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The Immunity-related GTPase M rs13361189 variant does not increase the risk for prevalent or incident steatosis; results from the Framingham Heart Study

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Abstract

Background & Aims.—Emerging data from pediatric populations suggest that variants in the autophagy-governing Immunity-related GTPase M (*IRGM*) gene may contribute to nonalcoholic fatty liver disease (NAFLD) susceptibility. We examined the relationship between *IRGM* rs13361189 variants and NAFLD in a community-based cohort of adults.

Methods.—We included all Framingham Heart Study participants with available data on the *IRGM* rs13361189 variant, undergoing study-directed computed tomography (CT) scans of the abdomen (2002-2005). Using multivariable linear and logistic regression modeling, we evaluated cross-sectional associations between rs13361189 genotype and hepatic steatosis (HS). Among the subset of participants without baseline HS and who underwent follow-up CT scan between 2008-2011, we used multivariable logistic regression modeling to assess the longitudinal relationship between *IRGM* rs13361189 genotype and risk for incident HS.

Results.—Among 2,070 participants (50% women; mean age 51±11 years), 332 (16%) had one copy of the variant rs13361189 variant C allele, while 19 (1%) had the CC genotype. Compared to the TT genotype, there was no increased odds of prevalent HS with the CT or CC genotype (multivariable-adjusted odds ratio [OR] 0.93 [95% CI 0.68-128] and 0.86 [95% CI 0.46-1.63], respectively). Among individuals without baseline HS (n=1,052), 19.3% developed incident HS over median 6.1 years. Compared to the TT genotype, neither the CT nor the CC genotype were significantly associated with incident HS (all p>0.05).

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Conclusion.—In our community-based, longitudinal cohort of Caucasian adults, variants in the autophagy-governing *IRGM* gene at the rs13361189 locus were not associated with increased prevalent or incident HS.

Keywords

nonalcoholic fatty liver disease; steatosis; autophagy; gene variants

Introduction

Nonalcoholic fatty liver disease (NAFLD) now represents the most common cause of chronic liver disease in the United States, impacting an estimated 19-24% of the population[1]. NAFLD is closely linked to diabetes and obesity, including an excess accumulation of metabolically-active visceral adipose tissue (VAT)[2]. However, not all individuals with diabetes or obesity develop NAFLD, and genetic studies support the heritability of NAFLD[3]. Genome-wide association studies (GWAS) have identified several NAFLD susceptibility genes, including patatin-like phospholipase domain-containing 3 (*PNPLA3*)[4]. However, the proportion of variance in genetic risk explained by these loci remains modest (<5%), and it is possible that some of the "missing heritability" derives from low frequency or rare variants.

Autophagy is a highly conserved metabolic process whereby defective intracellular organelles are targeted for lysosomal destruction[5]. Defects in autophagy confer susceptibility to chronic inflammatory diseases including obesity and diabetes[6], and also to impair ß-oxidation of intrahepatic lipids[5]. Consequently, it has been hypothesized that impaired autophagy may contribute to the pathogenesis of NAFLD[7]. In preclinical models, inhibition of autophagy promotes hepatic triglyceride accumulation, while autophagy stimulation with rapamycin accelerates lipid clearance, improving both steatosis and central obesity[8]. In humans, two cross-sectional studies recently demonstrated that compared to the wild-type (CC genotype), the TT genotype at the rs10065172 locus of the autophagy-governing, immunity-related GTPase M (*IRGM)* gene is associated with prevalent NAFLD[9, 10], and with elevations in alanine aminotransferase (ALT)[10]. To date, however, the link between functional genetic defects in *IRGM* and NAFLD susceptibility has not previously been extended to an adult population.

Thus, we examined a functionally-annotated variant in *IRGM* at the rs13361189 locus, which has been shown in European populations to be in perfect linkage disequilibrium with the rs10065172 locus (r^2=1.0)[11]. In a prior study of a predominantly Han Chinese population, these two loci also were found to be in tight linkage disequilibrium[10]. Specifically, we evaluated the association between rs13361189 genotype and both prevalent and incident hepatic steatosis (HS), defined by multidetector computed tomography (MDCT), in a well-phenotyped and prospective community-based cohort, the Framingham Heart Study (FHS).

Methods

Detailed methods are outlined in the Supplementary file.

Results

Baseline demographics and clinical characteristics of the 2,070 participants according to rs13361189 genotype are shown in Table 1. Compared to participants with the rs13361189 TT genotype (n=1719, 83%) or the CT genotype (n=332, 16%), those with the CC genotype (n=19; 1%) were significantly older (mean±standard deviation [SD] age [years] CC vs. CT vs. TT = 54±9 vs. 52±11 vs. 51±11; p=0.016), and more likely to have hypertension (CC vs. CT vs. TT= 16% vs. 34% vs. 27%; p=0.007). However, mean±SD body mass index (BMI) was similar between groups (mean SD BMI for CC vs. CT vs. TT genotypes: 27±−6 vs. 28±5 vs. 28 ±5; p=0.24), and a similar proportion of participants had diabetes (CC vs. CT vs. TT = 10.5% vs. 4.2% vs. 6.8%; p=0.11).

Overall, we recorded 368 cases of prevalent HS, including 304 (18%) among participants with the rs13361189 TT genotype, 63 (19%) among those with the CT genotype, and 1 (5%) among those with the CC genotype (Table 1). Compared to the rs13361189 TT genotype, no significant difference in HS was found with the CT genotype (adjusted ß= −0.001, 95% CI −0.006,0.005) or the CC genotype (adjusted ß = −0.002, 95% CI −0.013,0.009; Table 2). These associations were not materially altered after accounting for BMI, diabetes, smoking status and alcohol intake. Next, we evaluated the relationship between *IRGM* variants and prevalent HS, defined dichotomously by LPR≤0.33 (Table 2). Compared to the TT genotype, neither the CT nor the CC genotype were associated with prevalent HS (adjusted OR 0.93 [95% CI 0.68-128] and 0.86 [95% CI 0.46-1.63], respectively. There was no evidence of effect modification by BMI (Table S1; p-interaction>0.05) or by VAT volume (Table S2; p-interaction>0.05). We also assessed the relationship between *IRGM* variants and elevations in serum ALT (Table 2). However, compared to the TT genotype, no significant association with elevated ALT was found with either the CT or the CC genotypes (adjusted OR 0.97 [95% CI 0.77-1.23] and 0.95 [95% CI 0.59-1.51], respectively).

In secondary analyses, we explored the association between rs13361189 genotype and prevalent HS using a dominant genotype model that compared the CT/CC genotypes to the TT genotype, and our findings were null (adjusted OR for HS=0.96, 95% CI 0.68-1.36). Similarly, there was no significant association between rs13361189 genotype and elevated ALT using a dominant model (adjusted OR=0.95, 95% CI 0.59-1.51).

Finally, in the exploratory longitudinal analysis (n=1,052), we identified 204 cases of incident HS between paired MDCT scans, over a median of 6.1 years. In both the minimally-adjusted and the full multivariable models, there were no significant associations between *IRGM* rs13361189 variants and incident NAFLD (Table S3).

Discussion

To our knowledge, this represents the first epidemiological study of the relationship between *IRGM* rs13361189 variants and HS risk in a prospective, community-dwelling adult population. Despite appropriate statistical power and careful accounting for important confounders, including *PNPLA3* variants and population stratification, the *IRGM*

rs13361189 gene variant did not influence hepatic fat content, nor was it associated with risk of prevalent or incident steatosis, in this population.

A body of recent data suggests that defects in autophagy may influence the pathogenesis of HS and steatohepatitis (NASH), through hepatic lipid dysregulation and inflammatory activation[5]. While the precise mechanisms remain uncharacterized, *IRGM* is known to influence mitochondrial function, where it plays a direct role in the co-assembly of core autophagy machinery, including ULK1, Beclin-1 and ATG16L1[12]. *In vitro*, the knockdown of *IRGM* expression decreases cellular levels of phospho-ULK1, Beclin-1, and LC3-II, which leads to accumulation of intrahepatic lipid droplets[10]. In contrast, this phenotype is rescued when *IRGM* is overexpressed, or when cells are treated with rapamycin, an inducer of autophagy[10]. Furthermore, preclinical models of *IRGM*-knockout mice show that impaired autophagy increases susceptibility to diabetes[6] as well as to NAFLD[13].

Clinical evidence regarding a relationship between *IRGM* gene variants and NAFLD susceptibility is scarce[9, 10]. Within this community-based cohort of adults, we found no association between the *IRGM* rs13361189 variant and risk of either prevalent or incident steatosis. This is supported by previous population-based adult cohort studies, in which neither the *IRGM* rs10065172 or rs13361189 variants conferred susceptibility to NAFLD at GWAS significance thresholds[4], despite a MAF of at least 10% in European populations[14]. Although our findings appear to contrast with two recent cross-sectional candidate gene studies[9, 10], there are several potential explanations for these discrepant findings. First, the prior studies were limited by small sample size, cross-sectional design, and included highly-selected, pediatric populations, with limited generalizability. Second, candidate gene studies carry a well-recognized risk for false positive findings and lack of reproducibility, particularly when conducted in small, selected populations[15]. While a role remains for classical candidate gene studies in NAFLD, particularly for mechanistic or functional associations, our findings highlight the continued need for GWAS, exome-, and whole genome-wide association studies as well as investigations of epigenetic and transcriptional regulation of NAFLD.

Finally, it is also possible that the influence of *IRGM* gene variants on NAFLD risk is specific to pediatric populations. Pediatric NASH remains a challenging diagnosis, as it is marked by unique histopathological features rarely found in adults[16]. In a retrospective study of 100 pediatric NASH liver biopsy specimens, only 17% of children had adult-type NASH, defined by steatosis, lobular inflammation and hepatocyte ballooning[1], while more than 50% had “Type 2” NASH, with macrovesicular steatosis, portal inflammation with or without fibrosis, and little to no ballooning degeneration[16]. Children with Type 2 NASH are more likely to be male, younger in age, and obese, compared to children with adult-type NASH[16]. To date, it remains unknown whether these two distinct patterns reflect underlying differences in the etiopathogenesis or evolution of disease; similarly, it is not known whether variants in *IRGM* could account for a proportion of these differences. To test this hypothesis, future prospective natural history studies are needed in well-phenotyped adult and pediatric populations.

We highlight several important limitations. First, our study population was limited to Caucasians, thus our results may not be directly translatable to persons of other ancestries. Second, only 19 participants in our cohort had the rs13361189 variant CC genotype; this highlights the need for future large-scale studies in diverse populations, particularly given the potential impact of ethnic variability in *IRGM* genotypes on NAFLD susceptibility[9, 10], as well as the possibility of effect modification by *PNPLA3* genotype[9]. Finally, liver biopsy remains the gold standard for the assessment of nonalcoholic steatohepatitis (NASH) and fibrosis, and future studies will be needed to characterize the impact of *IRGM* variants on NAFLD histology. Nevertheless, CT is well-validated for the assessment and quantification of hepatic steatosis, which has demonstrated clinical and prognostic significance for persons with NAFLD.

In conclusion, within a large, prospective community-based population of U.S. adults, the *IRGM* rs13361189 variant was not significantly associated with prevalent or incident HS. Additional prospective studies are needed to validate our findings, and to determine if this association is unique to pediatric NAFLD/NASH.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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List of Abbreviations:

IRGM	Immunity-related GTPase M
NAFLD	nonalcoholic fatty liver disease
CT	computed tomography
HS	hepatic steatosis
OR	odds ratio
CI	confidence interval
VAT	visceral adipose tissue
GWAS	Genome-wide association studies
PNPLA3	patatin-like phospholipase domain-containing 3
ALT	alanine aminotransferase

MDCT	multidetector computed tomography
FHS	Framingham Heart Study
BMI	body mass index
LPR	liver-phantom ratio
MAF	mean allelic frequency
NASH	nonalcoholic steatohepatitis

REFERENCES

1. Chalasani N, Younossi Z, Lavine JE, et al. The Diagnosis and Management of Nonalcoholic Fatty Liver Disease: Practice Guidance from the American Association for the Study of Liver Diseases. Hepatology 2017.
2. Speliotes EK, Massaro JM, Hoffmann U, et al. Fatty liver is associated with dyslipidemia and dysglycemia independent of visceral fat: the Framingham Heart Study. Hepatology 2010;51:1979–87. [PubMed: 20336705]
3. Schwimmer JB, Celedon MA, Lavine JE, et al. Heritability of nonalcoholic fatty liver disease. Gastroenterology 2009;136:1585–92. [PubMed: 19208353]
4. Speliotes EK, Yerges-Armstrong LM, Wu J, et al. Genome-wide association analysis identifies variants associated with nonalcoholic fatty liver disease that have distinct effects on metabolic traits. PLoS Genet 2011;7:e1001324. [PubMed: 21423719]
5. Singh R, Kaushik S, Wang Y, et al. Autophagy regulates lipid metabolism. Nature 2009;458:1131–5. [PubMed: 19339967]
6. Lee HY, Kim J, Quan W, et al. Autophagy deficiency in myeloid cells increases susceptibility to obesity-induced diabetes and experimental colitis. Autophagy 2016;12:1390–403. [PubMed: 27337687]
7. Singh R, Xiang Y, Wang Y, et al. Autophagy regulates adipose mass and differentiation in mice. J Clin Invest 2009;119:3329–39. [PubMed: 19855132]
8. Lin CW, Zhang H, Li M, et al. Pharmacological promotion of autophagy alleviates steatosis and injury in alcoholic and non-alcoholic fatty liver conditions in mice. J Hepatol 2013;58:993–9. [PubMed: 23339953]
9. Bellini G, Miraglia Del Giudice E, Nobili V, et al. The IRGM rs10065172 variant increases the risk for steatosis but not for liver damage progression in Italian obese children. J Hepatol 2017;67:653–655. [PubMed: 28483681]
10. Lin YC, Chang PF, Lin HF, et al. Variants in the autophagy-related gene IRGM confer susceptibility to non-alcoholic fatty liver disease by modulating lipophagy. J Hepatol 2016;65:1209–1216. [PubMed: 27417217]
11. Broad Institute Single Nucleotide Polymorphism Annotation and Proxy Search (SNAP). http://archive.broadinstitute.org/mpg/snap/ldsearch.php; accessed September 4, 2017.
12. Chauhan S, Mandell MA, Deretic V. IRGM governs the core autophagy machinery to conduct antimicrobial defense. Mol Cell 2015;58:507–21. [PubMed: 25891078]
13. Hur JH, Park SY, Dall'Armi C, et al. Phospholipase D1 deficiency in mice causes nonalcoholic fatty liver disease via an autophagy defect. Sci Rep 2016;6:39170. [PubMed: 27976696]
14. McCarroll SA, Huett A, Kuballa P, et al. Deletion polymorphism upstream of IRGM associated with altered IRGM expression and Crohn's disease. Nat Genet 2008;40:1107–12. [PubMed: 19165925]
15. Border R, Keller MC. Commentary: Fundamental problems with candidate gene-by-environment interaction studies - reflections on Moore and Thoemmes (2016). J Child Psychol Psychiatry 2017;58:328–330. [PubMed: 28194762]

16. Schwimmer JB, Behling C, Newbury R, et al. Histopathology of pediatric nonalcoholic fatty liver disease. Hepatology 2005;42:641–9. [PubMed: 16116629]

Table 1.

Baseline characteristics of included Framingham Heart Study participants (N=2,070), according to IRGM rs13361189 genotype

Variable*	rs13361189 genotype			P-value[1,2]
	TT N=1719	CT N=332	CC N=19	
Age, SD	50.6+/−10.5	52.2+/−11.0	54.1+/−9.2	0.0162
Female sex, %	858 (49.9%)	169 (50.9%)	8 (42.1%)	0.746
White, %	1719 (100%)	332 (100%)	19 (100%)	-
Alcohol (drinks / week), SD	3.0+/−3.4	2.8+/−3.7	3.2+/−4.0	0.658
Cohort, %				0.076
• Offspring	663 (38.6%)	147 (44.3%)	10 (52.6%)	
• Generation 3	1056 (61.4%)	185 (55.7%)	9 (47.4%)	
BMI, kg/m^2, SD	27.7+/−5.2	28.1+/−5.0	26.5 +/− 5.7	0.241
Waist circumference (cm), SD	96.7+/−13.9	97.9+/−14.5	92.7+/−14.6	0.167
Diabetes, %	117 (6.8%)	14 (4.2%)	2 (10.5%)	0.106$
Hypertension, %	455 (26.5%)	114 (34.3%)	3 (15.8%)	0.007
Hypertriglyceridemia, %	479 (27.9%)	103 (31.3%)	2 (10.5%)	0.099
HDL cholesterol, mg/d, (SD)	51.9 (15.5)	51.0 (16.2)	53.1 (12.1)	0.636
Low HDL cholesterol, %	558 (32.5%)	122 (36.7%)	3 (15.8%)	0.087
Smoking, %				0.019$
• Current	872 (50.7%)	164 (49.4%)	9 (47.4%)	
• Former	643 (37.4%)	143 (43.1%)	5 (26.3%)	
• Never	204 (11.9%)	25 (7.5%)	5 (26.3%)	
Liver phantom ratio, SD	0.4 (0.1)	0.4 (0.1)	0.4 (0.03)	0.129
Liver phantom ratio ≤ 0.33, %	304 (17.7%)	63 (19.0%)	1 (5.3%)	0.338
VAT median (range), cm^3, SD	1634.0 (172.7-6410.0)	1691.0 (227.3-6207.0)	1335.0 (325.5-5850.0)	0.635
VAT volume, cm^3, SD	1776.7 (1004.7)	1827.0 (1010.1)	1703.2 (1384.2)	0.666
ALT, IU/L, SD	25.4 (19.7)	24.6 (15.7)	20.6 (6.9)	0.43
Elevated ALT, %	613 (35.7%)	120 (36.1%)	4 (21.1%)	0.407
AST, IU/L, SD	23.5 (14.0)	22.9 (8.2)	20.4 (3.9)	0.472

*All variables represented as mean (standard deviation, SD) unless stated otherwise.

Abbreviations: *IRGM, immunity-related GTPase M gene;* VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; SD, standard deviation

1: P-value is from Fisher's exact test for categorical data.

2: Mood's median test is used to compare 3 medians for continuous variables.

Table 2.

Cross-sectional association between the *IRGM* rs13361189 variant (C) allele[1] and hepatic steatosis[2] or elevated ALT[3], among Framingham Heart Study participants (n=2,070)

	***IRGM* Genotype**			
Outcome	**rs13361189 TT (Ref.) n=1719**	**rs13361189 CT n=332**	**rs13361189 CC n=19**	**P-value**
Difference in mean Liver Phantom Ratio[2]				--
Model 1[*]; β' [95% CI][◊]	1	0.002 [−0.004, 0.009]	0.005 [−0.008, 0.018]	0.444
Multivariable model 2[¥◊]; β' [95% CI]	1	0.003 [−0.003, 0.009]	0.007 [−0.006, 0.019]	0.288
Hepatic Steatosis[2]				--
Model 1[*]; OR [95% CI]	1	0.93 [0.67, 1.28]	0.86 [0.45, 1.65]	0.653
Multivariable Model 2[¥]; OR [95% CI]	1	0.93 [0.68-1.28]	0.86 [0.46, 1.63]	0.653
Elevated ALT (IU/L)[3]				--
Model 1[*]; OR [95% CI]	1	0.97 [0.77, 1.22]	0.94 [0.59, 1.50]	0.796
Multivariable Model 2[¥]; OR [95% CI]	1	0.97 [0.77-1.23]	0.95 [0.59-1.51]	0.822

Abbreviations: OR, Odds Ratio; CI, confidence interval; ALT, alanine aminotransferase; *IRGM*, Immunity-Related GTPase M

[1]Variant allele assessed using an additive model.

[2]Measures of hepatic steatosis included (1) continuous liver phantom ratio, in which a lower ratio corresponds to increased hepatic fat accumulation, and (2) hepatic steatosis defined dichotomously by liver-phantom ratio ≤ 0.33.

[3]Elevated ALT level defined as ALT > upper limit of normal (ULN), which was defined as ALT > 19U/L in women and ALT >30U/L in men.

[◊]Beta coefficient representing the difference in mean liver attenuation between genotype of interest compared to the rs13361189 TT genotype (referent)

[*]Model 1: adjusted for age (years), sex, cohort (Third Generation vs. Omni), hypertension, hypertriglyceridemia, low-HDL-C, the number of *PNPLA3* rs738409 variant alleles, and eigenvectors #1-#3.

[¥]Model 2: Model 1 + smoking status (current, prior, never), alcohol intake (drinks/week), body mass index (BMI, kg/m^2) and diabetes (yes/no)