Duodenal inflammation in common variable immunodeficiency has altered transcriptional response to viruses

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Background: A substantial proportion of common variable immunodeficiency (CVID) patients has duodenal inflammation of largely unknown etiology. However, because of its histologic similarities with celiac disease, gluten sensitivity has been proposed as a potential mechanism.

Objective: We aimed to elucidate the role of the duodenal microenvironment in the pathogenesis of duodenal inflammation in CVID by investigating the transcriptional, proteomic, and microbial signatures of duodenal biopsy samples in CVID.

Methods: DNA, total RNA, and protein were isolated from snap-frozen pieces of duodenal biopsy samples from CVID (with and without duodenal inflammation), healthy controls, and patients with celiac disease (untreated). RNA sequencing, mass spectrometry–based proteomics, and 16S ribosomal DNA sequencing (bacteria) were then performed.

Results: CVID separated from controls in regulation of transcriptional response to lipopolysaccharide and cellular immune responses. These differences were independent of mucosal inflammation. Instead, CVID patients with duodenal inflammation displayed alterations in transcription of genes involved in response to viral infections. Four proteins were differently regulated between CVID patients and healthy controls—DBNL, TRMT11, GCHFR, and IGHA2—independent of duodenal inflammation. Despite similar histology, there were major differences in CVID with duodenal inflammation and celiac disease both at the RNA and protein level. No significant difference was observed in the bacterial gut microbial signature between CVID, celiac, and healthy controls.

Conclusion: Our findings suggest the existence of altered functions of the duodenal epithelium, particularly in response to lipopolysaccharide and viruses. The latter finding was related to duodenal inflammation, suggesting that viruses, not gluten sensitivity, could be related to duodenal inflammation in CVID. (J Allergy Clin Immunol 2022;■■■:■■■-■■■.)

Key words: *CVID, RNA sequencing, proteomics, microbiome, gut microbiota, microbiota, gastrointestinal tract, duodenum, celiac disease, Primary immunodeficiency, IgA*

Common variable immunodeficiency (CVID) is the most common symptomatic immunodeficiency in adults, with a prevalence of 1:25,000 in White subjects.[1] Approximately, 10% to 20% of CVID patients have a monogenic cause, whereas the remaining patients probably have a polygenic etiology influenced by environmental factors.[2] Patients with CVID have a B-cell defect with reduced serum levels of IgG, IgA, and/or IgM,[3] leading to respiratory tract infections with capsulated bacteria.[4] In addition, a large proportion of patients (70-75%) has signs of immune dysregulation, particularly involving macrophages and T cells, resulting in inflammatory and immune-related complications such as autoimmunity and enteropathy.[2]

We have previously found that approximately 50% of CVID patients have heterogenous inflammation in their gastrointestinal (GI) tract, where the most consistent findings are in the proximal part of duodenum.[5] Histologically, the inflammation in the duodenum resembles celiac disease, with increased intraepithelial lymphocytes (IEL) and sometimes villous blunting; there has been great controversy regarding the etiology of this inflammation and whether it could be related to gluten sensitivity.[5-11] Moreover, we have reported that the presence of increased IEL was not associated with GI symptoms or markers of systemic

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Abbreviations used

CVID: Common variable immunodeficiency
CVID_all: All CVID patients
CVID_IEL: Subclassification of duodenal biopsy samples from CVID patient with increased IELs
CVID_N: Subclassification of duodenal biopsy samples from CVID patient with no increased IELs (normal)
DEG: Differently expressed gene
DEP: Differential expressed protein
GI: Gastrointestinal
GO: Gene Ontology (geneontology.org)
IEL: Intraepithelial lymphocyte
LPS: Lipopolysaccharide
MS: Mass spectrometry
RNA-Seq: RNA sequencing
rRNA: Ribosomal RNA

inflammation,[5] and the pathogenesis of IEL in subgroups of CVID patients is still not clear.

Microbial products originating from the commensal bacteria in the gut can, through various mechanisms, initiate systemic inflammatory responses involving activation of innate immunity and potentially also adaptive.[12,13] We have previously shown that gut microbial changes are associated with systemic inflammation and disease severity in CVID.[14] However, to our knowledge, the role of the gut microbiota in relation to increased IEL in CVID is so far not known.

Only a few studies have explored the transcriptome profile of duodenal biopsy samples from CVID patients,[5,15,16] and the literature is almost devoid of proteomics-based and gut microbial analyses from this part of the GI tract. To elucidate the role of duodenal microenvironment in the pathogenesis of CVID, we aimed to investigate the duodenal transcription and protein signature as well as the bacterial microbiota of the duodenal biopsy samples, particularly in relation to the presence of IEL. Given the histologic similarities between duodenal pathology in CVID with increased IEL and celiac disease, duodenal biopsy samples from untreated celiac patients were included in addition to healthy controls.

METHODS

Study design

The objective of the study was to compare regulation of RNA, protein levels, and the bacterial microbiome in duodenal biopsy samples from patients with CVID (with and without GI inflammation), untreated celiac disease (histologically similar duodenal inflammation as CVID patients), and healthy controls. Extended Methods are available in this article's Online Repository at www.jacionline.org. The duodenal biopsy samples from the CVID patients were subgrouped according to the presence of increased IEL (CVID_IEL), or CVID patients with no inflammation (normal) (CVID_N), as previously described.[5] If the comparison were made for CVID patients as a whole (CVID_IEL + CVID_N), we used the prefix CVID_all. CVID subgroups were classified as "infection only" or "complications," as previously defined.[17] CVID enteropathy was defined as persistent diarrhoea after exclusion of GI infection.[11] Here we use "gut microbiota" to refer to the bacteria in the gut.

Ethics

The study was approved by the Regional Committee for Medical and Health Research Ethics and conforms to the principles outlined in the Declaration of Helsinki. Written informed consent was obtained from all participants.

Extraction of RNA and protein

Total RNA and protein were isolated from snap-frozen pieces of duodenal biopsy samples using DNA, RNA, and protein AllPrep kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions with a few modifications (see the Methods in the Online Repository).

RNA sequencing

Total RNA extracted from biopsy samples was used for library preparation (NEBNext Ultra II non-directional RNA library kit with PolyA selection), then further sequenced by Illumina NextSeq v2 total RNA sequencing (RNA-Seq) (San Diego, Calif). The DESeq2 tool (R package, v1.30.1; R Project; www.r-project.org) was used to identify differently expressed genes (DEGs). A modified *t* test (Wald test) was used in statistical *P*-value analysis. Gene Ontology (GO; geneontology.org) and pathway enrichment analyses were performed by the R package clusterProfile, with the GSEA (gene set enrichment analysis) and DAVID (Database for Annotation, Visualization, and Integrated Discovery) tool, using the default settings[18] (see the Methods in the Online Repository).

RNA-Seq data were deposited in the Gene Expression Omnibus (GEO; www.ncbi.nlm.nih.gov/geo) archives under data accession no. GSE207243.

Protein mass spectrometry and proteomics analysis

Precipitated protein pellets, obtained using the AllPrep DNA/RNA/Protein Mini Kit (Qiagen, Hilden, Germany), were subjected to enzymatic digestion; the resulting peptides were analyzed on a liquid chromatography–tandem mass spectrometry platform consisting of an EASY-nLC 1200 ultra-high-performance liquid chromatography system coupled to a Q Exactive HF mass spectrometer operating in FullMS-ddMS2 mode (Thermo Fisher Scientific, Waltham, Mass). The proteins were quantified by processing mass spectrometry (MS) data using MaxQuant (MQ) v1.6.17.0.[19] The raw data were further analyzed, mainly by the R package DEP (Differential Enrichment analysis of Proteomics data, v1.12.0). The differential expressed proteins (DEPs) were identified using the protein-wise linear model ('limma' package inside DEP) combined with empirical Bayes statistics. GO and KEGG (Kyoto Encyclopedia of Genes and Genomes; www.genome.jp/kegg) pathway enrichment analysis were performed using the R package clusterProfile v3.18.1 (see the Methods in the Online Repository).

16S ribosomal RNA microbial analyses

Bacterial DNA was extracted using an established protocol[20] and subjected to high-throughput sequencing of the 16S ribosomal RNA (rRNA) gene with dual-indexed barcodes according to an established protocol.[21] Taxonomic classification of amplicon sequence variants (ASVs) was done in Qiime2 using a naive Bayes classifier[22] trained on the V3-V4 region of a preclustered version (99% sequence similarity) of Silva database v138[23] (see the Methods in the Online Repository).

Statistical analysis

Statistical analyses for RNA-Seq, proteomics, and 16S are described separately above as well as in the Methods in the Online Repository. For the clinical characteristics, we used Kruskal-Wallis tests for continuous variables, and the Pearson chi-square or Fisher exact test for categorical variables, as appropriate. *P* values are 2 sided and are considered significant at <.05.

RESULTS

Patient characteristics

The number of individuals included in RNA-Seq analyses, proteomics analyses, and 16S (bacterial) microbiota analyses for

TABLE I. Patient characteristics for RNA-Seq, proteomic, and 16S analyses

Characteristic	CVID total	Controls	CVID_N	CVID_IEL	Celiac disease	*P* value
RNA-Seq						
No. of patients	12	5	5	7	4	—
Age (years), mean (min-max)	46 (35-62)	49 (32-67)	50 (38-62)	43 (35-58)	37 (26-59)	.225*
Male sex	5 (42)	2 (40)	2 (40)	3 (43)	1 (25)	.946†
CVID enteropathy‡	7 (58)	—	0	7	—	.001§
Infection only‡	1 (8)	—	1	0	—	.417§
Proteomics						
No. of patients	20	10	11	9	5	—
Age (years), mean (min-max)	45 (28-68)	46 (21-67)	48(28-68)	40 (29-58)	34 (25-59)	.194*
Male sex	8 (40)	2 (40)	4 (36)	4 (44)	2 (40)	.832†
CVID enteropathy‡	10 (50)	—	3	7		.070§
Infection only‡	3 (15)		2	1		1.000§
16S analyses						
No. of patients	20	10	11	9	10	
Age (years), mean (min-max)	47 (28-68)	46 (21-67)	50 (28-68)	43 (29-61)	46 (29-65)	.646*
Male sex	9 (45)	3 (30)	5 (46)	4 (44)	6 (60)	.611†
CVID enteropathy‡	11 (55)	—	4	7	—	.092§
Infection only‡	2 (10)	—	1	1		1.000§

Data are presented as nos. (%) unless otherwise indicated. CVID patients are further divided according to the presence of microscopic inflammation in the form of increased intraepithelial lymphocytes (CVID_IEL) or no inflammation (CVID_N) in duodenal biopsy samples.
*Kruskal-Wallis test between CVID_N, _CVID_IEL, healthy controls, and celiac disease.
†Pearson chi-square test between CVID_N, _CVID_IEL, healthy controls, and celiac disease.
‡Percentage of total CVID cohort.
§Fisher exact test.

CVID_IEL, CVID_N, celiac disease, and healthy controls is given in Table I. There was no significant difference in age or sex between the different cohorts. The proportion of the infection-only clinical subgroup was similar between the 2 CVID subgroups. However, there were more patients with enteropathy in the CVID_IEL group compared to CVID_N in the RNA-Seq analyses, but not in the proteomic and 16S rRNA analyses. The overlap of different individuals in each analysis is provided in Table E1 in the Online Repository at www.jacionline.org. All the CVID patients had reduced IgA levels in serum at the time the biopsy samples were taken. Also, all duodenal biopsy samples from CVID patients included in the RNA-Seq and proteomic analyses were negative for norovirus (PCR test; see the Methods in the Online Repository).

Duodenal biopsy samples from CVID patients cluster separately from healthy controls and celiac disease

With regard to RNA-Seq and proteomics, duodenal biopsy samples from CVID patients clustered together, irrespective of the presence of IEL, whereas both healthy controls and celiac disease formed separate clusters (Fig 1, *A* and *B*). For 16S rRNA analyses of bacterial microbial composition from duodenal biopsy samples, 6 CVID patients (3 CVID_IEL and 3 CVID_N) formed a separate cluster compared to healthy controls and celiac disease (Fig 1, *C*).

Differential transcriptome profile in CVID versus healthy controls and celiac disease

Seven DEGs were identified between CVID_all and healthy controls and 30 DEGs between CVID_IEL and healthy controls. When comparing the 2 CVID subgroups, we found that 21 RNAs were differently regulated between CVID_N and CVID_IEL. We found 16 DEGs when we compared CVID_IEL to patients with verified celiac disease (Fig 2, and see Table E2 in the Online Repository at www.jacionline.org).

Enrichment analyses using GO: Different profiles in the CVID subgroups versus celiac disease and healthy controls

CVID patients have altered cellular immune response and response to lipopolysaccharide in duodenum compared to healthy controls. We then applied GO enrichment analysis to the RNA-Seq data obtained from duodenal biopsy samples. When comparing transcription regulation between CVID_all and healthy controls, we found that CVID patients showed different levels of factors involved in cellular responses to IL-1, lipopolysaccharide (LPS), and protein homodimerization activity, with the latter involved in biophysical interaction between 2 proteins that may significantly influence their function, compared to healthy controls (Fig 3, *A*).

CVID patients with duodenal inflammation have altered immune responses to virus in duodenum compared to other CVID patients and healthy controls. When comparing CVID_IEL, a potentially more severe CVID phenotype, with healthy controls, the same findings as above were replicated, with altered cellular response to LPS and protein homodimerization activity. In addition, we found altered transcription profiles in factors related to the type 1 IFN signaling pathway, immune response, defense response to virus, immunoglobulin receptor binding, and chemokine activity/receptor binding (Fig 3, *B*). The majority of these subsets of differently regulated genes had significantly higher transcript levels in CVID_IEL compared to healthy controls, except genes related to immunoglobulin receptor binding, which had significantly lower levels of transcription compared to healthy controls (Fig 2, *B*). Moreover, CVID_IEL had increased levels of transcription

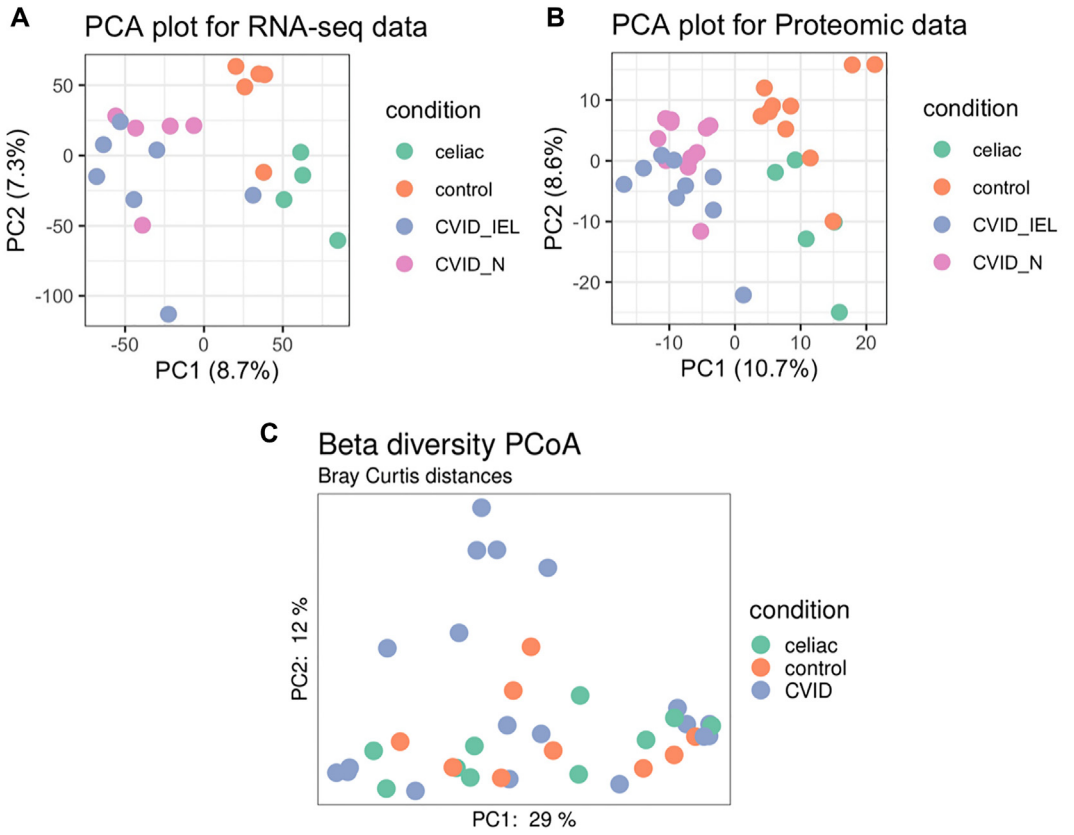


FIG 1. **A** and **B,** Principal component plot for RNA-Seq and proteomics data from duodenal biopsy samples showing the sample distance in 2D, by plotting the first 2 principal components of experimental covariates. Each point represents 1 sample and is colored according to disease phenotype. Samples from celiac disease patients and healthy controls formed separate clusters, whereas samples in CVID_N and CVID_IEL form 1 cluster. **C,** Beta diversity plot (Bray-Curtis) showing microbial diversity between different duodenal samples. *PCA/PCoA,* Principal component plot.

involved in defense responses to virus also when compared to CVID_N, although, to a lesser extent, the chemokine activity and RNA coding for Hsp90 protein-binding protein were also different between the 2 CVID subgroups (Fig 3, *C*).

Celiac-like disease in CVID differs from true celiac disease in terms of RNA regulation. When applying GO enrichment analysis to CVID_IEL and celiac disease, we found differences in transcript profiles involved in pyruvate metabolism, IgG receptor binding, and antigen binding compared to celiac disease (Fig 3, *D*).

Protein network analyses based on RNA-Seq

We next used RNA-Seq data to generate protein network analyses. Instead of using the DAVID tool for enrichment analyses (Fig 3), we first used the clusterProfile package, which can also be used to generate protein interaction networks (see the Methods in the Online Repository). The protein network analyses for CVID_IEL versus healthy controls confirmed the RNA-Seq data by showing increased transcription of mRNAs coding for proteins involved in response to LPS. These analyses also showed increased transcription of factors coding for proteins involved in responses to molecules of bacterial origin and response to oxygen-containing compound (Fig 4, *A*). Of note, further comparisons (ie, CVID_all vs healthy controls and CVID_IEL vs CVID_N) were not possible because of the low number of DEGs.

When applying another protein interaction network, gene set enrichment analysis, we found that celiac disease biopsy samples had enriched factors involved in adaptive immune response, immunoglobulin complex, immunoglobulin receptor binding, and antigen binding (Fig 4, *B*), suggesting reduced function of this part of the immune system in CVID_IEL in the duodenal mucosa also. Again, other comparisons were not possible (not significant) because of the low number of DEGs

Proteome analyses

IGHA2 is one of the major downregulated proteins in CVID compared to healthy controls. In addition to transcript analyses, as well as protein networks based on these analyses, biopsy samples were analyzed using MS for differences in protein regulation between the different groups. First, we compared duodenal biopsy samples from CVID and healthy controls, which showed a different proteomic profile and a clear cluster for disease phenotype (Fig 1, *B,* and Fig 5, *A*). Four proteins were differently regulated between CVID_all and healthy controls: Drebin-like protein (DBNL), TRNA methyltransferase 11 homolog (TRMT11), immunoglobulin heavy constant alpha 2 (IGHA2) and guanosine-5′-triphosphate cyclohydrolase I

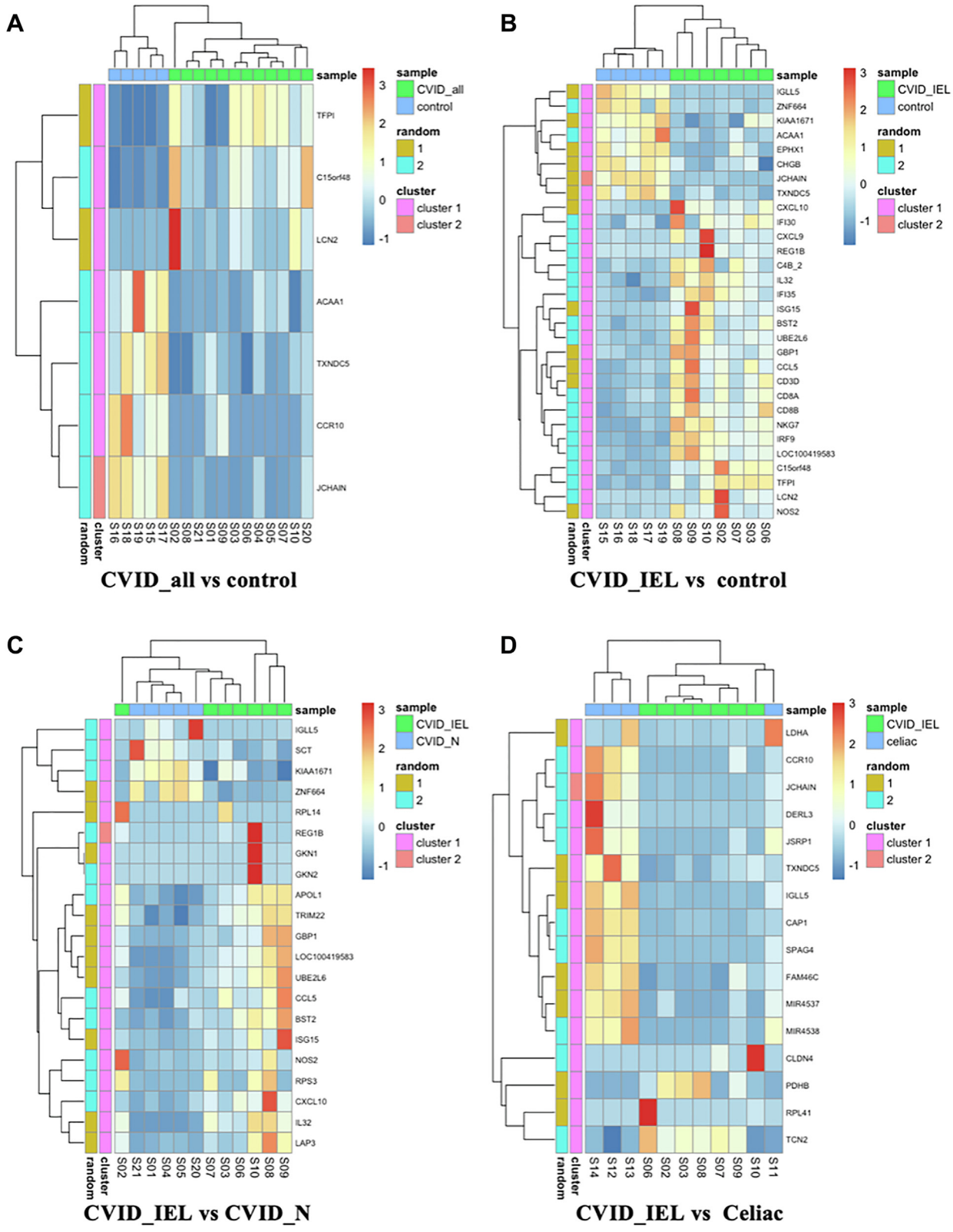


FIG 2. Heat map for DEGs in duodenal biopsy samples comparing **(A)** CVID_all to healthy controls, **(B)** CVID_IEL to healthy controls, **(C)** CVID_IEL to CVID_N, and **(D)** CVID_IEL to celiac disease.

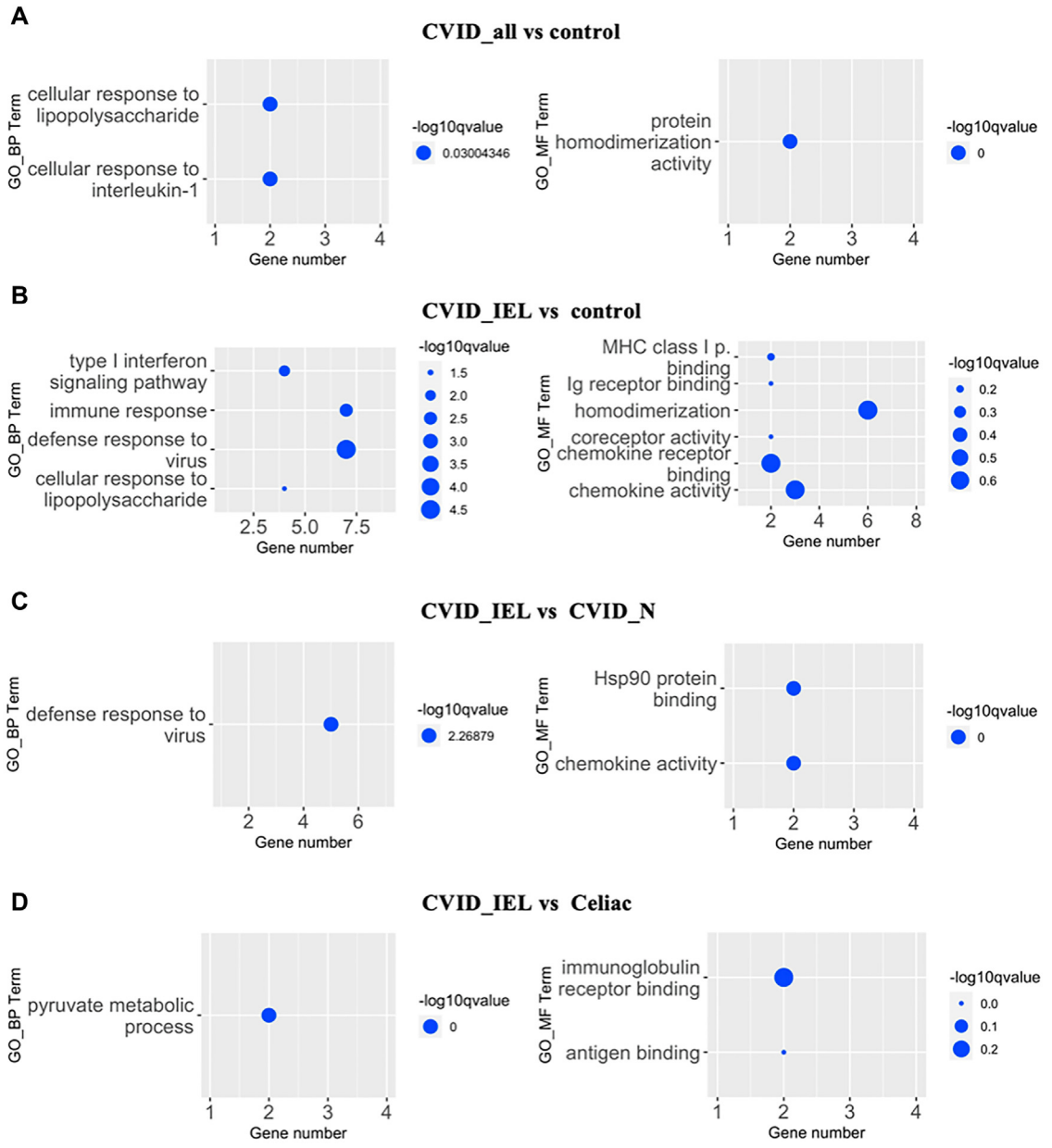


FIG 3. GO (geneontology.org) enrichment analysis based on RNA-Seq (DAVID [Database for Annotation, Visualization, and Integrated Discovery] tool) comparing **(A)** CVID_all and healthy controls, **(B)** CVID_IEL and healthy controls, **(C)** CVID_IEL and CVID_N, and **(D)** CVID_IEL and celiac disease. The *blue dot* refers to number of genes in that pathway, and the *size* refers to the *P* value. *Go_BP Term* and *Go_MF term* refer to GO biological process terms and GO molecular function terms, respectively.

feedback regulator (GCHFR) (Fig 5, *B*), involved in processes like antigen-receptor signaling, methyl transferase network, and biopterin signaling. Importantly, IGHA2 is part of the IgA immunoglobulin complex in the duodenum.

Large differences exist in protein regulation between CVID with IEL and celiac disease. Whereas we did not identify any differently regulated protein when comparing CVID_IEL to controls, or CVID_IEL to CVID_N (Fig 5, *B*), we found several differences between CVID_IEL and celiac disease. The top downregulated proteins when in CVID_IEL were TRMT11, IGHA2, immunoglobulin J polypeptide (IGJ), and immunoglobulin lambda-like polypeptide 5 (IGLL5), most of them related to immunoglobulin regulation. In contrast, CVID_IEL had upregulated protein levels of tripartite motif containing 3 (TRIM3) and B-box and SPRY domain–containing protein (BSPRY), related to, among others, cytokine regulation and stem cell function, respectively (Fig 5; see Table E3 in the Online Repository at www.jacionline.org).

Microbial signature in duodenal biopsy samples in CVID

Of 40 tissue samples, 38 samples (19 CVID, 10 celiac disease, 9 healthy controls) passed quality control and were analyzed for

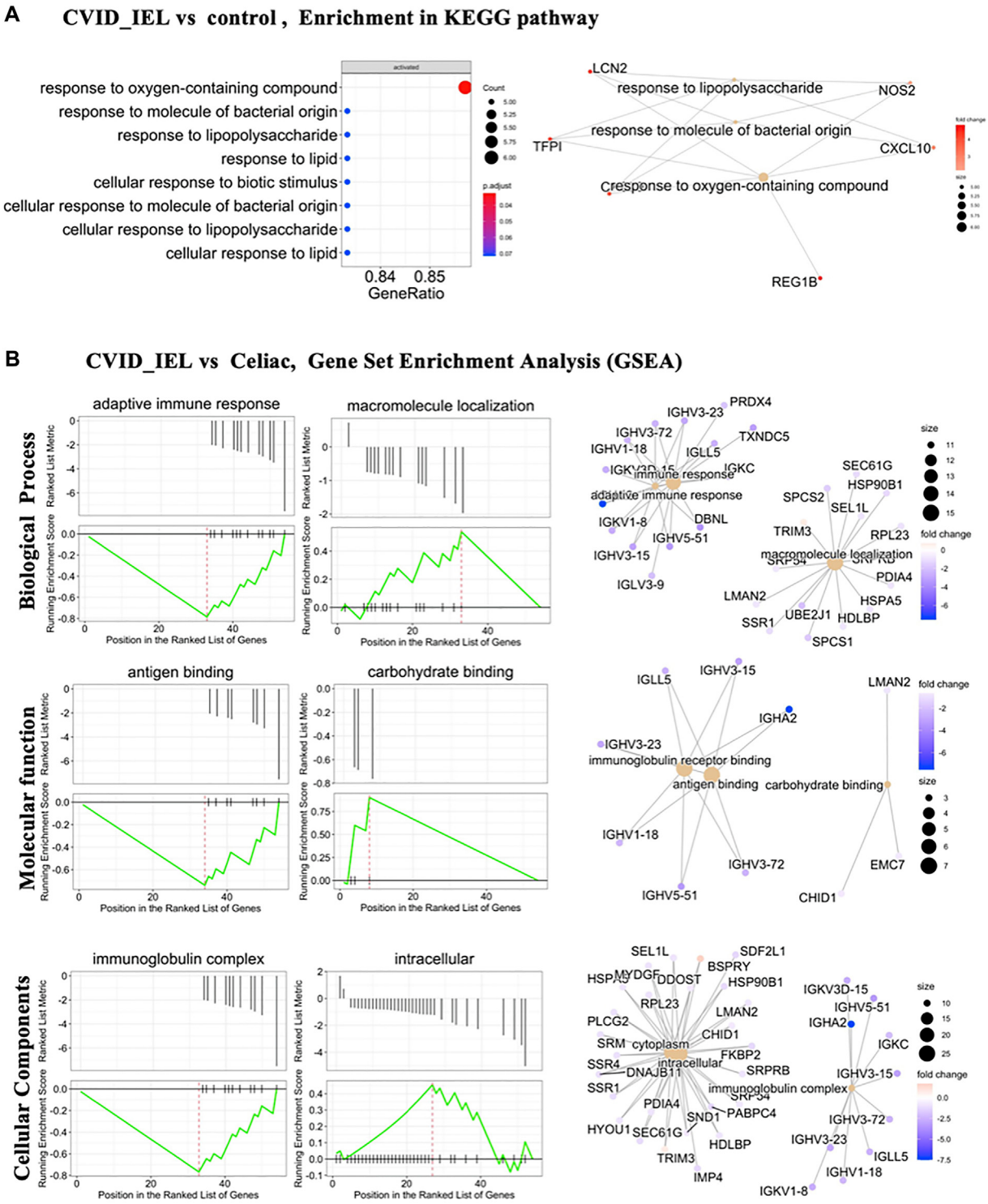


FIG 4. Protein interaction networks based on DEG enrichment in RNA-Seq (clusterProfile package). **(A)** KEGG (www.genome.jp/kegg) pathway enrichment for CVID_IEL versus healthy controls. *GeneRatio* indicates fraction of DEGs found in the gene set of a pathway. **(B)** *Left,* Protein network in top 3 pathways, with gene set enrichment analysis for CVID_IEL versus celiac disease. *Right,* Gene-concept network (cnetplot) in correlated enrichment.

microbial composition. There were no significant differences in alpha diversity (intraindividual diversity) between CVID, celiac disease, and healthy controls ($P = .54$, Kruskal-Wallis), or when comparing the alpha diversities between 2 groups to each other (CVID vs healthy controls, $P = .59$; CVID vs celiac disease, $P = .38$; celiac disease vs healthy, $P = .33$; Fig 6, *A*). Moreover, subgroup analyses of CVID_IEL versus CVID_N showed no significant difference in alpha diversity between groups (Fig 6, *B*).

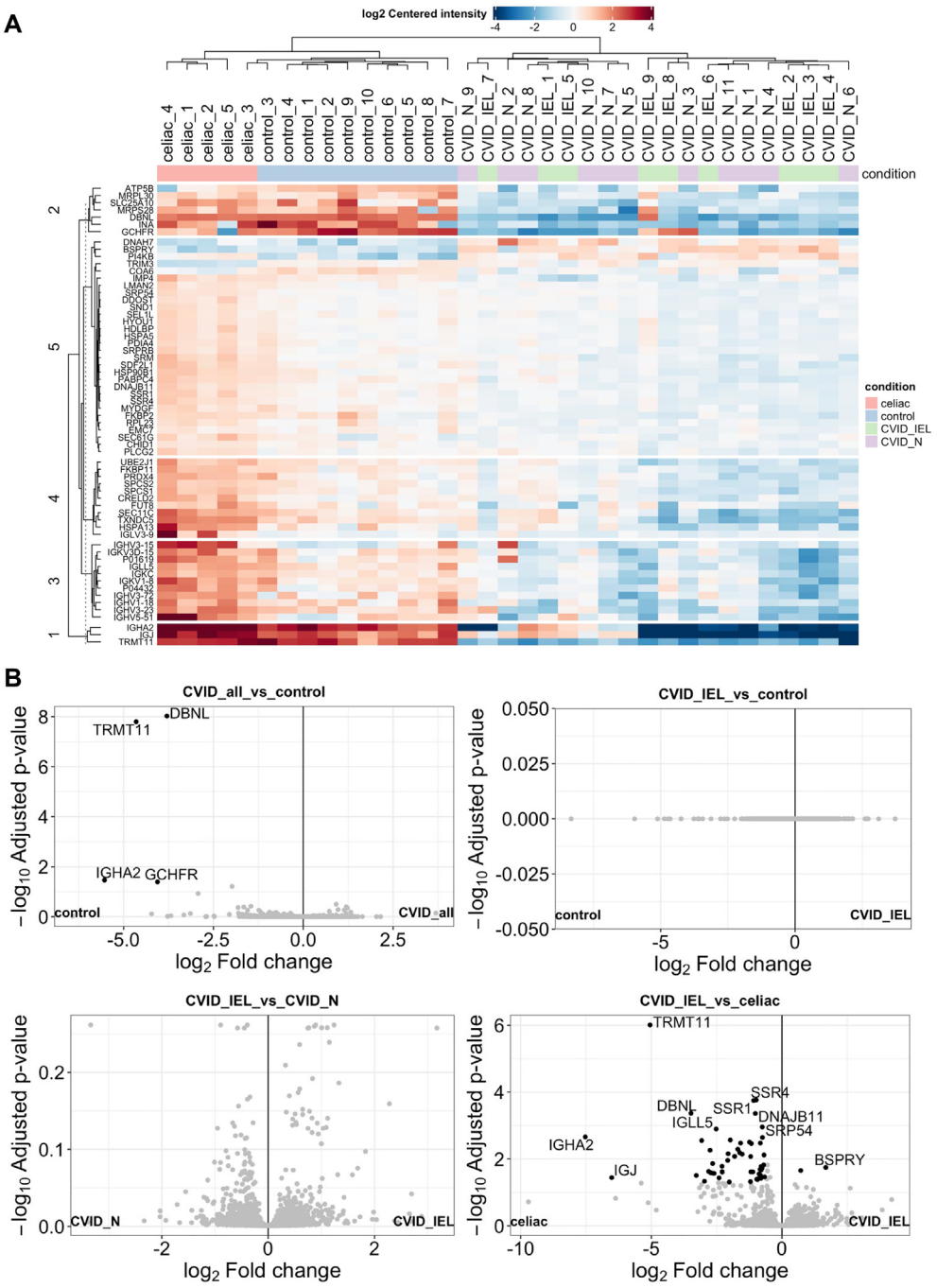
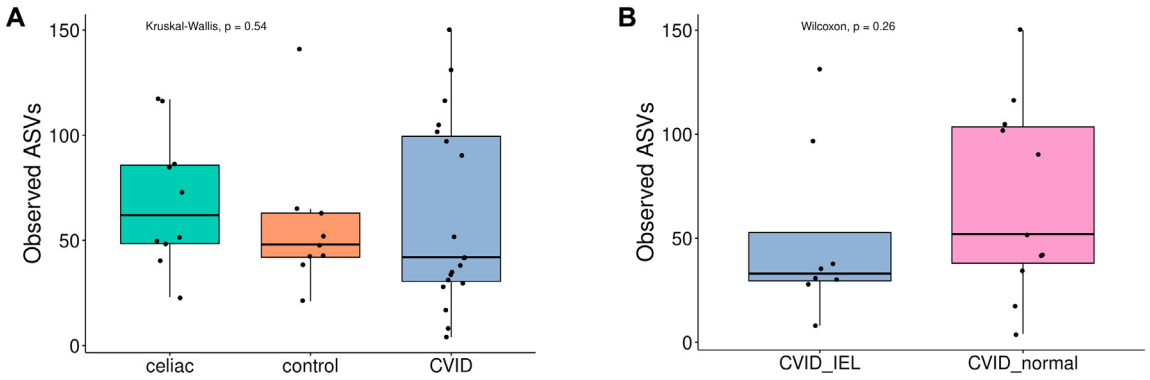


FIG 5. **(A)** Heat map for DEPs. Most DEPs showed higher levels in celiac and lower levels in CVID compared to control. CVID, celiac disease, and healthy controls showed a clear cluster of different protein level profile by group. **(B)** Volcano plot showing protein expression changes in CVID_all versus control, CVID_ IEL versus control, CVID_IEL versus CVID_N, and CVID_IEL versus celiac disease. *Black dots* indicate statistically significant changes, with the names of the main DEPs annotated; *gray dots,* not statistically significantly regulated proteins.

Beta diversity (ie, the degree to which different taxa are shared between individuals or groups of individuals) showed no significant differences between CVID and controls ($P = .58$, $R = 0.05$; Fig 1, *C*), or CVID_IEL versus CVID_N ($P = .32$, R = 0.06). The bacterial taxa, at the genus level, with $P < .05$ (before correcting for false discovery rate) are provided in Fig 6. An overview of all taxa is provided Darebin in Table E4 in the Online Repository at www.jacionline.org. The 4 genera that were upregulated in CVID_IEL compared to CVID_N were all part of the *Gammaproteobacteria*; however, when comparing *Gammaproteobacteria* at the class level, there was no significant difference between CVID_IEL (median 0.74) and CVID_N (median 0.48, $P = .17$).



C

Wilcoxon (rarefied): CVID vs CTR

Genus	median_CVID	median_control	Wilcoxon_pvalue	Wilcoxon_pvalue_fdr
Streptomycetales;f__Streptomycetaceae;g__Streptomyces	0	3.72e-4	0.0171	0.6526
Pasteurellales;f__Pasteurellaceae;g__Actinobacillus	0	1.86e-4	0.0263	0.6526
Burkholderiales;f__Comamonadaceae;g__Tepidimonas	0	0	0.0416	0.6526
Peptostreptococcales-Tissierellales;f__Peptostreptococcaceae;g__Filifactor	0	0	0.0416	0.6526
Bifidobacteriales;f__Bifidobacteriaceae;g__Alloscardovia	0	0	0.0416	0.6526

D

Wilcoxon (rarefied): CVID_IEL vs CVID_normal

Genus	median_CVID_IEL	median_CVID_normal	Wilcoxon_pvalue	Wilcoxon_pvalue_fdr
Xanthomonadales;f__Xanthomonadaceae;g__Stenotrophomonas	6.35e-2	1.86e-4	0.0150	0.6838
Pseudomonadales;f__Moraxellaceae;g__Acinetobacter	0	0	0.0375	0.6838
Burkholderiales;f__Comamonadaceae;g__Xylophilus	0	0	0.0375	0.6838
Xanthomonadales;f__Rhodanobacteraceae;g__Dyella	0	0	0.0375	0.6838

FIG 6. **(A)** Alpha diversity (observed ASVs) in CVID, celiac disease, and healthy control. **(B)** Alpha diversity in subgroup analyses of CVID_IEL versus CVID_N. **(C)** Comparison of taxa between CVID and healthy controls. **(D)** CVID_IEL and CVID_N, using Mann-Whitney test on rarefied genes.

DISCUSSION

In the present study, we examined the transcriptome and proteome, as well as bacterial microbiota, in duodenal biopsy samples from CVID patients, celiac patients, and healthy controls. The main findings were as follows. First, all CVID patients were clearly separated from controls, in particular by regulation of transcriptional response to LPS and cellular immune responses. Second, CVID_IEL patients in addition displayed alterations in transcription of genes involved in response to viral infections compared to the other CVID patients. Third and last, despite histologic similarities, there were major differences between CVID_IEL and celiac disease at both the RNA and protein level, characterized in particular by increased adaptive immune response in celiac disease, clearly suggesting that these are different entities.

RNA-Seq analysis suggested that CVID patients have altered response to LPS in the duodenum. LPS is part of the gram-negative cell wall and is used as marker of microbial translocation from gut to blood. LPS is thought to activate the innate immune system by its interaction with Toll-like receptor 4, an important mediator of innate immunity.[12] We have previously shown that CVID patients have increased LPS in the plasma compared to healthy controls, associated with systemic inflammation, correlating with increased macrophage and T-cell activation. In addition, the level of LPS in blood correlated with gut microbial dysbiosis in stool samples in CVID.[14] It has earlier been suggested that the increased LPS in the plasma of patients, including CVID patients, is the result of local gut inflammation, and thereby loss of tight junction between epithelial cells.[11,24] However, the present data suggest that duodenal gut mucosa of CVID patients has altered response to LPS irrespective of the presence of gut inflammation. Our observation of increased numbers of organisms of genera belonging to *Gammaproteobacteria* in the duodenal gut mucosa of CVID_IEL, in combination with altered gene transcript for LPS and its potential link to systemic inflammation, should be explored in future studies.

The finding of altered viral response in the duodenum in the CVID_IEL patients compared to CVID_N patients and to healthy controls in the RNA-Seq analyses is interesting and may add to our understanding of why a substantial proportion of CVID patients have inflammation in the duodenum. Moreover, whereas it is well established that celiac disease is characterized by altered duodenal IFN-γ response, CVID_IEL patients had altered type I IFN response, which is also supported in a very recent publication

by Strohmeier et al.[25] This may further support an altered response to virus in the duodenum of these patients. Although CVID is considered mainly a B-cell defect, characterized by increased susceptibility to bacterial infections, some previous publications have suggested an increased presence of viruses in the GI tract of CVID patients.[5,26] All of the samples in our study were negative for norovirus at the time of inclusion; however, previous exposure to viruses might be more important than the current presence of the actual virus. Preceding viral infections may have induced epigenetic and epitranscriptomic changes in the epithelial cells in the gut, initiating a chronic inflammatory state in the duodenal mucosa. We hypothesize that an altered virus response in epithelial cells of the duodenum, and not gluten, contributes to duodenal inflammation in CVID_IEL patients.

One of the major downregulated proteins in CVID compared to healthy subjects was IGHA2. IGHA2 is part of the monomeric IgA immunoglobulin complex in the duodenal mucosa. Previously, targeted analyses of messenger RNA levels of *IGHA1* and *IGHA2* genes (IgA subclasses) in duodenal biopsy samples showed that CVID patients with enteropathy had reduced levels of IGHA1 and IGHA2 messenger RNA in the duodenal biopsy samples compared to CVID patients without enteropathy. However, here we found that the IGHA2 protein is also differently regulated in global proteome analysis, where it involves whole CVID group, not only those CVID patients with enteropathy. Another differently regulated protein was DBNL, which is thought to act as a common effector of antigen receptor–signaling pathways in leukocytes and is also a key component of the immunologic synapse that regulates T-cell activation. It has previously been reported that LPS in CVID patients could involve T-cell exhaustion,[27] and the upregulation of DBNL could further support a link between LPS and T-cell activation/exhaustion.

In contrast to transcription profiles and proteomics analyses, the 16S data did not reveal any specific sample cluster for the different disease phenotypes. This is most likely because of the large intraindividual variation in the duodenal bacterial microbiota, which has also been observed in other disease phenotypes, such as inflammatory bowel disease.[28]

The present study has some limitations, such as the relatively small sample size. On the basis of the heterogeneity of the CVID patients, this could have contributed to the relatively low number of differently regulated proteins in the MS analyses. However, the sensitivity of this analyses may also be due to the limited material available from small duodenal samples. A strength of this study was its carefully matched individuals for age, sex, and ethnicity. Moreover, biopsy samples were collected in the same hospital by the same gastroenterologists, from the same part of the duodenum, using a well-defined protocol, thus avoiding anatomic bias.

In conclusion, these data show that altered function of the duodenal epithelium, particularly in response to LPS and virus, as well as downregulated IgA and related proteins, may play a major role in CVID pathogenesis. Instead of gluten, we suggest that an altered response to viruses may contribute to the duodenal inflammation in CVID_IEL.

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Key messages

- **CVID patients have altered transcriptional response to LPS in duodenal biopsy samples compared to controls.**
- **Altered transcriptional response to virus differentiates CVID patients with and without duodenal inflammation.**
- **Transcriptome and proteome profile distinguish between CVID with duodenal inflammation and celiac disease, implying that these are different entities.**
- **Virus and not gluten sensitivity could be related to duodenal inflammation in CVID.**

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