HHS Public Access

Author manuscript

Inflamm Bowel Dis. Author manuscript; available in PMC 2017 December 01.

Published in final edited form as:

Inflamm Bowel Dis. 2016 December ; 22(12): 2794–2801. doi:10.1097/MIB.0000000000000966.

Genetic Risk for Inflammatory Bowel Disease is a Determinant of Crohn's disease Development in Chronic Granulomatous Disease

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Abstract

Background—Approximately one third to one half of children with chronic granulomatous disease (CGD) develop gastrointestinal inflammation characteristic of idiopathic inflammatory bowel disease (IBD), usually Crohn's disease (CD). We hypothesized that overall IBD genetic risk, determined by IBD genetics risk score (GRS), might in part determine IBD development in CGD.

Methods—We reviewed medical records to establish IBD diagnoses in CGD subjects seen at NIAID. IBD risk SNP genotypes were determined using the Immunochip and GRS were estimated by Mangrove.

Results—Among 157 Caucasian CGD patients 55 were confirmed, 78 excluded and 24 were uncertain for IBD. 201 established, independent European IBD risk SNPs passed quality control. After sample quality control and removing non-IBD CGD patients with perianal disease, mean

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Conflicts of Interest: No authors have conflicts of interest to declare.

LIST OF SUPPLEMENTAL DIGITAL CONTENT

CGD_Supplemental Digital Content 1.tiff
CGD_Supplemental Digital Content 2.docx

GRS for 40 unrelated CGD-IBD patients was higher than 53 CGD non-IBD patients (in $\log_2$-scale 0.08±1.62 vs. −0.67±1.64, p=0.026) but lower than 239 IBD Genetics Consortium (IBDGC) young-onset CD cases (0.76±1.60, p=0.025). GRS for non-IBD CGD was similar to 609 IBDGC controls (−0.69±1.60, p=0.95). Seven established IBD SNPs were nominally significant among CGD-IBD vs. CGD non-IBD, including those near *LACC1* (p=0.005), *CXCL14* (p=0.007) and *TNFSF15* (p=0.016).

Conclusions—The weight of common IBD risk alleles are significant determinants of IBD in CGD. However, IBD risk gene burden among CGD children with IBD is significantly lower than that in non-syndromic pediatric CD, congruent with the concept that defective superoxide production in CGD is also a major IBD risk factor. Individual IBD genes might interact with the CGD defect to cause IBD in CGD.

Keywords

chronic granulomatous disease; inflammatory bowel disease; genetic risk score; immunodeficiency disorder; genetic interaction

INTRODUCTION

Chronic Granulomatous Disease (CGD) is a primary immunodeficiency disorder (PID) affecting at least 1 in 200,000 Americans with more than 20 new cases each year.[1] CGD is caused by absence or impaired production of superoxide in neutrophils due to mutations in phagocyte NADPH oxidase (PHOX) enzyme complex genes.[2] The most common form of CGD (70% of cases in North America) is X-linked recessive (XLR), with almost all affected being male, due to mutations in the cytochrome B-245 gene *CYBB* (gp91^{phox}). The other forms of CGD are autosomal recessive (AR), affecting males and females equally, primarily due to mutations in *NCF1* (p47^{phox}) (25% of cases), and less frequently *NCF2* (p67^{phox}), *CYBA* (p22^{phox}), or very rarely *NCF4* (p40^{phox}).[1–3]

CGD is characterized by repeated episodes of bacterial and fungal infections. Children are usually maintained on trimethoprim-sulfamethoxazole[4] and itraconazole[5](or other oral antifungal agents) to prevent these infections, and a subset of patients may also receive recombinant interferon-gamma to boost the immune system.[4] Many of those who have inflammation and autoimmune complications of CGD may be on chronic treatment with corticosteroids. CGD can be cured by allogeneic bone marrow transplant (BMT),[6, 7] which is increasingly being offered to patients with minimum residual oxidase activity or who have severe disease.

CGD complications frequently involve the gastrointestinal tract, with case series reporting more than a third of patients having gastrointestinal complications.[8–11] The most common and disabling gastrointestinal complications are recurrent perianal abscesses or chronic proctocolitis, with features characteristic of Crohn's disease (CD), one of the two main phenotypes of inflammatory bowel disease (IBD).[9] Endoscopic features and disease location may also be typical of ulcerative colitis (UC) (the other major IBD phenotype), although granulomas will inevitably be present on histology, typical of inflammatory responses in CGD, and hence per IBD phenotyping guidelines[12] the IBD phenotype will typically be

classified as CD. CD complicating CGD is often particularly difficult to manage, in part because anti-tumor necrosis factor-α (anti-TNF) therapy is contraindicated due to unacceptable infectious disease complication risks.[13] Development of IBD in patients with CGD tends to occur in childhood. The rate of IBD among CGD patients is truly remarkable: Jones et al. reported that among 94 United Kingdom and Ireland CGD patients, 37% were diagnosed with colitis,[11] a rate nearly 100 times greater than the estimated IBD prevalence in these countries at that time.[14] The median age of colitis onset was 5.2 years. Marciano et al reported on the NIH cohort and found rates of 43% in X-linked patients but much less in recessive forms of CGD.[8]

It is not known why some CGD patients develop chronic idiopathic intestinal inflammation and other features consistent with IBD while others do not. In this study we performed a chart review on a large set of set of CGD patients evaluated at the National Institute of Allergy and Infectious Diseases (NIAID) to identify CGD patients that met clinical criteria consistent with a diagnosis of IBD, and then we tested the hypothesis that CGD patients with IBD have a greater burden of IBD genetic risk variants than CGD patients without IBD. We also examined whether IBD development can be attributed to specific established IBD risk alleles interacting with the CGD genetic defect.

MATERIALS AND METHODS

Study Subjects

Study subjects were limited to those self-described as non-Hispanic white or Caucasian to reduce confounding from a mixed genetic population and because the odds ratios (ORs) we used for IBD risk variants were determined in Caucasians.[15] CGD subjects were patients evaluated at the NIH under NIAID protocols. All study subjects were confirmed as having CGD by the dihydrorhodamine flow cytometry assay of oxidase activity and/or the ferricytochrome C assay of superoxide generation. The genetic subtype of CGD was confirmed by a combination of assessments that included evidence of maternal mosaicism of oxidase activity (characteristic of X-linked CGD carrier), Western blot assessment of presence or absence of specific oxidase subunits or having documented mutations in one of the PHOX genes (*CYBB, NCF1, NCF2, CYBA* or *NCF4*). For comparison purposes, we used demographic and genotype data on NIDDK IBD genetics consortium (IBDGC) CD and non-IBD healthy controls (HC, without any personal or family history of IBD or chronic IBD symptoms), recruited and genotyped by the IBDGC as described.[12, 16] CD cases were limited to those with young-onset CD (diagnosed before age 17 years-old), for better comparison with CGD-IBD subjects who primarily have young-onset disease.

IBD determinations among CGD patients

Medical records of CGD patients, notably consultation, progress, operative, diagnostic imaging, endoscopic, and histopathology reports were reviewed by gastroenterologists experienced in IBD. Patients were considered as having IBD if medical records, notably having required findings on upper or lower endoscopies, surgical resections with related histopathology or radiological studies along with concurrent clinical histories, demonstrated features that established a diagnosis of IBD per the IBDGC phenotyping manual.[12]

CGD patients were classified into three phenotypic groups: Group I: “IBD,” having features diagnostic of CD or IBD type undetermined (i.e. indeterminate colitis); Group II: “no IBD,” either having no history of persistent IBD symptoms (e.g. chronic diarrhea, abdominal pain, rectal bleeding, anal fistula or abscess, or anal fissure), or having a history of IBD overlapping symptoms but with negative evaluations (e.g. negative colonoscopy for rectal bleeding); and Group III: “IBD uncertain”, having a history of chronic or recurrent IBD symptoms but either no or inconclusive evaluations or having no history of IBD but below the age of 17 years-old at last contact. Likewise, patients with a history of allogeneic BMT were classified as Group III as opposed to Group II if they had no history of IBD but had BMT prior to age 17 years-old. Patients with perianal abscesses or ulcers more significant than simple fissures but no other evidence for IBD were also excluded from final analyses.

Genotyping, Quality Control Measures and Statistical Analyses

DNA samples were derived from whole blood. All DNA samples were genotyped using the Immunochip (*Illumina*, Foster City, CA) and genotyped at the Feinstein Institute for Medical Research, New York. Multiple SNP-wise and sample-wise quality control measures were applied on CGD and IBDGC samples separately at first and then together.

For CGD data, we removed SNPs with genotyping missing rate (GMR) >10% or minor allele frequency (MAF) <5%. For IBDGC data, we removed samples with GMR >10% and SNPs with GMR >10%, or MAF <1%. For both datasets we deleted samples with genotypes inconsistent with subjects’ sex as recorded in the clinical databases. After this initial cleaning, we removed CGD group III individuals, then merged the CGD and IBDGC data sets and removed SNPs with A/T or C/G alleles to avoid strand issues, GMR >10%, or MAF <1%. We then removed additional samples with cryptic relatedness and outlier samples per a principle component analysis (PCA).

Cryptic relatedness was determined by plotting estimated probabilities of pairwise allele sharing identical-by-descent of 0 (Z0) and 1 (Z1) and identifying outliers. We retained one sample from each pair of related individuals, preferentially the individual with IBD for discordant pairs. For concordant pairs the individual with lower GMR was removed. For PCA, the first two estimated principal components (PCs) were plotted and outlier samples were removed. Most of the quality control (QC) steps were performed by using PLINK[17] and PCA was conducted in R by using the package snpStats.[18]

IBD genetic risk scores (GRS) were calculated using the R program, Mangrove,[19] with the list of IBD-associated SNPs and their odds ratios (ORs) for IBD, CD or UC (according to lowest reported P-value) and risk allele frequencies (RAFs) estimations in European ancestry individuals from the Liu et al. comprehensive IBD genome-wide association and immunochip meta-analysis.[15] (This study included data from 86,640 European ancestry and 9,846 Asian individuals, identifying 231 genome-wide significant independent SNP associations in 200 IBD loci.[6]) In CGD, even if there is no mucosal inflammation, granulomas are present in gastrointestinal biopsies.[20] Therefore, we included UC risk SNPs in our IBD GRS determinations as patients with CGD-IBD can have a continuous, distal pattern of mucosal inflammation limited to the colon and typical of UC but per IBD phenotyping guidelines, due to the inevitable presence of granulomas, would be called CD

(noting that non-CGD patients with a UC pattern always are classified as CD when multiple granulomas are present). Because our study was limited to individuals of European decent, we limited SNPs to those established as significant in Europeans.[15] For a given sample, if the sample had no call for one or more particular SNPs then the corresponding ORs were not used in the GRS calculation for that sample. Prior to performing statistical comparisons, GRS scores were log transformed, and the log transformed GRS were compared by Mann-Whitney test.

To determine if net IBD genetic risk influenced IBD development in CGD, the estimated IBD GRSs were compared among the following four groups: CGD patients with (Group I, *CGD-IBD*) and without IBD (Group II, *CGD-no IBD*), young onset CD patients from the IBDGC (IBDGC-CD YO), and non-IBD controls from the IBDGC (IBDGC-HC). Pair-wise comparisons were conducted using linear regression and adjusted for PC1 and PC2 for all comparisons and, for *CGD-IBD* vs. *CGD-no IBD*, adjusted for CGD genotypic groups (i.e. two dummy variables for the two most common types of CGD, gp91-phox and p47-phox), in addition to PC1 and PC2.

IBD-associated SNPs that passed QC were also examined for their individual contribution to risk of IBD among CGD patients. Study-wise association tests were conducted using logistic regression in PLINK by comparing the allele frequencies between Group I and Group II study subjects. Sex, CGD type (i.e. two dummy variables for the two most common types of CGD, gp91-phox and p47-phox) and the first 20 PCs were tested for correlation with IBD disease status. Only the first two PCs were significant ($p<0.05$) and therefore included as covariates in IBD association. Analysis was limited to SNPs that passed QC, had GMR less than 10% and minor allele frequency (MAF) greater than 5%.

ETHICAL CONSIDERATIONS

All study subjects (or their parents or guardians), whether recruited by NIAID or the IBDGC, gave informed consent for medical record review and for phlebotomy to obtain DNA for use in genetics research studies.

RESULTS

Clinical Features and QC pruning of the CGD patients

A total of 157 CGD patients with DNA samples were initially evaluated to determine their IBD phenotypic group. There were 135 patients that were males. One hundred and fourteen had XLR *CYBB* mutations (113 were XLR males and one extremely skewed to oxidase negative lyonized female *CYBB* mutation carrier), and the remaining 43 patients had AR gene mutations (22 males and 21 females). Upon chart review 55 were confirmed as having a diagnosis of IBD (Group I), 78 without IBD (Group II) and 24 were IBD uncertain (Group III). Characteristics of these patients are provided in Supplementary Table 1. Among these, 9 Group I, 5 Group II, and 1 Group III patients had inadequate or unsatisfactory DNA for genotyping. DNA from the remaining 142 CGD patients underwent *Immunochip* genotyping.

Following this procedure, we removed the 23 Group III IBD uncertain patients and also those 9 Group II patients who had perianal disease (8 with abscesses, 1 with perianal ulcers) from further analysis. Cryptic relatedness evaluation identified 11 pairs of related remaining CGD patients, all known to be siblings. Two pairs were discordant for IBD, 1 pair was concordant for Group I, and 8 pairs were concordant for Group II. After removal of one individual from each of these pairs (preferentially those without IBD), we removed an additional 5 Group I and 1 Group 2 patients for being PCA outliers (See Supplementary Figure 1).

Characteristics of the final analyzed set of 40 CGD patients with confirmed IBD and 53 patients with no IBD are shown in Table 1. Among the final set of Group II, *CGD-no IBD* patients, 2 patients upon colonoscopy evaluation had a non-specific colitis, and a third had a history of both radiation and *C. difficile* colitis. One patient had perianal disease limited to a perianal fissure.

Among the *CGD-IBD* cases, the colon was the portion of the intestinal tract most commonly involved (34 patients, Table 1). Fourteen patients had a history of perianal disease (all but one had disease also involving the colon or rectum). Interestingly, only 1 patient had disease involving the ileum and 1 patient had disease involving the jejunum. Sixteen patients had disease involving the esophagus, stomach or duodenum. Five of these patients had disease isolated to the upper GI tract disease and one had both upper GI and perianal disease.

Within the final analyzed dataset, XLR patients (i.e. those having *CYBB* mutations) were more likely to have IBD than AR patients (48% vs. 30%, respectively, OR=2.23, 95% CI 0.86, 5.82), but this trend was not significant (p=0.15). This trend appeared to be more related to the mutation than sex, as sex distribution for AR disease was similar among patients with and without IBD.

From the IBDGC a total of 1299 subjects, young-onset CD cases and controls without IBD (not age restricted), were selected. Samples from 238 subjects were removed for having GMR greater than 10%, 112 for having genotype conflicting with database sex designations, 15 for cryptic relatedness, and 97 for being PCA outliers. After QC 848 samples, 239 CD cases and 609 controls, remained and were used in final analyses.

Both CGD and IBDGC data included 196,524 SNPs before QC. After separate QC separately, 110,313 of the 196,524 SNPs remained in the CGD dataset and 130,223 SNPs remained in IBDGC dataset. After QC together 93,733 SNPs remained in the CGD and IBDGC combined dataset.

IBD Genetic Risk Score (GRS)

Two hundred and one (201) SNPs passed QC in both the CGD and IBDGC samples and hence were used to estimate an IBD GRS. The mean GRS for *CGD-IBD* cases was significantly greater (0.081 ± 1.62 [reported in $\log_2$ scale]) than that for *CGD-no-IBD* (−0.67 ± 1.64, p=0.026; Figure 1). The mean GRS for NIDDK healthy controls was nearly identical to that for the CGD-no-IBD patients (−0.69 ± 1.60, p=0.951). Interestingly, mean IBD GRSs among NIDDK young onset CD cases were significantly higher than the mean

CGD-IBD risk scores (0.759 ± 1.601, p=0.025). A histogram illustrating the distribution of GRSs among the different study groups is shown in Figure 2 and demonstrates a relatively large percentage of persons with GRS scores at or above 1.5 in the *CGD-IBD* group as compared to the *CGD-no-IBD* group.

Association with Established IBD Risk Variants

For the *CGD-IBD* vs. *CGD-no-IBD* SNP association analysis, 12 of the 201 SNPs showed nominal or greater significance ($p \leq 0.05$), although none met highly stringent significance Bonferroni corrected for multiple testing of 201 SNPs. Among these, 7 had ORs concordant with those from Liu et al. (Table 2). Most significant among these ($0.005<p<0.02$) were loci at or near Laccase domain containing 1 (*LACC1*), C-X-C motif chemokine ligand 14 (*CXCL14*), and Tumor necrosis factor superfamily member 15 (*TNFSF15*) genes.

DISCUSSION

IBD pathophysiology has been summarized as dysregulated immunity to gut microbes leading to chronic intestinal inflammation.[21] It has long been recognized that CGD mutations alone result in a tremendous tendency, roughly 100-fold, to develop IBD. However, it has not been known why IBD occurs in so many CGD patients, whereas the remaining patients do not develop IBD complications. In this study, we found that among ancestrally matched non-Hispanic Caucasians with CGD, the net IBD genetic load, as determined by presence of common established IBD genetic risk variants unrelated to CGD, were significantly associated with the high rate of IBD development. CGD patients with IBD on average had a significantly higher IBD GRS than CGD patients without either a history of IBD symptoms or with a diagnosis of IBD excluded clinically. Therefore, similar to that of non-syndromic IBD, the greater presence of IBD risk alleles and conversely the reduced presence of IBD protective alleles are partial determinants for the development of IBD. The percentage of individual CGD patients with high GRS appears to be much greater for those with IBD vs. those without IBD: the specific distribution of GRS among individual CGD patients (Figure 2) demonstrated that 53% of CGD-IBD patients had $\log_2$(GRS) scores greater than 0 with half of these ≥ 1.5, versus 30% of non-IBD CGD patients with $\log_2$(GRS) scores greater than 0 and only 25% ≥ 1.5. Additionally, very low GRS scores might be protective against IBD: GRS ≤ -3.0 were only present among the *CGD-no-IBD* cases.

A comparison of GRS among the CGD patients with and without IBD vs. non-syndromic younger-onset CD patients or controls without any history of IBD or chronic IBD symptoms is also revealing. Firstly, the modest presence of weighted-IBD risks alleles was nearly identical among CGD individuals who never developed IBD as compared to ancestry matched healthy controls excluded for a personal or family history of IBD. Also of great interest, was our finding that the mean GRS was significantly greater for young-onset non-syndromic (IBDGC) CD cases as compared to the *CGD-IBD* cases ($\log_2$GRS 0.76 vs. 0.08, respectively), noting that nearly all *CGD-IBD* cases developed IBD (with the granulomas pushing classification to CD) in childhood. The relative mean GRS amongst these four different groups seems intuitive: pediatric IBD *without* profound genetic defects that

markedly increase IBD risk often requires the presence of a high number of IBD risk alleles in order for IBD to develop, whereas in CGD the profound immune dysregulation requires only a more modest presence of IBD genetic risk factors for IBD to occur – although still on average significantly greater than that for persons that never develop IBD, whether they have CGD or not.

Our CGD subjects displayed a broad spectrum of CGD mutations although, as in most CGD cohorts, the majority were XLR *CYBB* males. We observed a non-significant trend for *CYBB*/gp91-phox mutations to increase risk for IBD as compared to the AR mutations (p=0.15). Foster et al.[22] similarly reported that a majority of CGD patients with gastrointestinal or genitourinary complications had XLR vs. AR CGD (respectively 49% vs. 20%, p=0.01). However, some of these patients had genitourinary complications alone, 9.5% of patients were not Caucasians, and whether some cases were related was not reported. In contrast, a British/Irish CGD registry reported only a slight, non-significant increase in colitis complications for XLR vs. AR mutation types (OR 1.18, 95% CI 0.67, 2.15).[11] We can only conclude that the relative effects of XLR vs. AR mutations on IBD development needs further study. Even if the development of IBD is influenced by the type of CGD mutation, our findings are robust and determined following adjustment for gp91-phox, p47-phox and other mutation status as well as for male sex.

Our study attempted to detect specific, established IBD risk alleles that individually might interact with the defect in phagocyte NADPH oxidase to provide a significant risk for IBD alone. We observed 12 SNPs that had nominal or greater evidence for increased risk of IBD among CGD patients. However, 5 of these associations were most likely spurious as their ORs for IBD among CGD patients were opposite those reported by Liu et al.[15] Among the 7 SNPs with concordant ORs, although none remained significant when corrected for multiple testing three showed relatively strong association ($0.005<p<0.02$) given our small study sample. Most strongly associated (and greatest CGD-IBD risk) was rs3764147 (OR=2.7, p=0.0054), located within *LACC1*. In addition to IBD, *LACC1* has been associated with risks for leprosy and juvenile idiopathic arthritis (JIA), and rare missense, putative loss-of-function variants have been found responsible for monogenic forms of early-onset CD and systemic JIA.[23] Although the specific function of mammalian laccase genes are unknown, laccases isolated from fungi have been observed to scavenge free radicals, upregulate expression of anti-oxidant enzymes (i.e. manganese superoxide dismutase and catalase) and protect pancreatic β–cells in-vitro from nitric oxide induced apoptosis.[24] Similar risk and association evidence (p=0.0071) was observed for rs254560 on chromosome 5, located near *CXCL14,* mitochondrial transport gene SLC25A14, and interleukin 9 (*IL9*) genes. Of particular interest may be the third strongest association, rs424560 at *TNFSF15* (OR=0.4, p=0.016), the gene most highly associated with CD in Asian populations, but also a major gene for Caucasian IBD.[25] *TNSFS15* encodes the TL1A cytokine, expressed in lamina propria T cells – notably increased in active IBD – and a potent stimulator of interferon-γ via TL1A receptor, death domain receptor 3 (DDR3).[26] While these associations are intriguing, all will require replication in additional cohorts before any of these loci can be considered as having specific risks for IBD among CGD patients. If replicated, it will be of interest to determine whether some CGD-IBD patients with low GRS are explained by specific loci that disproportionately increase IBD risk in CGD. The only other report of an

attempt to identify genetic factors predisposing CGD patients to gastrointestinal disease was a study of polymorphisms of 7 candidate genes reported by Foster et al., with 3 SNPs for myeloperoxidase (p=0.003), Fc-gamma receptor IIIb (p=0.007) and Fc-Gamma receptor IIa (p=0.05) found significant.[22] Among these, our immunochip genotyping only included the SNP for Fc-Gamma receptor IIa (rs1801274), but we observed no IBD association (p>0.5).

CGD patients are particularly predisposed to perianal disease in those with IBD but also among those without clear evidence of IBD. Nearly half (48%) of those confirmed with IBD had perianal disease, and 9 of 62 patients (15%) excluded for other evidence of IBD also had perianal disease (7 with abscess history, 1 with ulcers and 1 analyzed as a Group II patient having had only anal fissures and followed to age 40). We excluded all but the anal fissure patient in our analyzed set, uncertain as to whether the high rate of perianal disease development in CGD might be related to risk factors overlapping those of IBD as opposed to CGD predisposition to all types of infection, perhaps including perianal abscesses.

In addition to a relatively high rate of perianal involvement, our CGD-IBD cohort also had a relatively high rate of upper GI tract involvement. Although not necessarily limited to those patients who had IBD (and mucosal upper gastrointestinal disease was not reported), Jones et al. found that 11 of 94 CGD patients in their registry had esophagus obstruction or stricture, or had pyloric stenosis.[11] Foster et al. also noted a high rate of these complications (26 of 129 patients), and these were attributed to granulomata within these tissues.[22] It has been observed that young-onset CD is more highly associated with upper GI tract disease.[27] An effort to identify genetic and environmental factors underlying this predisposition among CGD and non-CGD children alike may be valuable.

IBD development in CGD may be associated with great suffering. This is in part because the most effective IBD therapies involve immunosuppression, which, especially as noted for anti-TNF medications, may have unacceptable rates of complications and because in CGD multiple immune dysregulation factors may be involved. Furthermore, CGD patients are traditionally managed initially on long-term corticosteroids, for either their IBD or for other auto-inflammation or granuloma manifestations of CGD. Allogeneic BMT has proven to be extremely effective in treating and potentially curing IBD in CGD patients by eliminating the CGD defect. Our findings suggest that established IBD genetic risk factors can help determine those CGD patients at increased risk for developing IBD and may have future potential for use in clinical algorithms for early BMT for CGD patients and their families who do not opt or are not eligible for BMT. A follow up to the present study might be able to improve this prediction, by replicating our SNP association findings and hence refining the risk SNPs to those that disproportionately play a role in increasing IBD risk among CGD patients. Such replication might also better define interactive molecular risk pathways and further unravel the pathophysiology underlying IBD in CGD specifically and perhaps have implications for non-syndromic IBD as well. Such pathways could potentially be targeted for IBD treatment or prevention in this genetic disorder with profound IBD risk.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the patients, those with CGD who have been followed at the NIH and those with IBD who joined the IBDGC research studies, as well as the healthy controls who volunteered to be a part of our genetic research studies. We thank the Robert E. Sadler Jr family for helping fund the research efforts at Johns Hopkins that made this study possible. This research was supported by the Intramural Research Program of NIAID, NIH, Intramural project numbers Z01-A1-00644 and Z01-A1-00988 (SSD and HLM), NIH Grant DK062431 (SRB) and the Robert E. Sadler, Jr. Family (SRB).

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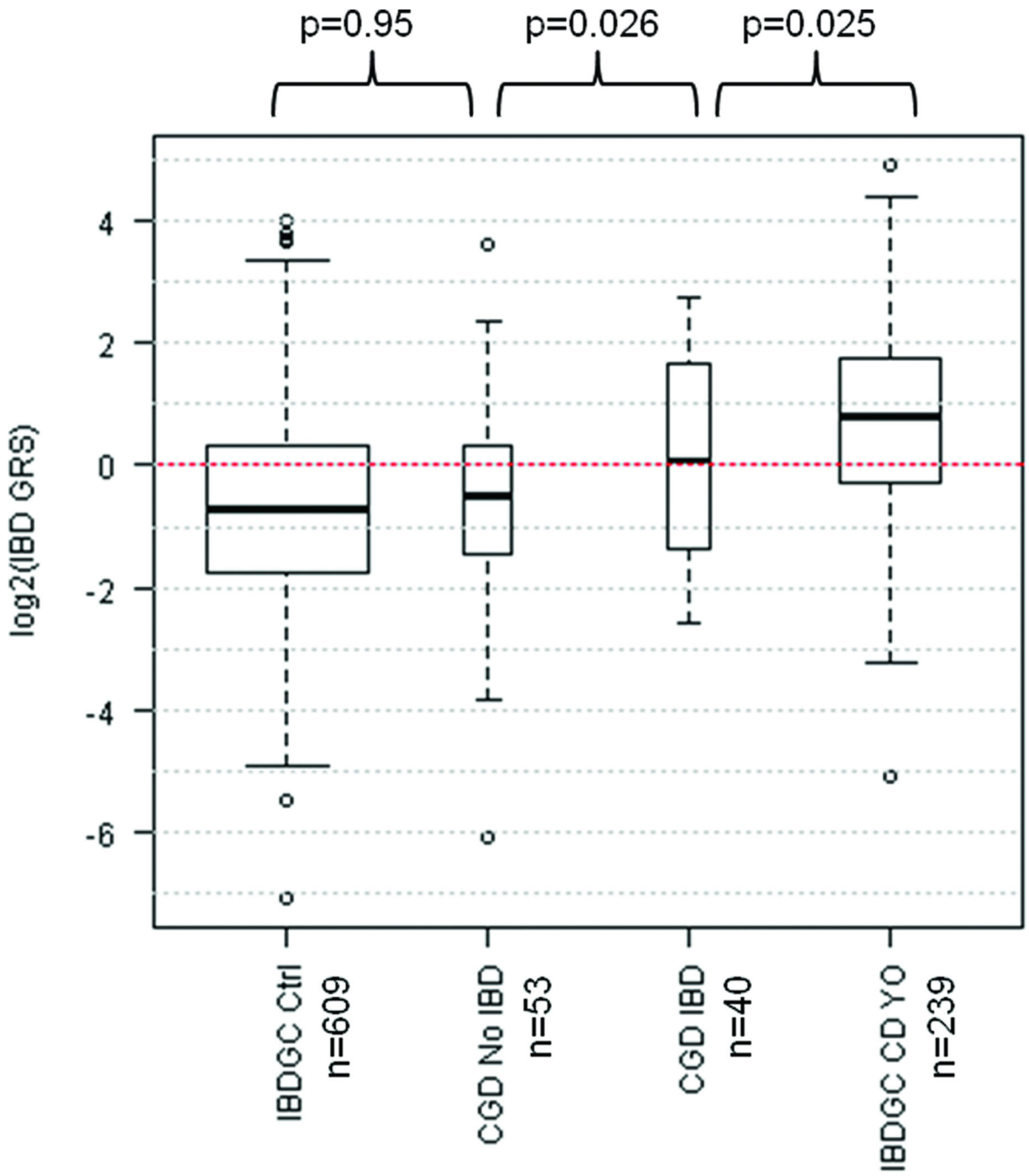

Figure 1.

Comparisons of mean IBD genetic risk scores (GRSs) in log2 scale among 609 IBDGC healthy controls (*IBDGC Ctrl*), 53 CGD patients without IBD (*CGD no IBD*), 40 CGD patients with IBD (*CGD IBD*), and 239 IBDGC young-onset CD cases (*IBDGC CD YO*)

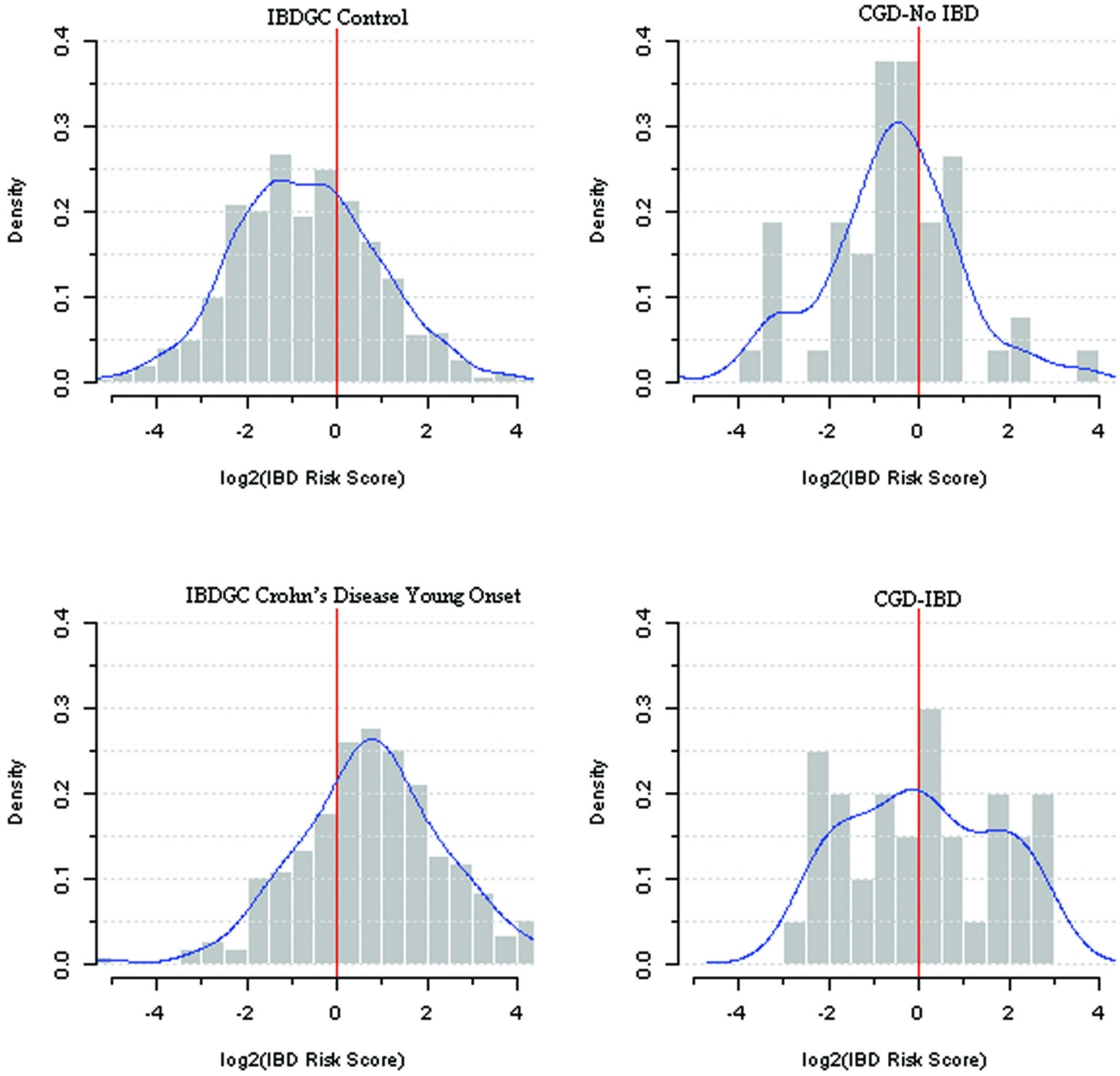

Figure 2.

Histograms showing distributions of IBD genetic risk scores (GRSs) in log2 scale for four sets of study subjects: IBDGC healthy controls and IBDGC Crohn's disease Young Onset (*left side*), and CGD-no IBD and CGD-IBD (*right side*).

TABLE 1

Demographic and Clinical Features of Analyzed Patient Dataset

	Group I, CGD-IBD (%)	Group II, CGD-No IBD (%)
N	40 (43.0)	53 (57.0)
Male	35 (87.5)	46 (86.8)
CYBB[1]	32 (80.0)	34 (64.2)
Sites of chronic inflammation		
Esophagitis	8 (20.0)	2 (3.8)
Gastritis	7 (17.5)	2 (3.8)
Duodenitis	4 (10.0)	3 (5.7)
EGD[2]	16 (40.0)	6 (11.3)
Jejunitis	1 (2.5)	0 (0)
Ileitis	1 (2.5)	0 (0)
Colitis	34 (85.0)	3 (5.7)[3]
Perianal disease[4]	14 (35.0)	1 (1.9)
Internal penetrating	2 (5.0)	
History of IBD surgery	7 (17.5)	

[1] All males;

[2] Inflammation in one or more sites in the esophagus, stomach or duodenum. Excluding perianal disease, inflammation was limited to EGD sites for 6 (37.5%) of Group 1 patients with EGD inflammation and 5 (83.3%) of Group 2 patients with EGD inflammation;

[3] Colitis in 1 patient attributed to radiation and *Clostridium difficile* infection;

[4] Perianal disease limited to anal fissures in 2 Group 1 and in 1 Group 2 patient and limited to anal stricturing disease in 1 Group 1 patient.

TABLE 2

Confirmed IBD Loci with Nominal Association Evidence in CGD with IBD vs. CGD without IBD*

SNP	Chr	BP	A1	A2	OR_IBD	SE	L95	U95	STAT	P	Candidate Genes
rs3764147	13	44457925	G	A	2.739	0.362	1.347	5.567	2.784	0.0054	LACC1
rs254560	5	134443606	A	G	2.576	0.351	1.294	5.129	2.693	0.0071	CXCL14
rs4246905	9	117553249	A	G	0.405	0.376	0.194	0.847	−2.401	0.0163	TNFSF15
rs10061469	5	72518148	G	A	0.454	0.342	0.233	0.887	−2.310	0.0209	FCHO2
rs13277237	8	130604563	G	A	2.067	0.318	1.108	3.857	2.281	0.0226	CCDC26
rs1363907	5	96252803	A	G	2.090	0.324	1.108	3.943	2.278	0.0228	ERAP1
rs7011507	8	49129242	A	G	0.319	0.554	0.108	0.946	−2.060	0.0394	UBE2V2

*Odds Ratios congruent with those from Liu et al., 2015