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Risk Variants in or Near *ZBTB40* AND *NFATC1* Increase the Risk of Both IBD and Adverse Bone Health Outcomes Highlighting Common Genetic Underpinnings Across Both Diseases

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ABSTRACT

Background: Inflammatory bowel disease (IBD) is associated with an increased risk of osteoporosis and bone fracture. The aims of this study were to (1) confirm the association between IBD and low bone density and (2) test for shared risk variants across diseases.

Methods: The study cohort included patients from the Michigan Genomics Initiative. Student's *t* tests (continuous) and chi-square tests (categorical) were used for univariate analyses. Multivariable logistic regression was performed to test the effect of IBD on osteoporosis or osteopenia. Publicly available genome-wide association summary statistics were used to identify variants that alter the risk of IBD and bone density, and Mendelian randomization (MR) was used to identify causal effects of genetically predicted IBD on bone density.

Results: There were 51 405 individuals in the Michigan Genomics Initiative cohort including 10 378 (20.2%) cases of osteoporosis or osteopenia and 1404 (2.7%) cases of IBD. Patients with osteoporosis or osteopenia were more likely to be older (64 years of age vs 56 years of age; $P <$ .001), female (67% vs 49%; $P <$ .001), and have a lower body mass index (29 kg/m^2 vs 30 kg/m^2; $P <$ .001). IBD patients with (odds ratio, 4.60; 95% confidence interval, 3.93-5.37) and without (odds ratio, 1.77; 95% confidence interval, 1.42-2.21) steroid use had a significantly higher risk of osteoporosis or osteopenia. Twenty-one IBD variants associated with reduced bone mineral density at $P \leq .05$ and 3 IBD risk variants associated with reduced bone mineral density at $P \leq 5 \times 10^{-8}$. Of the 3 genome-wide significant variants, 2 increased risk of IBD (rs12568930-T: *MIR4418;ZBTB40*; rs7236492-C: *NFATC1*). MR did not reveal a causal effect of genetically predicted IBD on bone density (MR Egger, $P = .30$; inverse variance weighted, $P = .63$).

Conclusions: Patients with IBD are at increased risk for low bone density, independent of steroid use. Variants in or near *ZBTB40* and *NFATC1* are associated with an increased risk of IBD and low bone density.

Lay Summary

Patients with inflammatory bowel disease (IBD) are at an increased risk of osteopenia, osteoporosis, and bone fracture. Herein, we identify risk variants in or near *ZBTB40* and *NFATC1* which associate with risk of both IBD and low bone density. Therefore, a subset of patients with IBD may be at risk for osteopenia and osteoporosis regardless of steroid use.

Key Words: inflammatory bowel disease, osteoporosis, genetics

Introduction

Preserving bone health is an important part of the care plan for patients with inflammatory bowel disease (IBD).[1] In IBD, the incidence of osteopenia ranges from 32% to 36%, and the incidence of osteoporosis ranges from 7% to 15%.[2] Correspondingly, IBD patients have an increased risk of bone fracture compared with control subjects.[3,4] Unfortunately, bone fractures significantly impair quality of life in addition to increasing morbidity and mortality.[5] Therefore, it is important to understand and mitigate the contributors to decreased bone strength in IBD patients. The most well-known risk factor for the development of osteoporosis in IBD is the use of corticosteroid therapy. However, other risk factors include the presence of chronic inflammation, reduction in muscle mass, and nutrient deficiencies.[6]

Corticosteroids are often used to achieve symptomatic control in active IBD and to bridge patients to effective long-term therapy. Unfortunately, corticosteroids have been linked to a number of adverse effects including an increased risk of osteoporosis and bone fracture.[7-9] While reduction of steroid

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Summary

What is already known?

- Patients with inflammatory bowel disease (IBD) are at an increased risk of osteopenia, osteoporosis, and bone fracture.

What is new here?

- We identify risk variants in or near *ZBTB40* and *NFATC1* which associate with risk of both IBD and low bone density.

How can this study help patient care?

- Patients with IBD should be considered at risk for osteopenia and osteoporosis regardless of steroid use. Patients with IBD who are genetically more susceptible to bone density loss may benefit from enhanced bone density surveillance.

use remains an important strategy in the treatment of IBD, published data suggest that the risk of osteoporosis is not related to corticosteroid use alone. In a study designed to evaluate the impact of IBD-related inflammation on bone metabolism, investigators measured the mean serum concentrations of interleukin-6, interleukin-1β, and tumor necrosis factor α in adult Crohn's disease (CD) patients then applied a cocktail of cytokines, at these measured concentrations, to osteoblasts and osteoclasts to observe changes in gene expression and cell function.[10] The cytokine cocktail was found to increase the expression of *RANKL*, which is a nuclear factor kappa B receptor responsible for induction of osteoclast activity.[11] Moreover, the addition of dexamethasone to the cytokine cocktail further increased expression of *RANKL*. These results suggest that IBD-related inflammatory changes directly alter bone function, and the effect can be amplified by concurrent steroid use. Despite the knowledge that IBD-related inflammation can directly impact bone health, there is a paucity of data investigating shared genetic risk across IBD and osteoporosis.

One study tried to determine if a genetic predisposition to IBD had a causal effect on osteoporosis.[12] The authors used Mendelian randomization (MR) and data from published genome-wide association studies (GWASs) to identify alleles that increase IBD risk then created an IBD exposure group and a no-IBD control group. Next, the authors determined the risk of osteoporosis in the IBD vs no-IBD risk group. Their results showed that IBD genetic risk was significantly inversely associated with total bone mineral density (BMD). However, there was no clear association with femoral neck, lumbar spine, or forearm bone density. Subtype analysis did not show consistent trends across CD and ulcerative colitis (UC), limiting the conclusions of the prior study.

In our study, we aim to build on this previous work by not only confirming the independent association between IBD and negative bone outcomes, but also identifying the genetic determinants that affect risk of both IBD and bone health.

Methods

Study cohort

Michigan Genomics Initiative

The Michigan Genomics Initiative (MGI) is an ongoing prospective institutional cohort that serves to advance precision health research in the medical sciences.[13] Clinical data were extracted from the electronic medical record, in conjunction with the Data Office for Clinical and Translational Research. A diagnosis of IBD was established using the following criteria: at least 1 encounter with an IBD diagnosis AND at least 1 of the following: (1) 1 or more encounters at a gastroenterology clinic; (2) 1 or more encounters with a gastroenterologist; and (3) 1 or more IBD entries in the problem list, entered by a gastroenterologist. IBD diagnoses were classified based on the presence of International Classification of Diseases–Ninth Revision (ICD-9) and/or International Classification of Diseases–Tenth Revision (ICD-10) codes (CD: 555.x, K50.00, K50.01x, K50.10, K50.11x, K50.80, K50.81x, K50.90, K50.91x; UC: 556.x, K51.00, K51.01x, K51.20, K51.21x, K51.30, K51.31x, K51.40, K51.41x, K51.50, K51.51x, K51.80, K51.81x, K51.90, K51.91x).[14] The outcome of interest was a composite outcome including osteoporosis or osteopenia. Osteoporosis was defined using the ICD-9 or ICD 10 codes 733.0x, M80.xxxx, and M81.x. Osteopenia was defined using the ICD-9 or ICD-10 codes 733.9 and M85.8xx.[15] Steroid use was defined as any prescription for prednisone, methylprednisolone (oral or intravenous), or hydrocortisone (oral, rectal, injection, or intravenous) that was captured in the electronic health record. Body mass index (BMI) was reported as the most recent BMI at the time of data extraction.

This study was conducted with University of Michigan Institutional Review Board approval (HUM00159951). MGI study participants' consent forms and protocols were reviewed and approved by the University of Michigan Medical School Institutional Review Board (as described in Zawistowski et al).[13] Opt-in written informed consent for broad research purposes was obtained.

Statistical analyses

Patients with outlier BMI values of <10 or >100 kg/m^2 were excluded from analyses. Univariate analyses were completed using the *gtsummary* package in R version 4.1.0 (R Foundation for Statistical Computing, Vienna, Austria).[16] The mean ± SD is reported for continuous variables and absolute number and percentage reported for categorical variables. For continuous variables, Student's *t* tests were used to determine statistically significant differences between groups. For categorical variables, chi-square tests were used to determine statistically significant differences between groups. Variables that were significantly associated with outcome of interest at a *P* value of ≤.05 were included in a multivariable logistic regression model evaluating the impact of IBD with or without steroid use on risk of osteoporosis or osteopenia. The reference categories for the final model included females, never smokers, and non-IBD patients with no steroid use. All analyses were performed in R.[17]

Genetic analyses

IBD risk variants

IBD risk variants were identified using published literature.[18,19] Details of the selection process have been outlined in previous work.[20] Briefly, publicly available GWAS summary statistics from the largest meta-analysis to date[18] and results from the IBD fine mapping study[19] were used to create a list of IBD risk variants. For any single nucleotide polymorphisms (SNPs) that were not considered independent, defined as <1 megabase from each other, a ranking algorithm was employed

to identify a priority SNP from the area. Variants that were fine mapped to single variant resolution were given the highest priority, followed by lead variants in the fine mapping study, then those with the most significant *P* value in the meta-analysis. A total of 188 IBD risk variants were included in the final SNP set.

BMD variants

Variants with effects on BMD were selected using publicly available GWAS summary statistics[21] given the substantial improvement in power compared with the MGI dataset. Kemp et al[21] tested for genome-wide effects on heel BMD in 142 487 individuals enrolled in the UK Biobank. IBD risk variants were parsed from the summary file and *P* values and betas were interrogated for each variant. Variants with a *P* value of $<5 \times 10^{-8}$, with a negative beta estimate (ie, associated with a lower BMD) were considered significant for both increasing IBD and decreasing BMD. Variants were oriented to the CD risk–increasing allele.

Mendelian randomization

MR was performed to test for a causal effect of genetically predisposed IBD on the outcome of BMD. Thus, the exposure of interest was genetically predicted IBD and the outcome of interest was BMD. Instrumental variables (ie, SNPs) were selected based on summary statistics from the combined GWAS/Immunochip analysis in Liu et al.[22] The outcome (ie, BMD) dataset utilized was ebi-a-GCST006288, which included 16 959 184 SNPs in a cohort of 142 487 European individuals.[21] The TwoSampleMR package (version 0.5.6) in R was utilized to complete MR.[23] Only genome-wide-significant ($P < 5 \times 10^{-8}$) instrumental variables with an *F* statistic >10 were included in the analysis. The clumping method in the TwoSampleMR package was used to refine the instrumental variables to independent only variants. MR Egger and inverse variance–weighted (IVW) tests were performed to assess for potential causality of genetically predisposed IBD on BMD. Each test was evaluated for heterogeneity and the Egger intercept was calculated for assessment of directional pleiotropy. Plots including a scatter plot showing the SNP effect on the exposure by the SNP effect on the outcome, forest plots showing SNP effect sizes on the outcome and leave-one-out analysis, and funnel plots that assess for heterogeneity were generated and are included in the Supplementary Material. Sensitivity analysis was performed using MR using the robust adjusted profile score (MR-RAPS), which accounts for weak instrument bias, pleiotropy, and extreme outliers. Parameters were set to consider overdispersion and robust loss was calculated using the Huber method. Finally, each disease subtype (CD and UC) was evaluated as an independent exposure as the diseases have genetically distinct features.

The data underlying this article are available in the article and in the Supplementary Material.

Results

Study cohort

There were 56 987 participants in the MGI cohort including 1404 patients with IBD and 55 583 control subjects. A total of 5499 patients of non-European ancestry were excluded, and an additional 83 patients with outlier BMIs were excluded. The final cohort size was 51 405. The mean age was 57 years, and there was a female predominance (53% female, 47% male). There were 10 378 (20.2%) individuals with a diagnosis of osteoporosis or osteopenia compared with 41 027 patients without a diagnosis of osteoporosis or osteopenia (**Table 1**). Patients with osteoporosis or osteopenia were more likely to be older (64 years of age vs 56 years of age; $P < .001$), were more likely to be female (67% vs 49%; $P < .001$), and were more likely to have a lower BMI (29 kg/m^2 vs 30 kg/m^2; $P < .001$). Patients with osteoporosis or osteopenia were more likely to be former smokers (40% vs 36%) and less likely to be current smokers (8% vs 11%). A history of steroid use was more common in those with osteoporosis or osteopenia than those without (44% vs 22%; $P < .001$). Among patients with steroid use, the mean duration of steroid use was 270 days compared with 220 days ($P < .001$). The highest rates of osteoporosis or osteopenia occurred in patients who had both IBD and a history of steroid use (n = 298 of 801 [37.2%]), followed by non-IBD patients with a history of steroid use (n = 4253 of 12 783 [33.3%]), IBD patients with no history of steroid use (n = 117 of 490 [23.9%]), and non-IBD patients with no history of steroid use (n = 5710 of 37 331 [15.3%]) (**Figure 1**).

Association between IBD and osteoporosis or osteopenia

A multivariable logistic regression model controlling for age, sex, BMI, and smoking history demonstrated an independent association between IBD and osteoporosis or osteopenia (**Table 2**). When comparing with the reference group (non-IBD patients with no steroid exposure), there was a statistically significant increase in the risk of osteoporosis or osteopenia among IBD patients who did not use steroids (OR, 1.77; 95% CI, 1.42-2.21) suggesting a disease-specific effect. In IBD patients with prior steroid use, there was a substantially higher risk of osteoporosis or osteopenia (OR, 4.60; 95% CI, 3.93-5.37). A model including the covariates age, sex, BMI, smoking, and the main effects of IBD diagnosis and history of steroid use with an interaction term between IBD and steroid use (IBD * steroid use) revealed no multiplicative effect ($P = .55$), suggesting that the risk of IBD and steroids is additive, rather than multiplicative.

Genetic overlap

Of 188 independent IBD risk variants, 21 were significantly associated with reduced BMD at a *P* value of <.05, and 3 were significantly associated with reduced BMD at a *P* value of $<5 \times 10^{-8}$ (**Supplementary Table 1**). Of the 21 risk variants, 14 were associated with an increased risk of CD (**Table 3**) and 7 were associated with a decreased risk of CD. Of the 14 variants with concordant risk of CD and BMD, all had congruent directions of effect across CD and UC with the exception of rs113010081 (*CCRL2*;*LTF*, which increased CD risk but decreased UC risk), rs864745 (*JAZF1*, which increased CD risk but decreased UC risk), and rs6651252 (*LINC00824*, which increased CD risk but decreased UC risk). Of these 14 variants, 2 reached genome-wide significance, rs12568930-T (*MIR4418*;*ZBTB40*) and rs7236492-C (*NFATC1*), for affecting BMD.

Mendelian randomization

Of the 232 variants which associated with IBD in the meta-analysis, 159 reached a combined *P* value $\leq 5 \times 10^{-8}$. Of these

Table 1. Demographic and clinical characteristics in patients with or without osteoporosis or osteopenia in the Michigan Genomics Initiative cohort.

	No Osteoporosis or Osteopenia (n = 41 027)	Osteoporosis or Osteopenia (n = 10 378)	*P* Value
Age, y	56 ± 16	64 ± 14	<.001
BMI, kg/m^2	30 ± 7	29 ± 7	<.001
Gender			<.001
Female	20 122 (49)	6993 (67)	
Male	20 905 (51)	3385 (33)	
History of smoking			<.001
Unknown	94 (0.2)	2 (<0.1)	
Current	4530 (11)	807 (7.8)	
Former	14 939 (36)	4190 (40)	
Never	21 464 (52)	5379 (52)	
Steroid use			<.001
No	31 994 (78)	5827 (56)	
Yes	9033 (22)	4551 (44)	
Total days of steroid use	220 (531)	270 (645)	<.001
Osteoporosis			<.001
No	41 027 (100)	6214 (60)	
Yes	0 (0)	4164 (40)	
Osteopenia			<.001
No	41 027 (100)	1804 (17)	
Yes	0 (0)	8574 (83)	

Values are mean ± SD or n (%).
Abbreviation: BMI, body mass index.

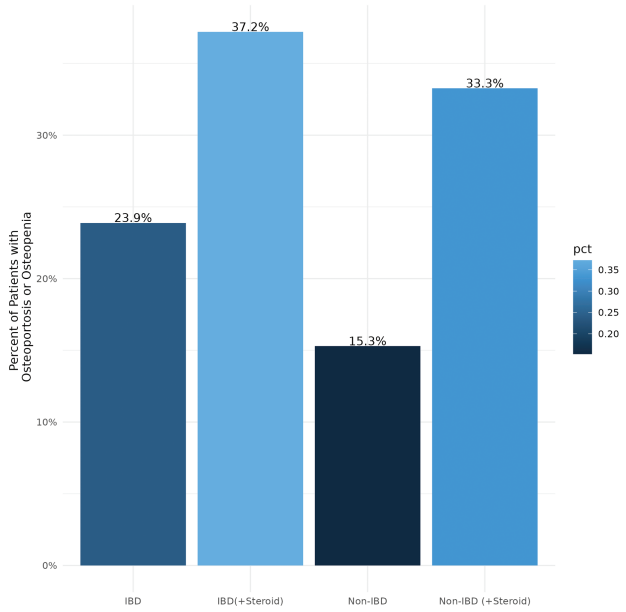

Figure 1. Percent of patients with osteoporosis and osteopenia by inflammatory bowel disease (IBD) status and history of steroid use.

159 variants, there were none that had an *F* statistic <10. After clumping, 106 independent variants remained for analysis. There was no significant causal effect of genetically predisposed IBD on BMD by the MR Egger (OR, 0.98; 95% CI, 0.96-1.01) or IVW (OR, 1.00; 95% CI, 0.98-1.01) methods (Table 4). There was significant heterogeneity observed in both the MG Egger ($P = 5.88 \times 10^{-26}$) and IVW ($P = 4.09 \times 10^{-26}$) tests. There was no significant pleiotropy observed when evaluating the Egger intercept ($P = .36$). Sensitivity analysis with MR-RAPS again showed no significant effect of genetically predisposed IBD on BMD ($P = .47$).

When evaluated by disease subtype, there were 142 SNPs that associated with CD at genome-wide significance and had an *F* statistic >10. After clumping, 99 independent variants remained. There was again no significant causal effect on BMD (MR Egger: OR, 0.98; 95% CI, 0.94-1.01; IVW: OR, 1.00; 95% CI, 0.99-1.01), there was significant heterogeneity in the methods (MR Egger: $P = 1.10 \times 10^{-36}$; IVW: $P = 7.24 \times 10^{-38}$), and there was no significant pleiotropy (Egger intercept, $P = .14$). MR-RAPS was consistent in demonstrating no causal effect ($P = .53$). For UC, there were 89 SNPs that associated at genome-wide significance and had a *F* statistic >10. After clumping, 62 independent variants remained. There was again no significant causal effect on BMD (MR Egger: OR, 0.99; 95% CI, 0.96-1.02; IVW: OR, 0.99; 95% CI, 0.98-1.01), significant heterogeneity in the methods was observed (MR Egger: $P = 2.54 \times 10^{-9}$; IVW: $P = 3.08 \times 10^{-9}$), and there was no significant pleiotropy (Egger intercept, $P = .55$). MR-RAPS was consistent in demonstrating no causal effect ($P = .29$).

Discussion

Patients with IBD have an increased risk for adverse bone outcomes such as osteopenia, osteoporosis, and bone fracture. Investigation into the association between IBD and adverse bone outcomes has revealed corticosteroid use as a major contributing risk factor. This has led to the recommendation that patients with IBD who have been exposed to more than 3 months of steroid therapy undergo BMD testing.[1]

However, functional work has also showed that the inflammatory changes that occur in IBD may directly impact bone turnover, suggesting a disease-specific effect.[10] Furthermore, genetic analyses using MR suggest a possible causal effect of IBD on osteoporosis.[12] In this study, we aimed not only to test the steroid independent relationship between IBD and osteoporosis or osteopenia, but also to identify shared genetic causes that confer risk for both diseases.

In this study, we show that IBD is independently associated with the risk of osteoporosis or osteopenia. Given this independent relationship, consideration should be given to screening all IBD patients for osteoporosis or osteopenia regardless of additional risk factors. Currently, guidelines limit screening recommendations to those IBD patients with prolonged steroid use or other established risk factors.[1] We also confirmed the steroid-related risk for osteoporosis or osteopenia and highlighted the additive risk of steroids and IBD. Given the substantial risk associated with steroid use in IBD patients, clinicians should continue to limit steroid use and screen steroid-exposed patients for osteoporosis or osteopenia.

We then sought to determine if there were overlapping genetic causes that alter the risk for both IBD and osteoporosis or osteopenia. To answer this question, we utilized publicly available GWAS data for IBD and BMD. Publicly available data provided substantially larger sample sizes than our institutional cohort and thus improved the power to detect small- to moderate-sized effects. With these data, we found that rs12568930-T (*MIR4418;ZBTB40*) and rs7236492-C (*NFATC1*) were associated with an increased risk of IBD and negatively impacted BMD. We further examined genetic contribution to BMD risk by utilizing a MR to determine if genetically predicted IBD is potentially causal for BMD. We found no significant causal effect from IBD, or any disease subtype, on BMD. However, the interpretation of these results was substantially limited by the significant heterogeneity observed. An extension of MR that accounts for instrument bias, pleiotropy, and outliers also showed no significant causal effect providing replication of the nonsignificant relationship observed in the original MR. Based on our results, we propose that genetically predisposed IBD is not causal for BMD loss, but rather is a subset of overlapping risk variants contributes to shared risk across the diseases. We highlight 2 genetic risk variants of particular interest, which reach genome-wide significance in analysis of both IBD and BMD.

ZBTB40 encodes for the protein zinc finger and BTB domain containing 40, a protein which is found in the nucleus and is involved in transcriptional regulation. In a 2008

Table 2. Multivariable logistic regression testing the effect of IBD on risk of osteoporosis or osteopenia.

	Odds Ratio	95% CI
Age	1.04	1.04-1.04
Sex	0.39	0.37-0.41
BMI	0.98	0.97-0.98
Smoking		
Unknown	0.10	0.02-0.31
Current	0.85	0.78-0.92
Former	0.94	0.89-0.98
Group		
Non-IBD patients with steroid use	2.82	2.68-2.96
IBD without steroid use	1.77	1.42-2.21
IBD with steroid use	4.60	3.93-5.37

Reference categories: females, never smokers, non-IBD patients with no steroid use.
Abbreviation: BMI, body mass index; IBD, inflammatory bowel disease.

Table 3. Crohn's disease risk increasing variants associate with reduced bone mineral density.

SNP	CHR	BP	Effect Allele	Other Allele	Effect Allele Frequency	Odds Ratio	Lower Bound 95% CI	Upper Bound 95% CI	*P*	Function	Gene
rs12568930	1	22702231	T	C	0.822	0.939	0.931	0.947	1.10×10^{-49}	Intergenic	*MIR4418;ZBTB40*
rs7236492	18	77220616	C	T	0.826	0.977	0.969	0.986	1.30×10^{-8}	Intergenic	*NFATC1*
rs11229555	11	58408687	G	T	0.761	0.986	0.978	0.993	.00011	Intergenic	*ZFP91-CNTF;GLYAT*
rs9297145	7	98759117	C	A	0.267	0.988	0.981	0.995	.00048	Intergenic	*SMURF1;KPNA7*
rs12942547	17	40527544	A	G	0.574	0.992	0.985	0.998	.0026	Intronic	*STAT3*
rs4703855	5	71693899	C	T	0.706	0.989	0.982	0.997	.0029	Intergenic	*PTCD2;ZNF366*
rs113010081	3	46457412	T	C	0.883	0.987	0.977	0.997	.0067	Intergenic	*CCRL2;LTF*
rs1260326	2	27730940	T	C	0.396	0.992	0.985	0.998	.0069	Exonic	*GCKR*
rs72810983	5	1.73E + 08	A	G	0.699	0.992	0.985	0.999	.0076	Intronic	*CPEB4*
rs11150589	16	30482494	T	C	0.484	0.991	0.985	0.998	.0084	Intergenic	*SEPHS2;ITGAL*
rs4802307	19	46849806	G	T	0.693	0.99	0.983	0.997	.0087	Upstream	*PPP5C*
rs864745	7	28180556	T	C	0.494	0.991	0.985	0.998	.011	Intronic	*JAZF1*
rs6651252	8	1.3E + 08	T	C	0.87	0.987	0.977	0.996	.014	ncRNA_intronic	*LINC00824*
rs1182188	7	2869985	T	C	0.7	0.993	0.986	1	.017	Intronic	*GNA12*

Extracted from Kemp et al.[21]
Abbreviations: BP, base position; CHR, chromosome; CI, confidence interval; ncRNA, noncoding RNA; SNP, single nucleotide polymorphism.

Table 4. MR identified no significant effect of IBD susceptibility variants on BMD.

Exposure	Outcome	Method	Beta	*P* Value	OR (95% CI)
IBD	BMD	MR Egger	-0.016	.30	0.98 (0.96-1.01)
IBD	BMD	Inverse variance weighted	-0.003	.63	1.00 (0.98-1.01)
IBD	BMD	MR-RAPS	-0.005	.47	
CD	BMD	MR Egger	-0.025	.14	0.98 (0.94-1.01)
CD	BMD	Inverse variance weighted	-0.002	.79	1.00 (0.99-1.01)
CD	BMD	MR-RAPS	-0.004	.53	
UC	BMD	MR Egger	-0.014	.37	0.99 (0.96-1.02)
UC	BMD	Inverse variance weighted	-0.006	.36	0.99 (0.98-1.01)
IBD UC	BMD	MR-RAPS	-0.006	.29	

Abbreviations: BMD, bone mineral density; CD, Crohn's disease; CI, confidence interval; IBD, inflammatory bowel disease; MR, Mendelian randomization; MR-RAPS, Mendelian randomization using the robust adjusted profile score; OR, odds ratio; UC, ulcerative colitis.

GWAS, a significant association between the 1p36 locus and BMD as well as bone fractures was identified.[24] The 1p36 locus includes the genes *ZBTB40 and WNT4*. Follow up meta-analyses confirmed the association between the 1p36 locus and BMD.[25,26] Interestingly, the 1p36 locus has been found to associate with increased *EPHB2* ($P = 1.72 \times 10^{-3}$) expression but not with increased *ZBTB40* expression ($P = .08$) in human bone tissue.[27] These results suggest that the primary effect of the 1p36 locus may be through modulation of nearby genes. In another study, the role of long noncoding RNA *ZBTB40-IT1*, which is generated from alternative splicing of the *ZBTB40* gene, was studied and shown to regulate bone turnover.[28] Specifically, the long noncoding RNA *ZBTB40-IT1* was found to reduce osteogenesis via decreased expression of *RUNX2*, *OSX*, *COL1A1*, and *ALP* and boost osteoclastogenesis via decreased expression of *OPG* and increased expression of *RANKL*. Interestingly, expression of the long noncoding RNA *ZBTB40-IT1* was influenced by drug therapy for osteoporosis (parathyroid hormone [PTH]) whereas *ZBTB40* expression was not. This study also highlighted the role of the JUN::FOS and CREB1 transcription factors in *ZBTB40-IT1* expression.

In IBD, there is a paucity of literature investigating the functional impact of *ZBTB40* in intestinal inflammation. However, the impact of this locus appears to be more important in UC than in CD given the larger effect size observed in this disease subtype.[22]

The other observed genetic association was with *NFATC1*. This gene encodes the protein nuclear factor of activated T cells 1 (NFATc-1), which is also found in the nucleus and involved in gene transcription. NFATc1 is induced through the RANKL/RANK signaling pathway, with associated activation of nuclear factor kappa B, and plays an important role in osteoclastogenesis.[29] Interestingly, short-chain fatty acids, which are known to have anti-inflammatory effects in the gut,[30] have also been shown to impede osteoclast differentiation through reduction of the expression of genes in the RANKL/RANK signaling pathway including NFATc1.[31]

In IBD, there is also a paucity of data explaining the role of *NFATC1* in intestinal inflammation. However, one study did implicate *NFATC1* as an HIF-dependent transcriptional regulator of *ITGA5* and *PLAUR* expression in the intestinal epithelium of IBD patients.[32] In contrast to the *ZBTB40* association, this locus has a larger effect size in CD than in UC.[22]

There are several strengths of this work. First, we carry out epidemiologic analyses testing the association between IBD and osteoporosis or osteopenia controlled for steroid exposure. As steroids are the most well-known risk factor for osteoporosis or osteopenia in IBD, controlling for exposure to this medication provides confidence in the finding of an independent association between IBD and osteoporosis or osteopenia. For the genetic analyses, we utilized publicly available GWAS summary statistics to identify overlapping variants (IBD and BMD), which allowed us to use data with the largest sample size available. Specifically, the Kemp et al study[21] testing of variants associated with BMD was performed in over 140 000 individuals. This power allowed us to adequately evaluate for IBD risk variants that may have small-to-moderate effect sizes on BMD and may not have been picked up on a smaller GWAS analyses, such as what was possible locally through the MGI cohort.

There are also several limitations of this work. First, there was a lack of available metadata on vitamin D and calcium levels in the existing institutional cohort. Therefore, we could not adequately control for these nutrient deficiencies in our analyses. However, given the retrospective nature of this study, the fluctuation in calcium and vitamin D levels over time, and the use of over-the-counter supplementation, which may not be adequately captured in the electronic medical record, adequate control for these variables would be difficult to achieve even if they had been available and accurate. Furthermore, we were able to control for overall nutrition by using BMI as a covariate in the model.

Discussion

IBD is associated with an increased risk of osteoporosis or osteopenia. This risk is independent of steroid exposure. Due to the disease-specific risk of osteoporosis or osteopenia in IBD, consideration of BMD screening in all IBD patients should be given. However, the appropriate frequency at which screening should be implemented remains to be determined. Furthermore, given the additive effects of steroids to the disease-specific risk, appropriate screening intervals may need to be shortened in those with additional steroid exposure, and the optimal length of these intervals also remains to be determined. In this study, we also showed that there are overlapping genetic risk factors for IBD and

osteoporosis or osteopenia, with the strongest association occurring with the variant near *ZBTB40*. Further studies evaluating the functional effects of the *ZBTB40* variant (as well as indirect gene effects due to variation at this locus) would be beneficial to advance our understanding of both intestinal inflammation and metabolic bone function. Not only would such work be beneficial to understand the biologic overlap across these disease states, but also it may help identify targets for drug therapy. The published work on short-chain fatty acids and *NFATC1* highlights a potentially interesting therapeutic overlap that could be further investigated. As we move toward precision medicine efforts in disease prognostication and individualized therapeutic decision making, these data offer an improved understanding of the overlap between IBD and osteoporosis or osteopenia.

Supplementary Data

Supplementary data is available at *Inflammatory Bowel Diseases* online.

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Conflicts of Interest

K.C.C., Y.C., X.D., V.C., A.K., and E.K.S. disclose no conflicts. PDRH reports personal fees from AbbVie, Eli Lilly, and Pfizer, outside of the submitted work.

References

1. Farraye FA, Melmed GY, Lichtenstein GR, Kane SV. ACG clinical guideline: preventive care in inflammatory bowel disease. *Am J Gastroenterol*. 2017;112(2):241-258. doi:10.1038/ajg.2016.537
2. Agrawal M, Arora S, Li J, et al. Bone, inflammation, and inflammatory bowel disease. *Curr Osteoporos Rep*. Dec 2011;9(4):251-257. doi:10.1007/s11914-011-0077-9
3. Szafors P, Che H, Barnetche T, et al. Risk of fracture and low bone mineral density in adults with inflammatory bowel diseases. A systematic literature review with meta-analysis. *Osteoporos Int*. 2018;29(11):2389-2397. doi:10.1007/s00198-018-4586-6
4. Bartko J, Reichardt B, Kocijan R, Klaushofer K, Zwerina J, Behanova M. Inflammatory bowel disease: a nationwide study of hip fracture and mortality risk after hip fracture. *J Crohns Colitis*. 2020;14(9):1256-1263. doi:10.1093/ecco-jcc/jjaa052
5. NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy. Osteoporosis prevention, diagnosis, and therapy. *JAMA*. 2001;285(6):785-795. doi:10.1001/jama.285.6.785
6. Lima CA, Lyra AC, Rocha R, Santana GO. Risk factors for osteoporosis in inflammatory bowel disease patients. *World J Gastrointest Pathophysiol*. 2015;6(4):210-218. doi:10.4291/wjgp.v6.i4.210
7. Balasubramanian A, Wade SW, Adler RA, Saag K, Pannacciulli N, Curtis JR. Glucocorticoid exposure and fracture risk in a cohort of US patients with selected conditions. *J Bone Miner Res*. 2018;33(10):1881-1888. doi:10.1002/jbmr.3523
8. Card T, West J, Hubbard R, Logan RF. Hip fractures in patients with inflammatory bowel disease and their relationship to corticosteroid use: a population based cohort study. *Gut*. 2004;53(2):251-255. doi:10.1136/gut.2003.026799
9. Compston JE, Judd D, Crawley EO, et al. Osteoporosis in patients with inflammatory bowel disease. *Gut*. 1987;28(4):410-415. doi:10.1136/gut.28.4.410
10. Blaschke M, Koepp R, Cortis J, et al. IL-6, IL-1β, and TNF-α only in combination influence the osteoporotic phenotype in Crohn's patients via bone formation and bone resorption. *Adv Clin Exp Med*. 2018;27(1):45-56. doi:10.17219/acem/67561
11. Hofbauer LC, Schoppet M. Clinical implications of the osteoprotegerin/RANKL/RANK system for bone and vascular diseases. *JAMA*. 2004;292(4):490-495. doi:10.1001/jama.292.4.490
12. Wu F, Huang Y, Hu J, Shao Z. Mendelian randomization study of inflammatory bowel disease and bone mineral density. *BMC Med*. 2020;18(1):312. doi:10.1186/s12916-020-01778-5
13. Zawistowski M, Fritsche LG, Pandit A, et al. The Michigan Genomics Initiative: a biobank linking genotypes and electronic clinical records in Michigan Medicine patients. medRxiv doi:10.1101/2021.12.15.21267864.
14. Cohen-Mekelburg S, Rosenblatt R, Gold S, et al. Fragmented care is prevalent among inflammatory bowel disease readmissions and is associated with worse outcomes. *Am J Gastroenterol*. 2019;114(2):276-290. doi:10.1038/s41395-018-0417-9
15. Khan N, Abbas AM, Almukhtar RM, Khan A. Prevalence and predictors of low bone mineral density in males with ulcerative colitis. *J Clin Endocrinol Metab*. 2013;98(6):2368-2375. doi:10.1210/jc.2013-1332
16. Sjoberg DW, Curry M, Lavery JA, Larmarange J. The R journal: reproducible summary tables with the gtsummary package. *R Journal*. 2021;13(1):570-580. doi:10.32614/RJ-2021-053
17. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing; 2022. https://www.R-project.org/
18. Liu JZ, van Sommeren S, Huang H, et al. Association analyses identify 38 susceptibility loci for inflammatory bowel disease and highlight shared genetic risk across populations. *Nat Genet*. 2015;47(9):979-986. doi:10.1038/ng.3359
19. Huang H, Fang M, Jostins L, et al. Fine-mapping inflammatory bowel disease loci to single-variant resolution. *Nature*. 2017;547(7662):173-178. doi:10.1038/nature22969
20. Cushing KC, Du X, Chen Y, et al. Inflammatory bowel disease risk variants are associated with an increased risk of skin cancer. *Inflamm Bowel Dis*. 2022;28(11):1667-1676. doi:10.1093/ibd/izab336
21. Kemp JP, Morris JA, Medina-Gomez C, et al. Identification of 153 new loci associated with heel bone mineral density and functional involvement of GPC6 in osteoporosis. *Nat Genet*. 2017;49(10):1468-1475. doi:10.1038/ng.3949
22. Liu JZ, van Sommeren S, Huang H, et al. Association analyses identify 38 susceptibility loci for inflammatory bowel disease and highlight shared genetic risk across populations. *Nat Genet*. 2015;47(9):979-986. doi:10.1038/ng.3359
23. Hemani G, Zheng J, Elsworth B, et al. The MR-Base platform supports systematic causal inference across the human phenome. *Elife*. 2018;7:e34408. doi:10.7554/eLife.34408

24. Styrkarsdottir U, Halldorsson BV, Gretarsdottir S, et al. Multiple genetic loci for bone mineral density and fractures. *N Engl J Med*. 2008;358(22):2355-2365. doi:10.1056/NEJMoa0801197
25. Rivadeneira F, Styrkársdottir U, Estrada K, et al. Twenty bone-mineral-density loci identified by large-scale meta-analysis of genome-wide association studies. *Nat Genet*. 2009;41(11):1199-1206. doi:10.1038/ng.446
26. Zhang L, Choi HJ, Estrada K, et al. Multistage genome-wide association meta-analyses identified two new loci for bone mineral density. *Hum Mol Genet*. 2014;23(7):1923-1933. doi:10.1093/hmg/ddt575
27. Nielson CM, Liu CT, Smith AV, et al. Novel genetic variants associated with increased vertebral volumetric BMD, reduced vertebral fracture risk, and increased expression of SLC1A3 and EPHB2. *J Bone Miner Res*. 2016;31(12):2085-2097. doi:10.1002/jbmr.2913
28. Mei B, Wang Y, Ye W, et al. LncRNA ZBTB40-IT1 modulated by osteoporosis GWAS risk SNPs suppresses osteogenesis. *Hum Genet*. 2019;138(2):151-166. doi:10.1007/s00439-019-01969-y
29. Park JH, Lee NK, Lee SY. Current understanding of RANK signaling in osteoclast differentiation and maturation. *Mol Cells*. 2017;40(10):706-713. doi:10.14348/molcells.2017.0225
30. Parada Venegas D, De la Fuente MK, Landskron G, et al. Short Chain Fatty Acids (SCFAs)-Mediated gut epithelial and immune regulation and its relevance for inflammatory bowel diseases. *Front Immunol*. 2019;10:277. doi:10.3389/fimmu.2019.00277
31. Lucas S, Omata Y, Hofmann J, et al. Short-chain fatty acids regulate systemic bone mass and protect from pathological bone loss. *Nat Commun*. 2018;9(1):55. doi:10.1038/s41467-017-02490-4
32. Knyazev E, Maltseva D, Raygorodskaya M, Shkurnikov M. HIF-Dependent NFATC1 activation upregulates ITGA5 and PLAUR in intestinal epithelium in inflammatory bowel disease. *Front Genet*. 2021;12:791640. doi:10.3389/fgene.2021.791640