symptoms caused by emotional conflicts.[7] In addition, multiple case studies point to adverse life events as causal factors in IBD relapses,[8] and psychosocial stress can induce and reactivate gut inflammation in animal models of colitis.[9] Finally, as many as a third of IBD patients also are affected by depression,[10] a mood disorder characterized by dysregulated stress responses.[11]

The hypothalamus orchestrates an intricate network of physiological and behavioral responses via processes mediated by the hypothalamus–pituitary–adrenal (HPA) axis and the sympathetic adrenomedullary network.[12] The HPA axis modulates cortisol release via coordinated feedback interactions between the hypothalamus and the pituitary and adrenal glands.[13]

The HPA axis also has been implicated in the pathogenesis of depression.[14,15] Furthermore, IBD patients are more susceptible to depression,[10] with the incidence increasing in association with the active phase of inflammation.[16] However, the possible physiological links between depression and IBD remain unclear.[17] Stress as a comorbid factor in both IBD and depression render the hypothalamus a possible mediator of IBD.

We performed genetic correlation analyses between IBD and depression to assess the degree of genetic commonality and followed this analysis by: (1) partitioned linkage disequilibrium (LD) score regression to measure the enrichment of IBD-associated risk variants in human Embryonic Stem Cell (hESC)-derived, hypothalamic-like neuron (HN), promoter-interacting, open chromatin regions (by intersecting publicly available IBD summary statistics with our Assay for Transposase-Accessible Chromatin (ATAC) sequencing and promoter-focused Capture C data); (2) variant-to-gene mapping to implicate putative causal IBD genes expressed in human embryonic stem cell–derived HNs and in primary hypothalamic tissue; (3) generating ATAC sequencing and promoter-focused Capture C libraries in epithelial-derived colonoids (whose barrier function can be impaired in IBD) to conduct the same analyses as in HNs; and (4) assessing the newly implicated genes, both in the HNs and colonoids, for pathway enrichment to implicate shared, as well as tissue-specific, pathways possibly influenced by IBD-associated genetic variants.

Results

Genetic Correlations

To explore genetic commonalities between IBD and depression, we conducted linkage disequilibrium score regression (LDSR) genetic correlation analyses,[18] leveraging the Psychiatric Genomics Consortium of UK Biobank depression summary statistics[19] and the summary statistics of 11 autoimmune diseases available in the LD Hub Test Center (http://ldsc.broadinstitute.org),[20] namely IBD, UC, CD, asthma, multiple sclerosis (MS), primary sclerosing cholangitis (PSC), rheumatoid arthritis, celiac disease, lupus, and eczema.

Among the 11 autoimmune diseases, IBD was the most statistically significant trait positively correlated with depression (Figure 1). IBD yielded a genetic correlation coefficient (rg) of 0.122 with an adjusted *P* value of 1.4×10^{-4}, followed by UC (rg = 0.122; adjusted *P* value [P_{adj}] = 2.5×10^{-3}) and CD (rg = 0.094 $P_{adj} = 2.5 \times 10^{-3}$). It also was noted that asthma and multiple sclerosis were highly correlated with depression. Although primary sclerosing cholangitis also was correlated significantly with depression, we did not observe such a relationship with celiac

Abbreviations used in this paper: **ACTH, adrenocorticotrophic hormone; ATAC, Assay for Transposase-Accessible Chromatin; CD, Crohn's disease; CNTF, –; cRE, cis-regulatory element; CRH, corticotrophin-releasing hormone; GWAS, genome-wide association study; hESC, human Embryonic Stem Cell; HN, hypothalamic-like neuron; HPA, hypothalamus–pituitary–adrenal; IBD, inflammatory bowel disease; LD, linkage disequilibrium; LDSC, linkage disequilibrium score regression; MS, multiple sclerosis; NE, norepinephrine; P_{adj}, adjusted *P* value; PSC, primary sclerosing cholangitis; rg, genetic correlation; RHOA, ras homolog gene family, member A; SNP, single-nucleotide polymorphism; UC, ulcerative colitis.**

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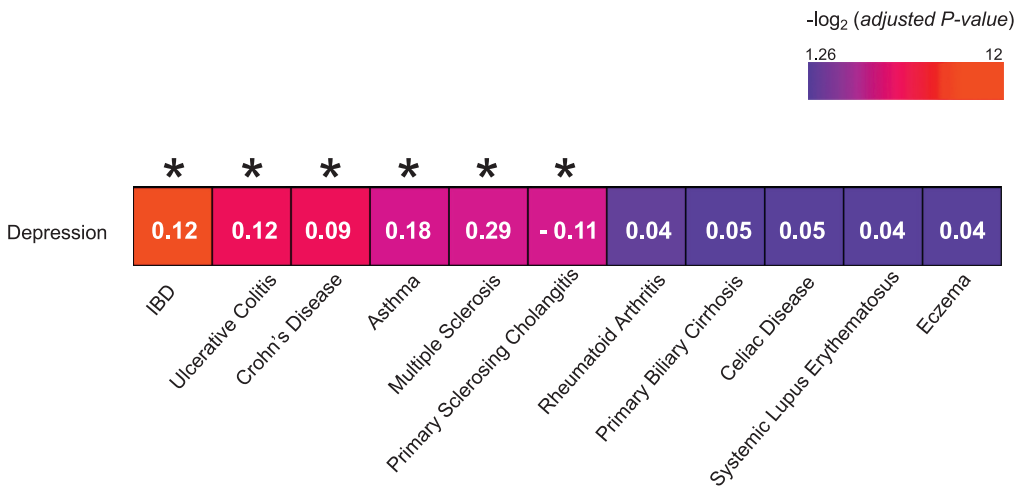

Figure 1. Genetic correlation estimates for 11 autoimmune diseases with depression. Numbers indicate the genetic correlation coefficients (rg) estimated with the LD-Hub test center, using precalculated LD scores. Colors indicate the statistical significance (-$\log_2$ [adjusted *P* value]) of the correlations. *Adjusted *P* value $<$ .05.

disease and rheumatoid arthritis, despite these traits being pathologically related to IBD.[21,22]

Genome-Wide ATAC Sequencing and Promoter-Focused Capture C

We compared 2 cell models: hESC-derived HNs and colonoids by leveraging ATAC sequencing–defined open chromatin maps and high-resolution, genome-scale, promoter-focused Capture C atlases.[23,24] The HNs data sets were previously derived in our laboratory.[23] Colonoids were derived from rectal biopsy specimens of 3 donors without pathologic disease noted at the time of colonoscopy.[25–27] From colonoids, we generated 3 ATAC sequencing libraries that were sequenced and subsequently analyzed with the ENCODE pipeline (https://github.com/kundajelab/atac_dnase_pipelines), yielding 72,440 open chromatin conservative peaks. The Capture C libraries yielded high coverage (an average of ~1.7 billion reads per each colonoid library), with an average of 54% valid reads pairs and 77% capture efficiency. Finally, similarly to what we did with the HNs,[23] we leveraged the colonoid data sets to call

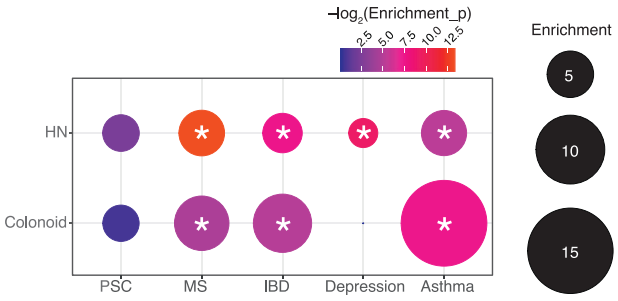

Figure 2. Heritability enrichment of IBD, depression, asthma, MS, and PSC loci within HNs and colonoid. The circle size represents the fold enrichment of IBD SNPs within hypothalamic or colonoid promoter-interacting open chromatin regions. Colors indicate the statistical significance (-$\log_2$ [*P* value]) of the enrichments. *P $<$ .05.

significant interactions using the CHiCAGO pipeline,[28] and performed analyses at 1-fragment resolution to identify short-distance interactions, and at 4-fragment resolution analyses to identify long-distance interactions, and then merged the results.

Partitioned LD Score Regression

We investigated if IBD-associated variants were enriched within promoter-interacting open chromatin regions of HNs. We intersected the ATAC sequencing and promoter-focused Capture C data from HNs with the International IBD Genetics Consortium IBD summary statistics and performed partitioned LD score regression (LDSR). We observed an approximately 4-fold significant enrichment (P = .005) of IBD single-nucleotide polymorphisms (SNPs) within hypothalamic promoter-interacting, open-chromatin regions (Figure 2).

We extended these analyses to depression and the other autoimmune traits significantly correlated with depression described earlier (Figure 1): namely, asthma, MS, and PSC. Depression yielded an approximately 2.5-fold significant enrichment (P = .001), while both asthma and MS had a significant approximate 5-fold enrichment (P = .028; P = 9.89×10^{-5}); in contrast, PSC correlated negatively with depression and there was no evidence of hypothalamic enrichment for the associated SNPs (Figure 2).

As a comparison, we also ran partitioned LDSR intersecting the same set of IBD SNPs with ATAC sequencing and promoter-focused Capture C data from colonoids, yielding an approximately 7-fold significant enrichment (P = .03). We extended these analyses to asthma, MS, PSC, and depression. Surprisingly, asthma showed a significant approximately 15-fold enrichment (P = .006) and MS showed an approximately 6-fold enrichment (P = .04); while there was no evidence for PSC or depression enrichment (Figure 2).

Partitioned LDSR therefore showed that IBD loci were enriched significantly both in HNs and colonoid promoter-interacting, open-chromatin regions. The enrichment in

Table 1. Variant-to-Gene Mapping Implicated IBD Putative Effector Genes in HNs

Implicated genes	PubMed hypothalamus/brain	TPM
CREM	Inhibition of *CRH* [31] Regulation of *PENK* expression[84] Circadian rhythm[85] Regulation of stress-induced corticosterone release in mice[86]	8.9
CNTF	Cortical stress responses[32] Neurogenesis in feeding centers of hypothalamus[87] Secretion of pituitary hormones (PRL, GH)[38] Neuroprotection and axonal regeneration in retina[88]	2.5
FAM111A	—	4
SLC9B2 (or *NHA2*)	—	54.9
SNAI2 (or *SLUG*)	—	3.5
LMAN2	—	52
PRR7	Specific removal of excitatory synapses and Wnt inhibition[41]	31.8
PRR7-AS1	—	8.4
FOXD1	Differentiation of anterior hypothalamic neurons[36] Pituitary luteinizing hormone expression in mice[37]	0.1
PRDM1 (or *BLIMP-1*)	—	1.7
UBAC2	—	13.8
GPR183 (or *EBI2*)	Central nervous system autoimmunity and T-cell migration Neuroinflammation[89]	2
SKAP2	—	16.2
TPD52L2	Invasiveness of glioblastomas[90]	54.2
ATG16L1	Neuroinflammation of central nervous system[55] Epilepsy[91]	19.2
NFKB2	Deficient anterior pituitary with variable immune-deficiency syndrome[39] Neuroprotection of dopaminergic neurons[92]	3.2
ACO2	Neurodegenerative disorders[93]	90.8
PHF5A	—	27.5
DENND1B	—	7.1
TCTA	—	64.7
RHOA	Ghrelin/leptin sensitivity in a subset of hypothalamic neurons[35] Social stress responses in dopaminergic neurons[33] Depression-like behaviors via dendritic remodeling of dopaminergic neurons[34]	261.5
STARD3	—	26.6
CBX3	Neural differentiation[94] Glioma proliferation[95]	43.7
HNRNPA2B1	—	82.1
GPR22	—	43.7

NOTE. Genes implicated leveraging the publicly available IBD summary statistics and ATAC sequencing and promoter-focused Capture C libraries generated in HNs; their reported known functions and their mean relative expression in HNs in TPM were according to RNA sequencing data.
GH, growth hormone; PRL, prolactin; TPM, transcript per million.

colonoids was higher with respect to the hypothalamic data, suggesting, not surprisingly, that IBD-associated loci operate more strongly in the gastrointestinal tract, but, importantly, our data also implicate the hypothalamus to a high degree.

Variant to Gene Mapping

To identify putative IBD functional effector genes, we leveraged published GWAS data, along with the ATAC sequencing and promoter-focused Capture C data described earlier.

We retrieved 332 statistically significant independent sentinel SNPs reported in published IBD GWAS and identified 10,035 proxy SNPs with an $r^2 > 0.8$.[1–3,5,29,30] To focus on just those variants residing within open-chromatin regions in either HNs or colonoids, and therefore putatively functional, we overlapped these proxies with ATAC sequencing peaks. This step identified a total of 471 open proxies for IBD (273 in colonoids, 198 in HNs).

To identify the IBD open proxies that contact the promoters of putative effector genes in HNs and colonoids, we constrained the Capture C data with the ATAC sequencing data for each cell type. We focused on open proxies with $r^2 > 0.8$ that contacted open gene promoter regions, given that this r^2 value represents strong linkage disequilibrium with each reported sentinel SNP. Indeed, approximately 70% of relevant proxies corresponded to members of previously described credible sets.[5]

Implicated Genes

We identified 25 gene promoters contacted by IBD open proxies in the HNs (Table 1, Supplementary Table 1). Gene expression was verified using both our RNA sequencing data generated in HNs[23] (Table 1) and data derived from hypothalamic tissue in the GTEx database (Supplementary Table 2).

Eleven of the implicated genes have known functions in the brain, although there is no current evidence to support a role for the other 14 genes. Among genes with known functions in the hypothalamus/brain, *CREM*, *CNTF*, and *RHOA* are directly involved in stress response regulation: *CREM* encodes a transcriptional repressor that regulates the secretion of corticotrophin-releasing hormone (CRH) and, in turn, corticosterone[31]; Ciliary neurotrophic factor (CNTF) is a polypeptide hormone that modulates cortical stress responses promoting norepinephrine release[32]; ras homolog gene family, member A is a monomeric guanosine triphosphatase that in mice mediates social stress responses and depressive-like behavior in dopaminergic neurons[33,34] and regulates food intake altering hypothalamic tyrosine hydroxylase neuron sensitivity to ghrelin and leptin and the expression of orexigenic hypothalamic neuropeptide agouti-related peptide and neuropeptide Y.[35]

Forkhead Box D1 is required for the development of hypothalamic cell populations responsible for the secretion of hormones such as CRH.[36] Both Forkhead Box D1 and CNTF regulate the secretion of pituitary hormones,[37,38] while mutations in *NFKB2* are causative for variable immunodeficiency and deficient anterior pituitary with variable immune deficiency syndrome, a condition characterized by immunodeficiency and anterior pituitary hormone deficiency, including adrenocorticotrophic hormone (ACTH).[39,40]

In the brain, *PRR7* encodes for a synapse-to-nucleus messenger, which regulates excitatory synaptogenesis by inhibiting Wnt.[41] Hypothalamic Wnt signaling participates in systemic glucose and energy homeostasis.[42]

The *ACO2*, *GPR183*, and *ATG16L1* gene products are associated with neuroinflammation and autoimmunity in the central nervous system, and GPR183 also enhances the migration/infiltration of autoreactive T cells in the brain.[43–45] *ATG16L1* encodes a key autophagy protein critical in handling bacteria in IBD pathogenesis and is associated strongly with CD.[46] The *DENND1B* locus is associated strongly with asthma,[47] a trait in which dysfunction of the HPA axis has been implicated.[48,49]

In colonoids, associated IBD loci contacted 43 genes (Table 2, Supplementary Table 3) that are expressed in the sigmoid and transverse colon, and the small intestine (GTEx database) (Table 2). Seven genes (*ACO2*, *FOXD1*, *LMAN2*, *PHF5A*, *PRR7*, *PRR7-AS1*, and *SKAP2*) were implicated in both HNs and colonoids; *FOXD1*, *PRR7*, and *SKAP2* encode proteins with known roles in immune regulation,[50–52] and *PHF5A* mediates stress resistance in a model of colorectal cancer.[53]

Pathway Analyses

The implicated IBD effector genes in both the HNs and the colonoids were assessed for pathway enrichment through ConsensusPathDB (http://consensuspathdb.org).[54] We initially ran enrichment analyses pooling the implicated genes from both cellular settings. We only considered pathways with an adjusted *P* value less than .05.

We observed an enrichment for immune-related pathways (eg, immune system development, hematopoietic/lymphoid organ development, leukocyte/myeloid cell differentiation, lymphocyte /T-cell/leukocyte activation, cytokine production); respiratory chain-related pathways (eg, tricarboxylic acid cycle, mitochondrial complex II deficiency, oxidative phosphorylation, nitrogen compound metabolic process); hormone-related pathways (eg, endocrine hormone secretion, endocrine process, exocrine system development); tissue development pathways (mesenchyme, connective tissue, columnar/cuboidal epithelial cells, gland); and symbiosis-related pathways (eg, symbiont entry into cells of other organism involved in symbiotic interaction) (Figure 3*A*, Supplementary Table 4).

We then ran the same analyses using the hypothalamic and colonoid implicated gene lists separately. We again considered only pathways with $P_{adj} < .05$. We observed similar enrichments (Figure 3*B* and *C*, Supplementary Tables 5 and 6). The 25 hypothalamic genes showed enrichment for various immune pathways, for the endocrine process, nitrogen compound regulation, gliogenesis, cell junction assembly, and branching structure morphogenesis, as also seen in the pooled analysis. The hypothalamic genes were enriched specifically for sterol binding (Figure 3*B*,

Table 2. Variant-to-Gene Mapping Implicated IBD Putative Effector Genes in Colonoids

Genes	TPM sigmoid colon	TPM transverse colon	TPM small intestine-terminal ileum
ACO2	70.53	82.89	82.42
ASCC2	27.81	29.43	32.96
C1orf106	0.52	14.09	18.08
C5orf56	5.881	4.29	6.56
CBLL1	16.63	10.98	11.46
CD6	0.47	2.54	14.41
CDH3	0.24	0.19	17.05
CEBPG	23.55	29.01	30.61
COG5	11.78	9.55	10.4
CUL2	19.02	12.54	12.61
DAG1	60.8	39.55	29.11
DLD	57.23	51.36	36.65
DOCK7	7.67	4.89	3.83
DUS4L	3.9	3.33	3.83
FAM213A	27.37	25.59	19.45
FNDC3A	26.5	20.54	23.5
FOXD1	0.12	0.07	0.58
GDF9	0.54	0.4	0.48
GNPDA1	25.58	17.39	21.6
HOXA-AS2	4.79	4.21	3.92
HOXA-AS3	0.65	1.54	1.29
HOXA3	9.73	11.47	9.45
HOXA5	23.7	29.19	22.48
HOXA9	25.13	27.34	19.39
LMAN2	77.46	96.25	94.96
MAML2	7.28	6.98	8.37
PHF5A	37.89	32.55	34.48
PRR7	1.13	1.42	2
PRR7-AS1	0.22	0.28	0.5
SATB1	26.37	14.94	7.84
SBNO2	33.46	30.04	41.32
SKAP2	14.97	15.56	19.82
SLC2A13	5.06	4.88	4.11
SPATA7	6.36	3.71	2.84
SPRED2	13.19	13.68	12.4
TEF	34.89	19.16	16.04
TMED10	87.99	80.77	75.48
TNFSF15	3.17	1.38	1.58
TRIM25	17.32	25.54	33.6
TSPAN14	8.97	10.93	13.33
UQCRQ	163.4	184.7	155.5
ZFP90	12.06	7.14	9.29
TBC1D5	12.42	8.78	10.91

NOTE. Genes implicated leveraging the publicly available IBD summary statistic and ATAC sequencing and promoter-focused Capture C libraries generated in colonoids and their relative expression in the sigmoid and transverse colon, and the small intestine in TPM according to GTEx.
TPM, transcript per million.

Supplementary Table 5). The 43 colonoid genes were enriched for various pathways associated with oxidative stress/respiratory chain reactions and symbiosis. They also were enriched for endocrine hormone secretion and immune-related pathways (Figure 3*C*, Supplementary Table 6).

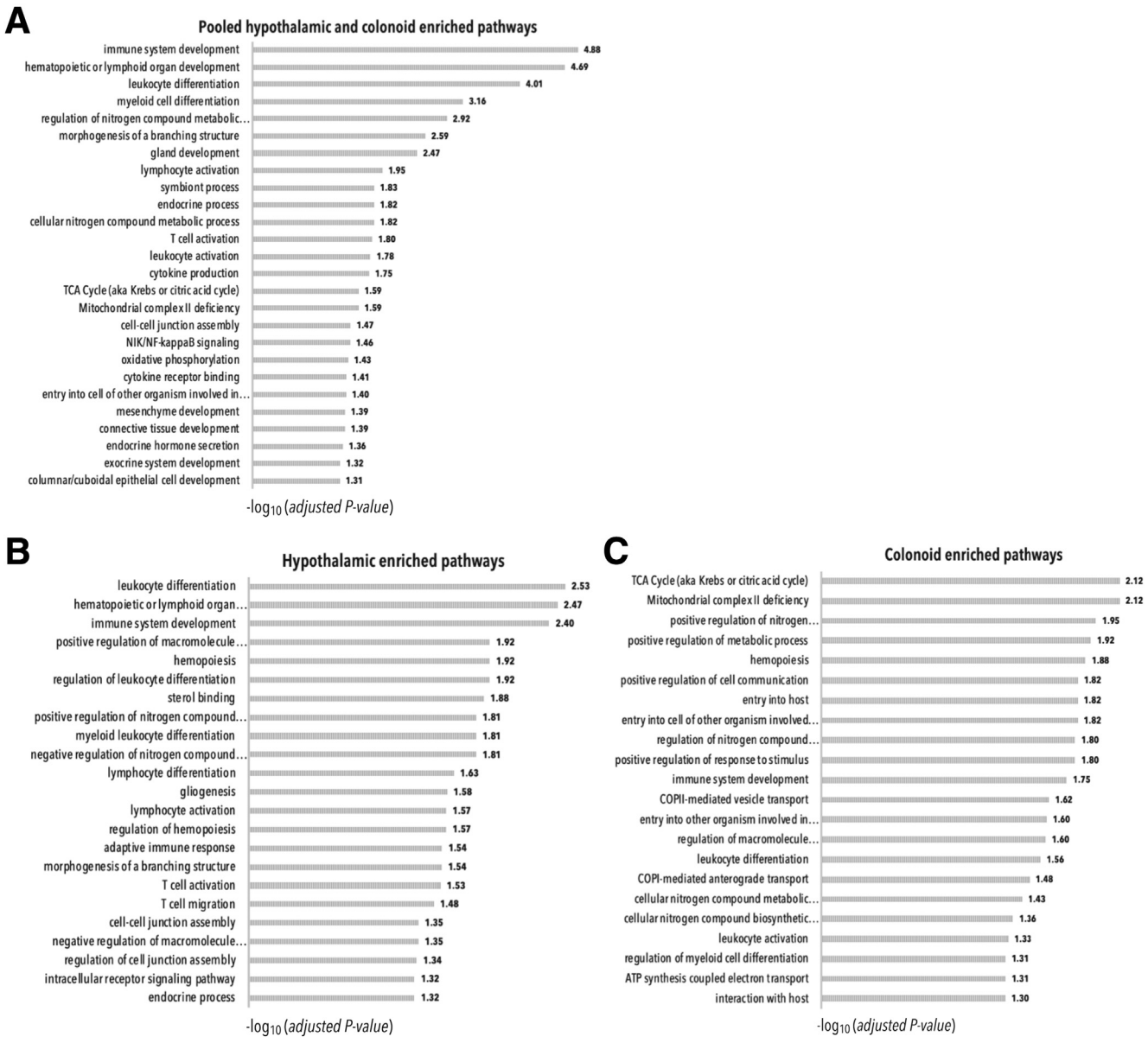

Figure 3. Pathway enrichment in HNs and/or colonoids. Enriched pathways for (*A*) hypothalamic- and colonoid-, (*B*) only hypothalamic-, or (*C*) only colonoid-implicated genes identified using ConsensusPathDB. The selection of significant enriched pathways (adjusted *P* value $< .05$) is reported. *Bars* indicate the statistical significance (-$\log_{10}$ [adjusted *P* value]) of the enrichments. ATP, adenosine triphosphate; COPI, coat protein; TCA, tricarboxylic acid cycle.

Taken together, the overall enrichment pathway results suggested a role for immunity, nitrogen compound metabolism, and hormones in IBD, with a specific role for symbiosis and host interaction in the colonoids and sterol binding in the HNs.

Discussion

Our results implicate a role for the hypothalamus in the genetic susceptibility to IBD. IBD is a complex polygenetic and multifactorial disease that principally affects the immune system and the gastrointestinal tract. However, the phenotype is also highly impacted by stress[6]: psychosocial stress is a known environmental trigger of IBD onset and flares[7,8] and IBD patients manifest high comorbidity with depression,[16,55] a stress-related disorder involving the hypothalamus and characterized by a deregulated HPA axis.[11,15]

We investigated how the genetics of IBD potentially might influence both the hypothalamus and the HPA axis by leveraging both public GWAS reports and our own genomic data. We investigated potential genetic relationships between IBD and stress using depression as a key proxy. We leveraged depression GWAS results, owing to the paucity of GWAS efforts focused on stress, with other stress-related GWAS also being relatively limited and largely focused on male military cases suffering from post-

traumatic stress disorder,[56,57] a condition triggered by a life-changing traumatic event rather than everyday psychosocial stress. Smaller studies on genetic determinants of stressful life events showed a significant and robust genetic association between stressful life events and depression.[58] Despite these correlations, the genetic studies of response to stress-related events are not uniform, with various different triggers being investigated. Each individual trigger study is much smaller than the large, relatively uniform studies of depression, thus supporting our use of depression as the best proxy for stress to run the most powered genetic correlation analyses possible. Using linkage disequilibrium score regression (LDSR) analyses based on selected GWAS, among 11 autoimmune diseases, IBD, CD, and UC were correlated the most significantly with depression (Figure 1). The genetic correlation scores were modest but robust (rg = 0.12, 0.12, and 0.09 for IBD, UC, and CD, respectively), given that in contrast the rg value between CD and UC themselves is 0.54 (http://ldsc.broadinstitute.org/lookup).

We conducted partitioned LDSR to assess the enrichment of IBD loci in promoter-interacting, open-chromatin regions of hESC-derived HNs and colonoids (Figure 2). Colonoids in this study were used principally to contrast with findings in the hypothalamic context. Not surprisingly, we observed a greater enrichment in colonoids (~7-fold enrichment), thus validating our approach, but also giving a sense of the relative degree of the hypothalamic contribution to the pathogenesis of IBD. The significant approximately 4-fold enrichment supports the hypothesis that HNs are indeed a relevant cell type for IBD genetics. We also observed significant enrichment for MS and asthma SNPs in HNs and colonoid putative regulatory elements. The HPA axis is dysregulated in some MS patients, and hypothalamic lesions are correlated with the disease severity and outcomes.[59–61] MS is associated with neurogenic bowel dysfunction,[62] and recent studies have implicated a role for microbial breeches in intestinal barrier integrity in its pathogenesis.[63,64] Asthma also is characterized by a dysregulation of the HPA axis[49] and by breeches in respiratory epithelium barrier integrity leading to exacerbation of inflammatory responses.[65] We speculate that similar abnormalities might occur in the gut, predisposing to a deregulation in the gut microbiome[66] and to characteristic inflammatory responses to food allergens.[67]

A variant-to-gene mapping–based search for IBD SNPs variants in HNs was used to investigate the potential role of IBD genetics within the hypothalamus. We implicated *CREM* (Table 1), which in the hypothalamus encodes an inducible cAMP early repressor,[68] which binds to the *CRH* promoter to terminate CRH expression in response to stressors.[31] However, in the pituitary gland, CRH promotes the expression of adrenocorticotrophic hormone, which in turn stimulates cortisol release from the adrenal gland. Thus, by repressing CRH release, inducible cAMP early repressor represses cortisol secretion and inhibits the HPA axis.[69]

We speculate that in IBD, hypothalamic expression of *CREM* is dysregulated in some genetically predisposed individuals, leading to suboptimal activation of the HPA axis with consequent overactivation of the immune response in the presence of a stressor. Supporting this hypothesis, in 1 small study, IBD steroid-naïve pediatric subjects showed a hyporeactive HPA axis and increased proinflammatory cytokine release in response to psychosocial stressors.[70]

Moreover, LEW/N rats, which have lower levels of hypothalamic CRH and circulating corticosterone, that is, murine cortisol, are more susceptible to both inflammatory disorders and induced colitis.[71] In experiments that aimed to assess a potential role for the neuroimmunoendocrine process mediated by adrenal glands in IBD, adrenalectomized experimental dextran sodium sulfate colitis mice show more severe inflammation and a higher clinical score. Administration of corticosterone to the adrenalectomized dextran sodium sulfate mice eliminates this phenotype.[72]

Another implicated hypothalamic gene is *CNTF*. *CNTF* encodes ciliary neurotrophic factor, which is released in response to acute stress and is necessary for the synthesis of cortical norepinephrine (NE) in norepinephrinergic neurons of the locus coeruleus. CNTF recruits secretagogin and extracellular signal-regulated kinase 1 to increase NE production. NE in turn activates and maintains neurons in the long-lasting excitatory cortical vigilance necessary to promote more favorable physiological and behavioral responses to stressors.[32] Therefore, dysregulation of *CNTF* expression might lead to an abnormal release of NE, affecting responses to stress. NE, epinephrine, and dopamine are stress-related catecholamines that affect nutrient and ion absorption rates in the gastrointestinal tract in addition to modulating the innate immune system and microbiome interactions.[73]

In mice, the guanosine triphosphatase RHOA mediates social stress responses and depressive-like behavior via dendritic remodeling of neurons responsible for motivation and reward[33,34]; specific knockout of *RHOA* in tyrosine hydroxylase hypothalamic neurons of the arcuate nucleus in mice leads to a higher sensitivity to peripherally administered ghrelin and to abolished response to leptin, promoting food intake and obesity-induced ghrelin resistance.[35] Similar modifications of paraventricular hypothalamic nucleus neurons participating in the HPA axis are possible.

NFKB2 mutations are associated with deficient anterior pituitary with variable immune deficiency syndrome, a rare condition characterized by immune and anterior pituitary deficiencies including ACTH.[39]

ATG16L1 is a known risk factor for CD[46] and encodes for a protein required for autophagy,[45] while *DENND1B* has been implicated in asthma.[47,48] Our results showed that asthma and IBD also both are correlated with depression (Figure 1*B*), and their associated SNPs are enriched in promoter-interacting open chromatin regions of HNs and colonoids (Figure 2), suggesting genetic overlap in functions beyond autoimmunity.

We also conducted a variant-to-gene mapping analysis in colonoids, identifying only 7 shared putative effector genes between the 2 cell models (Table 2). Among them, *FOXD1*, *PRR7*, and *SKAP2* gene products participate in regulating the immune system,[50–52] while *PHF5A* encodes a regulator of cellular stress resistance in colorectal

cancer.[53] Although we compared 2 completely different cell models, a number of shared functional genes between HNs and colonoids might have been expected. However, our results reflect the cell-type specificity of chromatin organization and gene regulation, and reflect a possible key aspect of IBD: the same set of genetic variants likely interacting with distinct genes in different cell types. For example, the IBD SNP rs34779708 in the HNs implicated *CREM*, while in the colonoids the same SNP implicated *CUL2* (Supplementary Tables 1 and 3).

We performed enrichment pathway analyses based on the variant-to-gene mapping results. Despite the relatively large number of pathways observed as a consequence of ConsensuPathDB annotations, such that repeated enriched genes showed pathways that are nested and not necessarily independent of each other, we observed a predictably broad enrichment for immune pathways, and enrichment for pathways involving hormonal and endocrine/exocrine systems and for symbiotic pathways and oxidative stress. These associations are consistent with a deregulation of the HPA axis and are in line with current knowledge supporting a role for the microbiome[74] and reactive oxygen/nitrogen species[75] in the development of IBD (Figure 3, Supplementary Tables 4–6).

Limitations of this study included the absence of a valid GWAS focused on stress/psychosocial stress–related disorders resulting from the complexity of defining and measuring the trait and the lack of in-depth characterization of HPA reactivity in an IBD population. Such data, in the context of currently available IBD, CD, and UC GWAS, could provide more direct insights about the relevance of IBD genetics in the hypothalamus and in hypothalamus-mediated stress responses. Our results warrant functional follow-up evaluation to validate these putative hypothalamic–IBD implicated genes, in particular, *CREM*, *CNTF*, and *RHOA*. Future studies are warranted, in which genes could be knocked out specifically in the PVH neurons of model mice for colitis and then the mice exposed to stress, to investigate how inflammation severity compares with model wild-type mice.

Our results are consistent with a role of the hypothalamus in IBD. We propose that IBD-associated SNPs alter the HPA axis and stress responses predisposing to and/or exacerbating this disease. Our data suggest that IBD genetic risk variants influence both general (eg, immunologic and endocrine) and specific (eg, symbiotic process) pathways in different cell types. These studies provide approaches to extending mechanistic analyses of GWAS data for complex phenotypic traits.

Materials and Methods

Genetic Correlation Analyses

We performed LDSR analyses[18] to calculate genetic correlations between pairs of traits using the LD-Hub web interface (http://ldsc.broadinstitute.org). We uploaded the Psychiatric Genomics Consortium of UK Biobank_depression summary statistics[19] and we used the GWAS summary statistic available in the LD-Hub test center[20] for IBD,[2] UC,[2] CD,[2] asthma,[76] MS (http://www.imsgenetics.org), PSC,[77] rheumatoid arthritis,[78] primary biliary cirrhosis,[79] celiac disease,[21] lupus,[80] and eczema.[81] We ran the analyses using default parameters. Quality control of the inputted summary statistics and heritability analysis were performed automatically. The SNPs were filtered automatically and selected on the basis of minor allele frequency >1%, and sample size ($X^2 > 80$). Only summary statistics with more than 450,000 SNPs and more than 5000 individuals were considered. Insertion or deletion of basans, strand-ambiguous SNPs, and SNPs whose alleles did not match those in the 1000 Genomic data were removed. All summary statistics excluded the major histocompatibility complex.[20] We adjusted the *P* values for multiple comparisons using the Benjamini–Hochberg false-discovery rate method and considered 2 traits to be correlated significantly with an adjusted P value $< .05$.

Partitioned LDSR

Partitioned LDSR estimates the heritability from GWAS summary statistics within a subset of regions of the genome after accounting for LD.[18] Partitioned heritability was measured using LDSR v1.0.0 (https://github.com/bulik/ldsc) to identify enrichment of GWAS signals among cis-regulatory elements (cREs) in HNs and colonoids as previously performed.[23] Briefly, the annotation and heritability estimates for HNs and colonoids were generated using bed files containing the position of the cRE (open chromatin regions located proximal to a promoter (-1500/+500 bp of transcription start site) + open chromatin regions located within overlapping regions with ±500 bp buffers. We considered a cRE as open if the normalized ATAC sequencing signal exceeded a cut-off value of fragments per kilobase >1. The HNs and the colonoids were compared with the baseline model for the European ancestry, downloaded from https://github.com/bulik/ldsc/wiki/Partitioned-Heritability. The results were visualized as bubble plots using ggplot2 (https://cran.r-project.org/web/packages/ggplot2/index.html), with the circle size representing the fold enrichment of cREs compared with the base annotation, and the color indicating the statistical significance (-log [*P* value]). We selected a subset of autoimmune GWAS data for partitioned LDSR based on genetic correlation results; we chose the autoimmune diseases that correlated significantly with depression. We tested asthma,[76] IBD (https://www.ibdgenetics.org), MS (http://www.imsgenetics.org), and PSC,[77] and also depression [19] We selected GWAS summary statistics available on LD-Hub.[20] Because the asthma GWAS was a meta-analysis, partitioned LDSR was conducted using the summary statistics from the fixed-effects model.

Cell Models

HNs were generated and validated previously.[23,82] Colonoids were derived following established protocols.[25,27] Briefly, at the time of endoscopy, 1–2 biopsy specimens from the rectum were placed in culture media with antibiotics and then incubated in chelation buffer to

isolate the epithelial crypts. The biopsy specimen was incubated in chelation buffer to isolate the epithelial crypts. Purified crypts were plated in Matrigel (Corning, Corning, NY) droplets and then overlaid with media. The overlaid media, partially produced by conditioned media, contained growth factors (epidermal growth factor, R-spondin, Noggin, and Wnt3a) for stem cell expansion. Approximately 100–200 colonoids were grown per well (~100,000 live cells/well). The colonoids were passaged weekly at a ratio of 1:2 or 1:3 based on density. At each passage, an aliquot of 100,000 cells was processed for ATAC sequencing and to control for passage effects. To obtain at least 2.5 million cells for Capture-C, the colonoids were passaged for expansion 4–6 times. Three replicates were processed from subjects with no pathologic disease.

ATAC Sequencing

ATAC sequencing libraries were generated and analyzed as previously described.[23,24] Briefly, 100,000 colonoids were collected and pelleted at 550 relative centrifugal force for 5 minutes at 4°C. The pellet was resuspended in 50 μL chilled lysis buffer and then centrifuged again at 550 rcf for 10 minutes at 4°C. The pelleted DNA then was tagged using Tn5 Transposase (cat. FC-121–1030; Illumina, San Diego, CA) and incubated for 45 minutes at 37°C. Next, the DNA was purified with the MinElute Kit (cat. 28004; Qiagen, Germantown, MD) and eluted in elution buffer. Purified tagged DNA fragments were polymerase chain reaction–amplified using Nextera primers and NEB-Next High-Fidelity Polymerase Chain Reaction Master Mix (cat. M0541; New England Labs, Woburn, MD) to generate libraries, which then were cleaned with Agencourt AMPureXP beads (cat. A63880; BeckmanCoulter, Brea, CA) and bioanalyzed. Finally, the libraries were paired-end sequenced using the Illumina NovaSeq platform. Open chromatin regions were called using the ENCODE ATAC sequencing pipeline (https://www.encodeproject.org/atac-seq), selecting the resulting irreproducibility discovery rate conservative peaks (with all coordinates referring to Homo sapiens genome assembly GRCh37). We defined a genomic region as open if it had a 1-bp overlap with an ATAC sequencing peak.

ATAC sequencing libraries for HNs were generated and analyzed previously in our laboratory.[23]

Promoter-Focused Capture C

We generated colonoid Capture C libraries following an established protocol.[23,24] Each library then was sonicated using a QSonica (QSonica, Newtown, CT) Q800R to obtain DNA fragments with an average size of 350 bp. DNA fragments were purified using AMPureXP beads (Agencourt) and measured via Qubit (Invitrogen, Carlsbad, CA) fluorometer. Fragment quality and sizes were assessed on a Bioanalyzer (Agilent Technologies, Carlsbad, CA) 2100 using a 1000 DNA Chip. DNA ends were repaired and adaptors were ligated using the SureSelect^{XT} Library Prep Kit (Agilent). After a clean-up step, the sizes and concentrations of DNA fragments were checked again. To generate high-complexity libraries, adaptor ligated DNA fragments were hybridized with a custom-designed capture panel (Agilent)[24] using the SureSelect^{XT} capture kit (Agilent). Each captured library was first paired-end sequenced on 1-lane HiSeq 4000 sequencing (100-bp read length) for quality control and then sequenced on an S2 flow cells on an Illumina NovaSeq (50-bp read length). Data were analyzed as previously described.[24]

Promoter-focused Capture-C libraries from HNs were generated and analyzed previously in our laboratory.[23]

Genetic Loci Included in Variant-to-Gene Mapping

We used 332 loci from published IBD GWAS studies.[1–3,5,29,30] To derive proxy SNPs, we used SNiPA[83] with Homo sapiens genome assembly GRCh37 as human reference assembly, 1000 Genomes phase 1v3 as the variant set, a European population; Ensembl 87 for genome annotation, and an LD threshold of $r^2 > 0.8$.

Enrichment Pathway Analyses

Enrichment pathway analyses were performed using the ConsensusPathDB platform over-representation analyses.[54] Our selected genes were searched in predefined Gene Ontology–based gene sets using a cut-off P value of $\leq$.05 using the default background gene set. ConsensusPathDB calculated P values using the hypergeometric test, which then were corrected by the false-discovery rate method. We considered pathways with at least 2 overlapping genes and with an adjusted P value less than .05 as significant.

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