IRGM Gene Variants Modify the Relationship Between Visceral Adipose Tissue and NAFLD in Patients With Crohn's Disease

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Background: Nonalcoholic fatty liver disease (NAFLD) is an increasingly recognized comorbidity in Crohn's disease (CD), but the mechanisms are poorly understood. Autophagy is a highly conserved process regulating innate immunity that contributes to CD susceptibility. Emerging data suggest that variants in the autophagy-governing *IRGM* gene may contribute to the accumulation of visceral adipose tissue (VAT) and hepatic fat. Our objective was to characterize the relationship between VAT, *IRGM* gene variants, and NAFLD risk in patients with CD.

Methods: We included all CD patients in the Prospective Registry in Inflammatory Bowel Disease Study at Massachusetts General Hospital (PRISM) without history of alcohol abuse or liver disease. Hepatic fat was quantified by liver attenuation (LA) on computed tomography, with NAFLD defined by the validated liver:spleen (L:S) ratio. NAFLD severity was estimated by the FIB-4 Index and alanine aminotransferase (ALT). Using logistic regression modeling, we examined the relationship between VAT, autophagy gene variants, and NAFLD risk.

Results: Among 462 patients, 52% had NAFLD. Increasing VAT quartile was associated with reduced LA (mean change, –7.43; 95% confidence interval [CI], –10.05 to –4.81; $P_{trend} < 0.0001$). In the fully adjusted model, patients in the highest VAT quartile had a 2.2-fold increased NAFLD risk (95% CI, 1.21 to 4.14; $P_{trend} = 0.032$) and a 4.2-fold increased risk of ALT>upper limit of normal (ULN) (95% CI, 1.19 to 14.76; $P_{trend} = 0.017$). The relationship between VAT and NAFLD was modified by *IRGM* variants rs4958847 and rs13361189 ($P_{interaction} = 0.005$ and $P_{interaction} < 0.001$, respectively).

Conclusions: In a large CD cohort, VAT was directly associated with prevalent NAFLD, and this relationship was augmented by functionally annotated *IRGM* variants associated with impaired autophagy.

Key Words: Crohn's disease, autophagy, lipophagy, hepatic steatosis, nonalcoholic fatty liver disease, NAFLD

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is increasingly recognized as an important hepatobiliary complication in patients with Crohn's disease (CD).[1,2] Whereas NAFLD is estimated to affect 19%–24% of the general US population,[3] emerging data suggest that 40%–50% of adults with CD may harbor undiagnosed NAFLD.[4–6] More striking is the finding that, among them, 20% will already have advanced steatohepatitis (NASH) or fibrosis at the time NAFLD is diagnosed.[2,7] To date, little is known about the specific risk factors that mediate the pathogenesis of NAFLD in CD. Historically, steatosis in inflammatory bowel disease (IBD) was attributed to disease-related factors, including sepsis, malnutrition, steroids, or sustained inflammation from poorly controlled luminal disease,[2,4,8] whereas more recently it has been posited that obesity and the metabolic syndrome may drive the co-occurrence of NAFLD and IBD.[2,9] Within the general population, the accumulation of metabolically active visceral adipose tissue (VAT) accurately predicts both incident and progressive NAFLD, and in patients with CD, increasing VAT has been linked to IBD severity.[10–14] However, whether VAT mediates the risk and progression of NAFLD in patients with CD remains unknown.

Not all patients with CD or increased VAT develop NAFLD; thus it is plausible that specific environmental and genetic factors modulate individual susceptibility. Autophagy

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is a highly conserved metabolic process whereby defective intracellular organelles are targeted for lysosomal destruction.[15] Defects in autophagy have been shown to confer susceptibility to CD,[16–18] to increase the risk of obesity and obesity-related diabetes,[19, 20] and, more recently, to impair hepatic ß-oxidation.[15, 21, 22] Within hepatocytes, lipid droplets are destroyed by an autophagy-related lysosomal degradation process that is termed "lipophagy," and defects in this process reflect a novel mechanism of NAFLD pathogenesis.[15] In preclinical models, administration of autophagy inhibitors promotes the accumulation of hepatic triglycerides, whereas autophagy stimulation with rapamycin accelerates lipid clearance,[15] improving hepatic steatosis and central obesity.[22, 23] Such evidence suggests that impaired autophagy could modify the relationship between CD, central adiposity, and NAFLD.

Through genome-wide association (GWA) studies, variants in both the immunity-related GTPase M (*IRGM*) gene and the autophagy-related 16-like 1 (*ATG16L1*) gene have been associated with susceptibility to CD.[16–18] More recently, *IRGM* gene variants have also been found to contribute to the pathogenesis of obesity and NAFLD.[19, 24] However, the mechanisms that underpin this relationship have yet to be fully characterized. Given the known association between *IRGM*, *ATG16L1,* and CD risk, and as the high prevalence of NAFLD among patients with CD, a large cohort of patients with CD offers an ideal opportunity to explore the relationship between genetic defects in autophagy, VAT, and NAFLD risk.

Using the Prospective Registry in Inflammatory Bowel Disease Study at Massachusetts General Hospital (PRISM), we sought to explore the relationship between VAT volume, previously characterized genetic variants that govern the autophagy pathway, and NAFLD risk.

METHODS

Study Population

Beginning in 2004, adults ≥18 years of age with a diagnosis of CD, ulcerative colitis, or indeterminate colitis were recruited into the PRISM cohort. At enrollment, detailed patient information was collected, including disease characteristics according to the Montreal classification and demographic and clinical information including smoking, body mass index (BMI), and comorbidities. Genetic data were available from 787 CD patients; among them, 482 had at least 1 abdominal noncontrast computed tomography (CT) scan using a 64-slice CT scanner (Siemens Medical Solutions, Andover, MA, USA) for the quantification of VAT, liver attenuation (LA) measured in Hounsfield Units (HU), and liver-to-spleen attenuation ratio (L:S). Patients were excluded for unreadable CTs (n = 12), primary sclerosing cholangitis (PSC) (n = 1), prior splenectomy (n = 2), and any current or prior methotrexate use (n = 5), leaving 462 eligible patients for analysis. As previously reported from PRISM, there were no significant differences in baseline demographics and clinical characteristics, when PRISM participants with and without abdominal CT scans were compared.[14] This study was approved by the Institutional Review Board at Massachusetts General Hospital.

Measurement of VAT Volume

VAT was quantified from the earliest available CT obtained after CD diagnosis, using the Aquarius 3D iNtuition viewer (TeraRecon Inc., version 4.4.12, San Mateo, CA, USA). By tracing the abdominal muscular layer, this software allows for quantification of VAT (including mesenteric adipose tissue and subcutaneous adipose tissue [SAT]), with volumes measured using predetermined reference points within the Fat Analysis 3D option. Using validated methods previously described by our group and others,[14, 25, 26] VAT was measured from the 11th thoracic vertebra to the 5th sacral vertebra. As we previously have described, a correlation between VAT and CD complications has previously been reported[2, 4, 8]; therefore, we obtained serial CT data from a subset of PRISM participants (n = 258) before and after confirmed CD-related complications and found no significant intrapatient differences in VAT volume over time.[14]

Assessment of Outcomes

The primary outcome was continuous LA, measured in HU.[27] With this validated method for quantifying hepatic fat, a lower LA value corresponds to a lower HU measurement, which in turn corresponds to relatively *more* hepatic fat. Hepatic and splenic attenuation were measured using regions of interest (ROIs) >100 mm^2. With this method, 1 ROI is placed in the right liver lobe, 1 ROI in the left lobe, and 1 ROI in the spleen, on 3 consecutive slices for each region, from which the average is calculated.[28]

Secondary outcomes included the following: (1) radiographic NAFLD, defined by the validated liver-to-spleen attenuation ratio (L:S) <1.0 [28]; (2) elevated alanine aminotransferase (ALT) >upper limit of normal (ULN) (40 IU/L), as a surrogate serum marker of hepatic inflammation; (3) among patients with radiographic NAFLD, we used a serum index of NAFLD severity, the FIB-4 index. The FIB-4 is a validated serum index for the identification or exclusion of clinically significant advanced fibrosis in NAFLD, with reported 100% sensitivity, 83% specificity, and negative predictive values (NPVs) >90%, rendering it an accurate and reliable tool for the diagnosis or exclusion of advanced NAFLD fibrosis.[29, 30] The FIB-4 was calculated by the following algorithm: FIB-4 score = age ([years]×AST [IU/L])/(platelets [10^9/L])×(ALT [IU/L]$^{1/2}$).

Assessment of Covariates

At enrollment, information on date of IBD diagnosis, IBD type (inflammatory, penetrating, stricturing), disease location (colonic, ileocolonic, small bowel, upper gastrointestinal tract, and perianal) and prior surgeries was collected and

confirmed by medical record review, with subsequent validation by primary gastroenterologists. Duration of IBD was defined as the elapsed time between confirmed date of IBD diagnosis and date of CT scan. Height was reported at enrollment, and weight was obtained from the time of CT scan. We also collected information on sex, race, smoking (never, prior, current), alcohol intake, history of chronic liver disease, ever-use of IBD medications, and any use of steroids within the 12 months preceding CT scan. Laboratory data were collected from the closest date (within 12 months) to the CT by manual chart review.

Genotyping and Population Stratification

Our group has previously described the genotyping of PRISM,[14, 31] which was performed on the Illumina Immunochip at the Broad Institute (Cambridge, MA, USA). From the full cohort of 462 CD patients, 427 had available genotype data for this analysis. We selected known variants in 2 autophagy genes (*ATG16L1* rs2241880, *IRGM* rs13361189, *IRGM* rs4958847), which have been previously identified as CD susceptibility loci, and whose functional significance in the autophagy pathway has been established.[32–34] We also considered the *IRGM* single nucleotide polymorphism (SNP) rs10065172 because of the recent report of its relationship with NAFLD risk.[24] However, this variant was found to be in perfect linkage disequilibrium (LD) with rs13361189 (r^2 = 1.00), and therefore was not included. Finally, we included *PNPLA3* rs738409 to explore its association with NAFLD in CD. The 4 SNPs were recoded (as 0, 1, or 2) by the number of risk variant alleles. Due to the known functional significance of the *IRGM* risk alleles and the limited number of participants who were homozygous for the risk allele, a dominant genotype model was constructed as follows: *IRGM* rs13361189 CT/CC vs TT genotype, *IRGM* rs4958847 GA/AA vs GG genotype, *PNPLA3* rs738409 GC/GG vs CC genotype, and *ATG16L1* rs2241880 GA/GG vs AA genotype. Eigenvalues were derived using PLINK 1.9 "PCA Function," and the first 3 eigenvectors, accounting for >80% of the population stratification, were used in genetic analyses.

Statistical Analysis

We created quartiles of VAT volume based upon overall VAT distribution. Baseline characteristics were compared across VAT quartiles, using chi-square tests for categorical variables and the Kruskal-Wallis H test for continuous variables. We used linear regression modeling to estimate the association between VAT quartile and LA. To test for presence of a significant linear trend, we used the median value for each quartile of VAT volume. To explore the degree of confounding by measures of CD severity and traditional predictors of NAFLD, a series of multivariable linear regression models were created; the fully adjusted model accounted for age in years, sex, BMI (kg/m^2), VAT volume (cm^3), diabetes, hypertension, hyperlipidemia, smoking status, disease duration in years, penetrating or stricturing disease type, any steroid use within the preceding 12 months, any current or prior biologic medication use, ≥1 *PNPLA3* rs738409 variant alleles, and the first 3 eigenvectors. We then explored whether the association between VAT volume and LA is modified by genetic variants in autophagy genes by introducing an interaction term (modeled as the product of genotype strata*VAT quartile) to the final multivariable models and by testing for significance using the Wald test.

Logistic regression modeling was used to evaluate the relationship between VAT volume and risk of NAFLD, defined by L:S <1, and also risk of elevated ALT>ULN. In exploratory analyses limited to the subset of patients with NAFLD (n = 242), we evaluated the relationship between VAT quartile, autophagy gene variants, and measures of NAFLD severity, including a validated radiographic measurement of severe steatosis (HU < 40), and the validated threshold cutoff FIB-4 >1.45 (n = 32). We tested for modification by genetic variants in autophagy genes using the log likelihood ratio test comparing models with interaction with those with only the main effect. All *P* values were 2-sided, and a *P* <0.05 was considered significant. Analyses were conducted using SAS, version 9.4 (SAS Inc., Cary, NC, USA).

RESULTS

Among 462 included patients, the mean age was 40 ± 15 years; 437 (95%) were Caucasian and 246 (53.3%) were female, with a mean overall BMI of 25.4 ± 5.0 kg/m^2. Compared with those in the lowest VAT quartile, patients in the highest quartile on average had higher BMI and ALT and were more likely to have diabetes and hypertension (Table 1).

Predictors of NAFLD (L:S ≤ 1.0)

The prevalence of radiographic NAFLD was 52% (n = 242). After adjusting for known NAFLD risk factors and variables associated with IBD severity, the factors that remained significantly associated with NAFLD risk included age (odds ratio [OR] per 1-year increment, 1.02; 95% confidence interval [CI], 1.00 to 1.05; *P* = 0.032), diabetes (OR, 8.91; 95% CI, 1.12 to 71.11; *P* = 0.041), VAT quartile (OR, 1.37; 95% CI, 1.03 to 1.81; *P* = 0.012), and *PNPLA3* (G) genotype (OR, 1.55; 95% CI, 1.07 to 2.49; *P* = 0.047) (Supplementary Table 1).

VAT Quartile and Hepatic Fat Accumulation (Continuous HU)

Increasing VAT volume in quartiles was associated with significantly increased hepatic fat accumulation, measured by continuous reduction in mean HU (Table 2). After adjusting for age, patients in the highest VAT quartile had a significantly lower mean HU compared with those in the lowest quartile, corresponding to significantly increased hepatic fat (mean difference in HU, –27.03; 95% CI, –33.04 to –21.01). The addition of IBD-related factors slightly diminished but did not significantly impact this association (mean HU, –24.07; 95% CI, –31.18 to –16.96; P_{trend} < 0.0001), nor was it

TABLE 1: Baseline Characteristics of Subjects According to Quartiles of VAT Volume

Variable	VAT Volume, cm^3			
	Quartile 1 (n = 115)	Quartile 2 (n = 116)	Quartile 3 (n = 116)	Quartile 4 (n = 115)
Median (range), cm^3	579.0 (136.0–806.0)	1143.7 (815.0–1565.0)	2210.0 (1567.0–3133.0)	4702.0 (3166.0–13,463.0)
Age, mean (SD), y	32.1 (9.2)	33.6 (10.8)	41.6 (14.9)	51.3 (14.7)
Male sex, No., %	32, 27.8	44, 37.9	56, 48.3	84, 73.0
Caucasian race, No., %	101, 88.6	112, 96.6	109, 94.0	115, 100.0
BMI, mean (SD), kg/m^2	21.9 (3.0)	23.8 (2.9)	26.2 (4.0)	29.7 (5.7)
VAT volume, mean (SD), cm^3	545.9 (162.7)	1143.7 (215.3)	2252.5 (447.3)	5109.9 (1706.3)
SAT volume, mean (SD), cm^3	2526.0 (1552.8)	4092.3 (1836.4)	6029.2 (2854.6)	7868.2 (4128.7)
Diabetes, No., %	2, 1.7	2, 1.7	4, 3.5	16, 13.9
Hypertension, No., %	1, 0.9	10, 8.6	18, 15.5	41, 35.7
Smoking, No., %				
Current	11, 10.0	12, 10.4	14, 12.3	11, 9.73
Former	24, 21.8	25, 21.7	37, 32.5	42, 37.2
Never	75, 68.2	78, 67.8	63, 55.3	60, 53.1
ALT, mean (SD), IU/L	20.5 (23.1)	20.6 (16.3)	24.5 (20.0)	33.1 (35.7)
AST, mean (SD), IU/L	23.5 (21.1)	23.2 (14.9)	21.9 (9.3)	28.4 (23.7)
Platelets, mean (SD)	361.5 (147.0)	367.7 (145.3)	335.2 (122.1)	300.8 (115.6)
Albumin, mean (SD)	3.8 (0.7)	3.9 (0.6)	4.0 (0.6)	4.0 (0.5)
IBD-related variables				
Age category at diagnosis, No., %				
1	34, 29.6	29, 25.0	21, 18.1	17, 14.8
2	72, 62.6	75, 64.7	80, 69.0	62, 53.9
3	9, 7.8	12, 10.3	15, 12.9	36, 31.3
Disease duration, mean (SD), y	9.7 (7.8)	9.8 (8.4)	14.2 (12.6)	14.2 (16.1)
Type of disease, No., %				
Inflammatory	35, 30.4	36, 31.0	45, 38.8	49, 42.6
Stricturing	37, 32.2	34, 29.3	29, 25.0	29, 25.2
Penetrating	43, 37.4	46, 39.7	42, 36.2	37, 32.2
Location of disease, No., %				
Ileal	24, 20.9	30, 25.9	35, 30.2	26, 22.6
Ileo-colonic	67, 58.3	58, 50.0	57, 49.1	59, 51.3
Colonic	15, 13.0	21, 18.1	19, 16.4	26, 22.6
Upper	2, 1.7	3, 2.6	1, 0.9	2, 1.7
Active penetrating disease at the time of CT, No., %	9, 7.8	17, 14.7	14, 12.1	5, 4.4
Prior IBD surgeries, No., %	59, 51.3	53, 45.7	65, 56.0	66, 57.4
Steroid use, No., %				
Any prior use	98, 85.2	94, 81.0	99, 85.3	99, 86.1
Recent IV steroids[a]	14, 12.2	16, 13.8	21, 18.1	13, 11.3
Current prednisone	26, 22.6	24, 20.7	27, 23.3	19, 16.5
Biologic use,[a] No., %	64, 55.7	71, 61.2	67, 57.8	63, 54.8
Immunomodulator use,[b] No., %	81, 70.4	80, 69.0	80, 69.0	80, 69.6
Any 5-ASA use, No., %	98, 85.2	93, 80.2	94, 81.0	91, 79.1

All variables are represented as mean (SD), unless stated otherwise.
Abbreviations: 5-ASA, 5-aminosalicylic acid; IV, intravenous.
[a]Recent IV steroid use was defined as administration of IV steroids within the preceding 12 months before baseline.
[a]Biologic use was defined as any current or known prior use of infliximab, adalimumab, tysabri, or cimzia.
[b]Immunomodulator use was defined as any current or known prior use of azathioprine or 6-mercaptopurine.

TABLE 2: Relationship Between VAT and Continuous Liver Attenuation Among Patients With Crohn's Disease (n = 462)

	Difference in Mean Liver Attenuation, HU				
	VAT Q1 (n = 115)	VAT Q2 (n = 116)	VAT Q3 (n = 116)	VAT Q4 (n = 115)	P_{trend}
Median (range), cm^3	579.0 (136.0–806.0)	1143.7 (815.0–1565.0)	2210.0 (1567.0–3133.0)	4702.0 (3166.0–13,463.0)	
Age-adjusted model	Reference				
β' (95% CI)[a]		–2.65 (–7.94 to 2.63)	–14.79 (–20.26 to –9.32)***	–27.03 (–33.04 to –21.01)***	<0.0001
Multiple model 2[a,b]					
β' (95% CI)		–1.78 (–7.34 to 3.80)*	–13.77 (–19.65 to –7.90)***	–24.07 (–31.18 to –16.96)***	<0.0001
Multiple model 3[a,c]					
β' (95% CI)		–2.92 (–8.82 to 2.97)*	–13.22 (–19.69 to –6.75)***	–21.79 (–30.06 to –13.52)***	<0.0001
Multiple model 4[a,d]					
β' (95% CI)		–3.00 (–8.85 to 2.84)*	–13.63 (–20.05 to –7.21)***	–22.13 (–30.34 to –13.92)***	<0.0001

*$P > 0.05$; **$P < 0.001$; *** $P < 0.0001$.
[a]Beta coefficient representing the difference in mean liver attenuation (HU) per VAT quartile increase.
[b]Model 2: multiple linear regression model, adjusted for age, sex, eigenvectors #1 through #3, smoking status, disease duration, penetrating or stricturing disease type, current or recent prior steroid use within the preceding 12 months, and use of any prior or current biologic medications.
[c]Model 3: model 2 + BMI, hypertension, hyperlipidemia, and the presence of ≥1 copies of the *PNPLA3* rs738409 (G) allele.
[d]Model 4: model 3 + diabetes.

materially affected by further adjustment for NAFLD risk factors (mean HU, –21.79; 95% CI, –30.06 to –13.52; $P_{trend} < 0.0001$). In the final fully adjusted model, compared with individuals in the lowest quartile of VAT volume, mean HU remained significantly reduced among those in the highest VAT quartile (mean HU, –22.13; 95% CI, –30.34 to –13.92; $P_{trend} < 0.0001$) (Table 2).

VAT Quartile and Prevalent NAFLD (L:S ≤ 1.0)

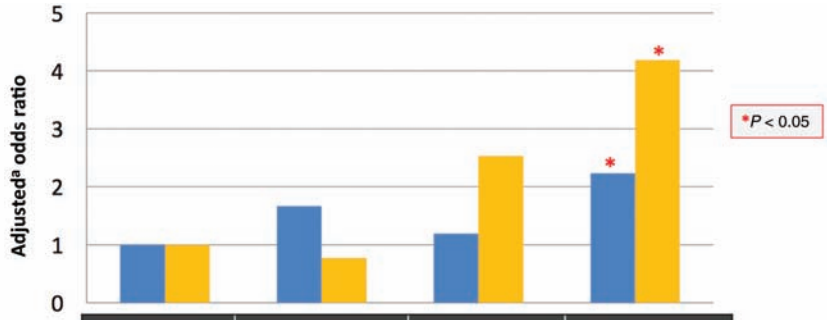
Increasing VAT quartile was associated with a significantly increased risk of radiographic NAFLD (L:S < 1.0) (Fig. 1). Compared with the lowest VAT quartile, patients in the highest VAT quartile demonstrated a 2.2-fold increased risk of NAFLD (95% CI, 1.21 to 4.14; $P_{trend} = 0.032$) and a 4.2-fold increased risk of elevated ALT>ULN (95% CI, 1.19 to 14.76; $P_{trend} = 0.017$) (Fig. 1).

We also examined whether increasing VAT was associated with severe steatosis, defined by the validated radiographic threshold (HU < 40).[28] Although the number of patients meeting this end point was small (n = 13), we found a consistent relationship between increasing VAT quartile and risk of severe steatosis in age- and sex-adjusted analyses (OR, 5.08; 95% CI, 2.00 to 12.88; $P_{trend} = 0.0006$).

Interaction Between Variants in Autophagy Genes and VAT Volume on Measurements of NAFLD

We examined whether the association between VAT volume and NAFLD is modified by variants in autophagy genes. Compared with patients with the TT genotype at the rs13361189 locus, patients with ≥1 C alleles had significantly increased hepatic fat per quartile increase in VAT (mean difference in HU, –22.98; 95% CI, –32.27 to –13.69; vs mean difference in HU, –19.30; 95% CI, –36.30 to –2.30; adjusted $P_{interaction} = 0.002$) (Fig. 2a; Supplementary Table 2). Similarly, patients with ≥1 variant A alleles at the rs4958847 locus displayed significantly more hepatic fat compared with those with the GG genotype (mean difference in HU, –23.94; 95% CI, –34.07 to –13.82; vs mean difference in HU, –18.43; 95% CI, –32.57 to –4.29; adjusted $P_{interaction} = 0.032$) (Fig. 2B; Supplementary Table 2). In contrast, the relationship between VAT quartile and hepatic fat was not modified by either variant in the *ATG16L1* (rs2241880) or *PNPLA3* (rs738409) genes (adjusted $P_{interaction} = 0.501$ and 0.083, respectively) (Supplementary Table 2).

Next, we tested whether these variants modify the relationship between VAT and radiographic NAFLD (Table 3). Due to the relatively small number of observed outcomes, VAT estimates were calculated per quartile increase, using the median value of each quartile. Patients with ≥1 C alleles at the rs13361189 locus had significantly increased risk of NAFLD per quartile increase in VAT, compared with the TT genotype (adjusted OR, 1.57; 95% CI, 1.11 to 2.23; vs adjusted OR, 1.01; 95% CI, 0.51 to 1.99; $P_{interaction} < 0.001$) (Table 3). Similarly, in comparison with patients with the GG genotype at the rs4958847 locus, those with ≥1 variant A alleles had a significantly increased risk of NAFLD per quartile increase in VAT (adjusted OR, 1.44; 95% CI, 0.87 to 2.36; vs adjusted OR, 1.30; 95% CI, 0.90 to 1.89; $P_{interaction} = 0.005$). In contrast, the association between increasing VAT and NAFLD did not vary by genotype at either the *ATG16L1* or *PNPLA3* locus ($P_{interaction} = 0.132$ and 0.061, respectively) (Table 3).



	VAT Quartile 1 (n = 115)	VAT Quartile 2 (n = 116)	VAT Quartile 3 (n = 116)	VAT Quartile 4 (n = 115)	P_{trend}
Median VAT (range), cm^3	579.0 (136.0–806.0)	1143.7 (815.0-1565.0)	2210.0 (1567.0-3133.0)	4702.0 (3166.0–13,463.0)	
Adjusted[a] odds ratio (95% CI)					
L:S < 1.0	Reference	1.67 (0.99 to 2.81)	1.19 (0.69 to 2.05)	2.24 (1.21 to 4.14)	0.032
ALT > ULN[b]	Reference	0.77 (0.40 to 1.47)	2.53 (0.95 to 6.79)	4.19 (1.19 to 14.76)	0.017

FIGURE 1. Association between VAT and radiographic NAFLD and elevated ALT. [a]Multivariable logistic regression model adjusted for age, sex, hypertension, hyperlipidemia, smoking status, disease duration, penetrating or structuring disease, steroid use within the preceding 12 months, biologic medication use, PNPLA3 rs738409, and eigenvectors #1 through #3. [b]ULN defined as ALT >40 IU/L.

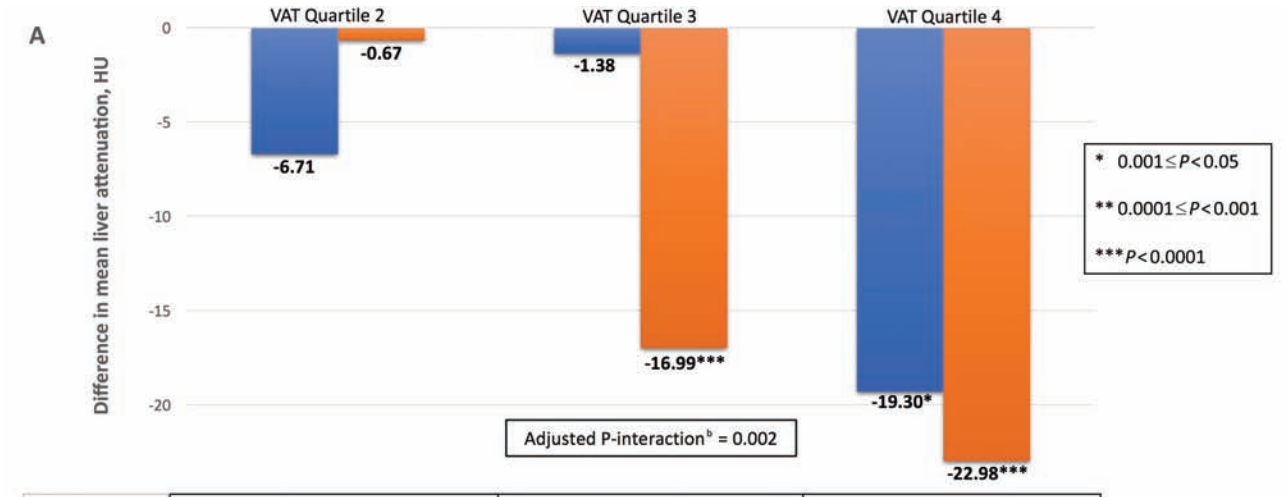
IRGM variants also modified the relationship between VAT volume and risk of elevated ALT (Table 4). Compared with the TT genotype, patients with *IRGM* rs13361189 CT/CC genotypes displayed an increased risk of ALT>ULN, with increasing VAT volume (adjusted OR, 2.30; 95% CI, 1.32 to 4.04; vs adjusted OR, 1.68; 95% CI, 0.81 to 3.50; $P_{interaction}$ = 0.044). Similarly, compared with the GG genotype, patients with *IRGM* rs4958847 AG/AA genotypes had an increased risk of having an elevated ALT, with increasing VAT (adjusted OR, 2.14; 95% CI, 1.09 to 4.22; vs adjusted OR, 1.92; 95% CI, 1.09 to 3.40; $P_{interaction}$ = 0.012). No effect modification at the *ATG16LI* or *PNPLA3* susceptibility loci was seen ($P_{interaction}$ = 0.275 and 0.741, respectively) (Table 4).

Finally, we tested whether *IRGM* gene variants modify the relationship between VAT volume and risk of advanced NAFLD fibrosis, estimated by the FIB-4 index (Supplementary Table 3). With increasing quartile of VAT, patients with the *IRGM* rs13361189 CT/CC genotype had significantly increased risk of FIB-4 >1.45 compared with those with the TT genotype (adjusted OR, 1.23; 95% CI, 0.59 to 2.56; vs adjusted OR, 0.21; 95% CI, 0.05 to 0.87; $P_{interaction}$ = 0.053). Similarly, patients with the *IRGM* rs4958847 AG/AA genotype also had increased risk of FIB-4 >1.45 with increasing VAT quartile, compared with those with the GG genotype (adjusted OR, 1.19; 95% CI, 0.55 to 2.60; vs adjusted OR, 0.32; 95% CI, 0.11 to 0.95; $P_{interaction}$ < 0.001) (Supplementary Table 3). As in previous models, no effect modification at the *ATG16L1* or the *PNPLA3* loci was observed (both $P_{interaction}$ terms > 0.05) (data not shown).

DISCUSSION

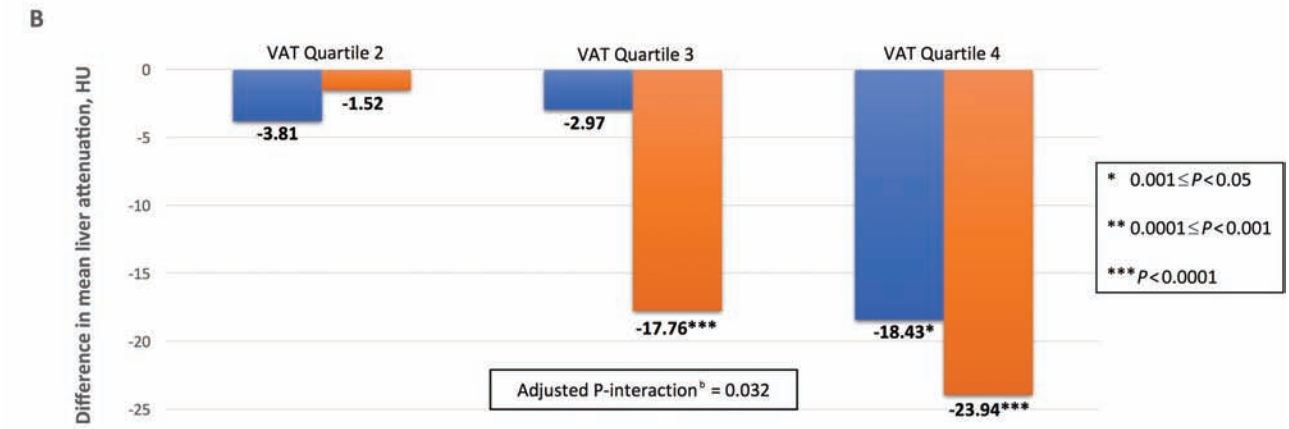
This study represents the first to describe the association between VAT volume, *IRGM*-mediated defects in autophagy, and NAFLD risk in a well-characterized population of patients with CD. In this cohort, increasing VAT volume was a strong independent predictor of hepatic fat accumulation and was associated with radiographic and serum markers of NAFLD severity. This association remained significant after full adjustment for known cardiometabolic risk factors and measures of IBD severity, biologic medication use or recent prednisone use, each of which have previously been hypothesized to explain the co-occurrence of NAFLD and IBD.[2, 4, 8] This study is also the first to demonstrate that the risk of NAFLD associated with VAT is significantly augmented in patients with functionally annotated *IRGM* gene variants, suggesting that defects in autophagy pathways governed by *IRGM* may modify the relationship between VAT and NAFLD risk.

Previous GWA studies have established *IRGM* as an important susceptibility gene for CD.[32–34] *IRGM*-mediated autophagy protects against intracellular bacteria, and defects in autophagy have been linked to the pathogenesis of multiple human diseases outside of inflammatory bowel disease, including cancer and cardiovascular disease.[35] Recently, data have suggested that defects in *IRGM*-mediated autophagy may also promote the pathogenesis of hepatic steatosis and steatohepatitis through dysregulation of hepatic lipid metabolism and systemic inflammatory activation.[23] Although the precise mechanisms have not been elucidated, *IRGM* has been shown to impact mitochondrial function[36] and plays a direct role in the co-assembly of core autophagy machinery, including ULK1, Beclin-1, and ATG16L1.[37] The knockdown of *IRGM* expression in human hepatoma HepG2 and PLC/PRF/5 cell lines decreases cellular levels of phospho-ULK1, Beclin-1, and LC3-II, which results in accumulation of intrahepatic lipid droplets[24]; this phenotype is rescued when



	VAT Quartile 2 (95% CI)	VAT Quartile 3 (95% CI)	VAT Quartile 4 (95% CI)
0 C alleles	(−18.21 to 4.79)	(−13.29 to 10.54)	(−36.30 to −2.30*)
≥1 C allele	(−7.28 to 5.94)	(−24.45 to −9.52***)	(−32.27 to −13.69***)

Abbreviations: HU, Hounsfield Units; CI, confidence intervals; *IRGM* gene, immunity-related GTPase M gene, VAT, visceral adipose tissue
[1]Difference in mean liver attenuation (HU) per quartile increase in VAT, compared to VAT quartile 1, adjusted for age, sex, race, BMI, diabetes, hypertension, hyperlipidemia, smoking status, disease duration, penetrating or stricturing disease, current or recent steroid use within the preceding 12 months, use of biologic medications and eigenvectors #1 through #3.
* P-value for patients with 0 vs. 1 or 2 IRGM rs13361189 (C) risk alleles, modeled using a dominant genotype model
[b] P-interaction for the relationship between the allele of the of interest and visceral adipose tissue (VAT) quartile on liver attenuation, measured as a continuous variable in HU



	VAT Quartile 2 (95% CI)	VAT Quartile 3 (95% CI)	VAT Quartile 4 (95% CI)
0 A alleles	(−14.02 to 6.40)	(−14.16 to 8.22)	(−32.57 to −4.29*)
≥1 A allele	(−8.60 to 5.57)	(−25.79 to −9.74***)	(−32.07 to −13.82***)

Abbreviations: HU, Hounsfield Units; CI, confidence intervals; *IRGM* gene, immunity-related GTPase M gene, VAT, visceral adipose tissue
[1]Difference in mean liver attenuation (HU) per quartile increase in VAT, compared to VAT quartile 1, adjusted for age, sex, race, BMI, diabetes, hypertension, hyperlipidemia, smoking status, disease duration, penetrating or stricturing disease, current or recent steroid use within the preceding 12 months, use of biologic medications and eigenvectors #1 through #3.
* P-value for patients with 0 vs. 1 or 2 IRGM rs4958847 (A) risk alleles, modeled using a dominant genotype model
[b] P-interaction for the relationship between the allele of the of interest and visceral adipose tissue (VAT) quartile on liver attenuation, measured as a continuous variable in HU

FIGURE 2. A and B, Difference in mean liver attenuation (HU) per quartile increase of VAT according to strata defined by *IRGM* variants. A, *IRGM* rs13361189 (C). B, *IRGM* rs4958847 (A). [a]*P* value for patients with 0 vs 1 or 2 IRGM rs13361189 (C) risk alleles, modeled using a dominant genotype model. [b]$P_{interaction}$ for the relationship between the allele of interest and VAT quartile on liver attenuation, measured as a continuous variable in HU.

cells are subsequently treated with rapamycin, an inducer of autophagy, or when *IRGM* is overexpressed within cultured hepatocytes.[24] Additionally, preclinical models of *IRGM*-knockout mice show that impaired autophagy increases susceptibility not just to diabetes and colitis,[19] but also to incident NAFLD.[38]

To date, only 1 published human study has reported an association between polymorphisms in *IRGM* and NAFLD

TABLE 3: Association Between VAT Volume and Risk of Radiographic NAFLD According to Strata Defined by Genetic Variants (n = 427)

	No. (%)	Adjusted[a] OR (95% CI) per Quartile Increase in VAT, cm^3	$P_{interaction}$[b]
IRGM rs13361189			
TT			<0.001
OR (95% CI)	313 (73.3)	1.01 (0.51 to 1.99)	
TC or CC			
OR (95% CI)	114 (26.8)	1.57 (1.11 to 2.23)*	
IRGM rs4958847			
GG			0.005
OR (95% CI)	270 (63.2)	1.30 (0.90 to 1.89)	
GA or AA			
OR (95% CI)	157 (36.8)	1.44 (0.87 to 2.36)	
ATG16L1 rs2241880			
AA			0.132
OR (95% CI)	342 (80.1)	1.79 (0.81 to 3.92)	
GA or GG			
OR (95% CI)	85 (19.9)	1.33 (0.96 to 1.83)	
PNPLA3 rs738409			
CC			0.061
OR (95% CI)	275 (64.4)	1.06 (0.63 to 1.78)	
CG or GG			
OR (95% CI)	152 (35.6)	1.54 (1.07 to 2.22)*	

*$0.01 \leq P < 0.05$.

[a]Multivariable logistic regression model, adjusted for age (years), sex, BMI, diabetes, hypertension, hyperlipidemia, smoking status, disease duration (years), penetrating or stricturing disease type, any steroid use within the preceding 12 months, any current or prior use of biologic medications, eigenvectors #1 through #3, the SNP of interest, and the respective interaction between that SNP*VAT volume quartile.

[b]The $P_{interaction}$ term obtained from the log likelihood ratio test, comparing the main multivariable logistic regression model with a model including an interaction term, modeled as the product of the SNP of interest*VAT volume quartile.

risk.[24] In a study of 832 obese Taiwanese pediatric patients, the *IRGM* rs10065172 SNP was associated with increased risk of ultrasound-defined NAFLD; however, in that analysis, the authors did not account for measures of body composition beyond BMI, and no interactions between obesity-related factors and *IRGM* variants were investigated.[24] Moreover, their population was comprised of obese patients who like had increased VAT volume, which could have augmented the relationship between *IRGM* and NAFLD risk. The authors found a direct relationship between the *IRGM* rs10065172 SNP and NAFLD susceptibility, an association not previously reported in GWAS.[39–41] We found that rs10065172 is in perfect linkage disequilibrium with the *IRGM* variant tested in the present study, rs13361189 (r^2 = 1.0), with an allelic frequency of 10% in European populations.[42] Interestingly, neither variant was found to confer susceptibility to NAFLD at GWA study significance thresholds.[43] Therefore, it is plausible that the link between *IRGM* and NAFLD may only be present in a specific subgroup of the population, where it modifies the relationship between VAT and NAFLD risk. Future studies in large, well-defined cohorts will be necessary to fully characterize this relationship.

Within PRISM, we observed a high prevalence (52%) of radiographic NAFLD, which lends support to recent cross-sectional studies, which have reported rates of co-occurrent NAFLD and IBD approaching 40%.[4–6, 44] Although such statistics point to a rising incidence of NAFLD in IBD, to date the specific mediators that drive this relationship remain uncharacterized. Some have hypothesized that traditional cardiometabolic risk factors may promote the pathogenesis of NAFLD in IBD,[2] whereas others point to IBD-specific factors, including disease severity,[4–6, 44] the use of systemic steroids,[45] or other medication-related effects.[44] In the present study, NAFLD was not attributable to CD-related factors, nor was an independent link found between BMI and NAFLD. Rather, we observed a strong and consistent association between VAT and NAFLD and found that increasing VAT also predicted the presence of more severe steatosis (defined by HU < 40) and elevation in ALT, which may indicate underlying hepatic inflammation. These findings suggest that metabolic factors including VAT may reflect a common underlying risk factor that predicts both CD severity and incident NAFLD, while also providing novel insight

TABLE 4: Association Between VAT Quartile and Risk of ALT Above the Upper Limit of Normal (40 IU/L), According to Strata Defined by Genetic Variants (n = 427)

	No. (%)	Adjusted[a] OR (95% CI) per Quartile Increase in VAT, cm^3	$P_{interaction}$[b]
IRGM rs13361189			
TT			0.044
OR (95% CI)	313 (73.3)	1.68 (0.81 to 3.50)	
TC or CC			
OR (95% CI)	114 (26.8)	2.30 (1.31 to 4.04)**	
IRGM rs4958847			
GG			0.012
OR (95% CI)	270 (63.2)	1.92 (1.09 to 3.40)*	
GA or AA			
OR (95% CI)	157 (36.8)	2.14 (1.09 to 4.22)*	
ATG16L1 rs2241880			
AA			0.275
OR (95% CI)	342 (80.1)	3.04 (0.98 to 9.46)	
GA or GG			
OR (95% CI)	85 (19.9)	1.78 (1.09 to 2.91)*	
PNPLA3 rs738409			
CC			0.740
OR (95% CI)	275 (64.4)	2.04 (0.90 to 4.63)	
CG or GG			
OR (95% CI)	152 (35.6)	2.16 (1.24 to 3.79)**	

*$0.01 \le P < 0.05$; **$0.001 \le P < 0.01$.

[a]Multivariable logistic regression model, adjusted for age (years), sex, BMI, diabetes, hypertension, hyperlipidemia, smoking status, disease duration (years), penetrating or stricturing disease type, any steroid use within the preceding 12 months, any current or prior use of biologic medications, eigenvectors #1 through #3, the SNP of interest, and the respective interaction between that SNP*VAT volume quartile.

[b]$P_{interaction}$ term obtained from the log likelihood ratio test, comparing the main multivariable model with that including the SNP of interest and the interaction term, composed of the SNP*VAT volume quartile.

into the autophagy-related genetic factors that modify this relationship.

This study is the first to demonstrate a strong, independent relationship between VAT and NAFLD risk within a well-characterized CD population and is the first to show that this relationship is modified by variants at the *IRGM* rs13361189 and rs4958847 loci. Our results suggest that genetic defects in IRGM-mediated autophagy may impact the relationship between VAT and NAFLD risk. We leveraged a large, well-characterized patient cohort, in whom all cases of CD, all recorded medications, and diagnoses of disease-related complications were confirmed by manual review of the medical record, rather than through diagnostic codes or self-report. We also used a validated and objective tool for the measurement of both VAT volume and LA, thus minimizing the risk of measurement error. Finally, we tested the consistency of our findings in a series of analyses using noninvasive measures of NAFLD severity, including FIB-4, ALT level, and the use of a second, strict radiographic cutoff that is highly specific to severe (>30%) steatosis. In each of those analyses, we observed consistent results, supporting our primary findings.

Several important limitations must be emphasized. First, due to the cross-sectional nature of this study, we were not able to account for changes in VAT, hepatic fat, or NAFLD severity over time. However, an assessment of serial CT scans in a subpopulation of patients with multiple CT scans (n = 258) demonstrated that VAT volume and liver attenuation were not impacted before and after recorded disease complications, thus arguing against the possibility of a proxy effect. We also carefully constructed our model to account for disease duration, penetrating or stricturing disease, and the use of biologic medications or steroids, which further minimizes the possibility that the observed association was related to IBD-associated factors. Second, this study represents an observational analysis that is subject to residual confounding and reflects data from a single tertiary center, which could limit generalizability. However, as our group has previously demonstrated, age at diagnosis, complication rate, and distribution of important contributory lifestyle factors are similar in our cohort to published data from large US and European populations.[46, 47]

Third, our study lacked hepatic histology, which remains the gold standard for the diagnosis of NAFLD. Although CT is

well validated and widely used for the diagnosis of steatosis, CT cannot reliably identify NASH or assess fibrosis. We attempt to address this by using noninvasive measures to estimate the severity of any underlying liver disease, including FIB-4, ALT level, and a second, strict definition of steatosis severity; in these analyses, we observed consistent results, lending support to the validity of our findings. Nonetheless, in this relatively young CD cohort, overall FIB-4 indices were low (mean, 0.81), and few patients (n = 32) met the threshold cutoffs for advanced NAFLD fibrosis (FIB-4 > 1.45). We also examined in an exploratory analysis the NAFLD Fibrosis Score, thus limiting our ability to use these scores in exploratory analyses. To address this, future studies with well-phenotyped populations are needed, with hepatic histology or modalities such as fibroscan or magnetic resonance elastography.

Finally, despite representing the first report of its kind, we acknowledge that the robust evaluation of gene-environment interactions requires the analysis of large populations, and therefore we eagerly await validation of our findings in large, well-phenotyped cohorts, including those from the general population.

CONCLUSION

Within a well-characterized cohort of patients with CD, increasing VAT volume was associated with a significantly increased risk of NAFLD and with markers of NAFLD severity. This risk was augmented by variants at the *IRGM* rs13361189 and rs4958847 loci, suggesting that genetic defects in *IRGM*-mediated autophagy may modify the relationship between VAT and NAFLD risk. Our results provide insight into a novel mechanism of NAFLD pathogenesis in patients with CD and carry potentially important clinical implications. If validated, they would support the adoption of targeted screening protocols for CD patients with central obesity, who might be at risk for NAFLD and cardiometabolic disease.

SUPPLEMENTARY DATA

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

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Guarantor of the article: Hamed Khalili is acting as the guarantor of this article and takes responsibility for the integrity of the work as a whole, from inception to publication.

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