Genetic Risk for Hepatic Fat among an Ethnically Diverse Cohort of Youth: The Exploring Perinatal Outcomes among Children Study

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Objective To assess the importance of genetic and nongenetic risk factors contributing to hepatic fat accumulation in a multiethnic population of youth.

Study design We investigated the relationship between genetic factors and hepatic fat fraction (HFF) in 347 children aged 12.5-19.5 years. We examined 5 single nucleotide polymorphisms previously associated with HFF and a weighted genetic risk score (GRS) and examined how these associations varied with ethnicity (Hispanic vs non-Hispanic white) and body mass index (BMI) category. We also compared how much variation in HFF was explained by genetic factors vs cardiometabolic factors (BMI z-score and the Homeostasis Model of Insulin Resistance) or diet.

Results *PNPLA3* rs738409 and the GRS were each associated with HFF among Hispanic ($\beta = 0.39$; 95% CI, 0.16-0.62; $P = .001$; and $\beta = 0.20$; 95% CI, 0.05-0.34; $P = .007$, respectively) but not non-Hispanic white ($\beta = 0.04$; 95% CI, −0.18 to 0.26; $P = .696$; and $\beta = 0.03$; 95% CI, −0.09 to 0.14; $P = .651$, respectively) youth. Cardiometabolic risk factors explained more of the variation in HFF than genetic risk factors among non-lean Hispanic individuals (27.2% for cardiometabolic markers vs 6.4% for rs738409 and 4.3% for the GRS), and genetic risk factors were more important among lean individuals (2.7% for cardiometabolic markers vs 12.6% for rs738409 and 4.4% for the GRS).

Conclusions Poor cardiometabolic health may be more important than genetic factors when predicting HFF in overweight and obese young populations. Genetic risk is an important contributor to pediatric HFF among lean Hispanics, but further studies are necessary to elucidate the strength of the association between genetic risk and HFF in non-Hispanic white youth. *(J Pediatr 2020;220:146-53)*.

The prevalence of nonalcoholic fatty liver disease (NAFLD) has been increasing rapidly, both in adults and children, in conjunction with increases in obesity, a major risk factor for fatty liver.[1] Genetics play a role in NAFLD, and heritability estimates range from 20% to 70%, depending on study design and ethnicity.[2] Previous genome-wide association studies in adults have identified numerous single nucleotide polymorphisms (SNPs) associated with NAFLD severity, liver fibrosis, and hepatic fat fraction (HFF).[3-9] Studies of pediatric populations have confirmed that many of these SNPs are associated with measures of fatty liver in children.[10]

There are racial and ethnic differences in NAFLD prevalence, with higher prevalence in Hispanics than in non-Hispanic whites or African Americans both in adults (45%, 33%, and 24%, respectively) and in children (11.8%, 8.6%, and 1.5% respectively).[11,12] The primary genetic risk factor for NAFLD is the *PNPLA3* rs738409G allele, and its frequency is likewise highest among Hispanics, then non-Hispanic whites, then African Americans.[13] Pediatric obesity prevalence is also highest among Hispanic children, followed by African Americans and then non-Hispanic whites.[14] NAFLD is less common among normal weight individuals, but it does occur and genetics likely contribute toward its development.[15] Furthermore, epidemiologic evidence suggests that nonalcoholic steatohepatitis (NASH), fibrosis, and mortality do not differ significantly between lean and obese individuals with NAFLD.[15,16] A better understanding of similarities and differences in the genetic associations with HFF between adult and younger populations, as well as across racial/ethnic and weight groups, may offer additional pathophysiologic insight into the condition and improve opportunities for early risk profiling before disease has been fully established.

Understanding the preclinical stages of hepatic fat accumulation, rather than focusing exclusively on individuals with established NAFLD, may help to improve our ability reverse this condition in young populations. Thus, the aim of this study was to

BMI	Body mass index
EPOCH	Exploring Perinatal Outcomes among Children
FFQ	Food frequency questionnaire
GRS	Genetic risk score
HFF	Hepatic fat fraction
HOMA-IR	Homeostasis Model of Insulin Resistance
NAFLD	Nonalcoholic fatty liver disease
NAH	Nonalcoholic steatohepatitis
SNP	Single nucleotide polymorphism

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examine the association between genetic risk factors and HFF among youth, while also evaluating the role of cardiometabolic risk factors and ethnicity.

Methods

In this study, we use a multiethnic cohort of youth (median age, 16.8 years; range, 12.6-19.6 years) from the Exploring Perinatal Outcomes among Children (EPOCH) study, to examine 5 SNPs (PNPLA3 rs738409, NCAN rs2228603, GCKR rs1260326, PPP1 R3B rs4240624, and LYPLAL1 rs12137855) that have been previously linked with HFF in adults, and some of which have also been associated in pediatric populations. We also use these SNPs to build a weighted genetic risk score (GRS) for HFF as measured by magnetic resonance imaging to assess how the associations vary across race/ethnicity and explore the role of obesity and other risk factors. EPOCH is a historical prospective study of more than 600 mother/child pairs identified through the Kaiser Permanente of Colorado Perinatal database based on child exposure to gestational diabetes mellitus during singleton pregnancies. The cohort used in this analysis includes the subset of the children (n = 347) with both a measure of HFF at an in-person study visit conducted at an average age of 16.7 ± 1.2 years, as well as genetic data. The study was approved both by the Colorado Multiple Institutional Review Board and Human Participant Protection Program. All participants provided written informed consent.

Data Collection

Hepatic imaging was performed using a modification of the Dixon method by Hussain involving multi-breath-hold double gradient echo sequences.[17,18] HFF was calculated from the mean pixel signal intensity data for each flip angle acquisition. NAFLD is categorized based on HFF with 5%-33% defined as mild, 34%-66% as moderate, and greater than 66% as severe.[19] Height was measured by SECA stadiometer (SECA, Hamburg, Germany), and weight was measured using an electronic SECA scale, as described previously.[20] Age- and sex-specific body mass index (BMI) z-scores were calculated using CDC reference standards, and weight groups were defined using percentiles of BMI-for-age: underweight (defined as <5th percentile); normal weight (5th-85th percentile); overweight (85th-≤95th percentile); obese (≥95th percentile).[21,22] Blood samples were obtained at the EPOCH study visit after an overnight fast, and glucose, lipids and insulin were measured, as described previously.[20] The Homeostasis Model of Insulin Resistance (HOMA-IR), calculated as fasting glucose (mmol/L) × fasting insulin (μU/mL)/22.5, was used as a marker of insulin resistance. Maternal gestational diabetes status was physician-diagnosed using a standard 2-step screening protocol and ascertained from the Kaiser Permanente of Colorado Perinatal database, an electronic database linking the neonatal and perinatal medical record.[23] Race/ethnicity was self-reported using 2000 US census definitions and categorized as Hispanic (any race), non-Hispanic white, non-Hispanic African American, and non-Hispanic other.

Data collection also involved completion of the Block Kid's Food Questionnaire, a semi-quantitative food frequency questionnaire (FFQ) developed for children aged 8 years and older, which assesses 83 food items consumed in the last week.[24] Participants reported the frequency of consumption of small, medium, or large portions of each food or beverage ranging from 1 day to every day.

Genetic Data

Peripheral venous blood was drawn from children at the study visit and stored at −80°C. DNA was extracted using the QIAamp kit (Qiagen, Germantown, Maryland). DNA samples were quantified and purity assessed using a NanoDrop spectrophotometer and a Qubit fluorometer (Thermo Scientific, Wilmington, Delaware). Samples were genotyped in two batches. The first batch of 336 (n = 223 in this study) was performed using the Illumina Infinium Omni2.5-8 v1.1 BeadChip (Illumina, San Diego, California). The second batch of 140 (n = 124 in this study) was performed using the Illumina Multi-Ethnic Global Array v1.0. Before any other quality control procedures, variants genotyped on the Omni2.5-8 v1.1 BeadChip were filtered to retain variants that are represented at the same chromosome and position on the most up-to-date version of the Omni 2.5 array (Omni2.5-8 v1.4). Beyond this initial filtering step, the same quality control procedures were applied to both the Omni 2.5 and Multi-Ethnic Global Array datasets. Individuals with more than 5% missing genotypes and variants with more than 2% missing genotypes were excluded.

Principal components for global ancestry and possible batch genotyping effects were calculated using variants that were directly genotyped and passed quality control on both BeadChips. We selected variants with a minor allele frequency of greater than 5% and performed linkage disequilibrium pruning to retain a subset of independent variants with a maximum pairwise correlation of 0.2. All calculations were completed using PLINK 1.9 (https://www.cog-genomics.org/plink/1.9).[25]

Genotypes in each dataset were aligned to the forward strand.[26] We then used the Michigan Imputation Server (v1.0.4) to phase and impute missing genotypes in each dataset.[27] Datasets were imputed separately to maintain the intended genotyping backbone of each BeadChip.[28] Genotypes were phased using Eagle and imputation was completed using the 1000 Genomes Phase 3 (version 5) reference panel.[29,30] Imputed genotypes were modeled as dosage in association models.

SNP Selection

We identified SNPs previously associated with a continuous measure of hepatic fat in adult or pediatric populations from previously published research. We included 5 SNPs with evidence of association in non-Hispanic whites or Hispanic populations because these groups form the majority of our cohort (*PNPLA3* rs738409, *GCKR* rs1260326, *PPP1 R3B* rs4240624,

LYPLAL1 rs12137855, and *NCAN* rs2228603).[3] Among the 5 chosen SNPs, 4 had previously reported beta estimates for regressions of HFF.[31] The exception was *GCKR* rs1260326, which is in high linkage disequilibrium with rs780094 ($R^2 \geq 0.91$). We chose to include rs1260326 rather than rs780094 because it had slightly higher risk allele prevalence in EPOCH (allele frequency of 1.1 vs 1.0), as well as a slightly stronger univariate association with NAFLD status ($P = .031$ vs $P = .113$). Although both *GCKR* SNPs have been repeatedly associated with NAFLD, we were only able to find reported effect estimates for association with a continuous measure of HFF for rs780094, which we used in the weighted GRS.[32] The GRS was calculated using standard methods as the weighted sum of the number of risk alleles at each of 5 loci, weighted by the previously reported effect size for association with HFF, and scaled so that each unit increase in GRS corresponds approximately with 1 additional risk allele.[5,31,33]

Dietary Patterns

We previously created dietary patterns from the FFQ data for this cohort. We consolidated the 83 items from the FFQ into 42 food groups based on their nutritional properties. We then estimated total daily energy intake using the US Department of Agriculture Food Composition Database and adjusted each food group by total energy intake using the residuals method.[34,35] Using principal components analysis (PROC FACTOR in SAS [SAS Inc, Chicago, Illinois]), we consolidated the food groups into principal components (factors) and rotated them orthogonally to maintain noncorrelation and facilitate interpretability. PROC FACTOR extracts as many factors as there are original variables—that is, the 42 food groups were converted into 42 factors, each of which represents a unique dietary pattern parameterized as a continuous, normally distributed score that can be interpreted as the extent to which an individual's diet resembles the combination of food groups within a given factor. We considered food groups with factor loadings |0.30| or greater to be a key contributor to a dietary pattern. Of the 42 factors, we retained the first two based on standard criteria of the Scree plot and eigenvalues of greater than >1, and interpretability of the dietary patterns.[36] We refer to the patterns as prudent, characterized by high fruit and vegetable intake, and Western, characterized by high levels of fried foods and refined carbohydrates; greater detail can be found in **Table I** (available at www.jpeds.com).

Statistical Analyses

We compared cohort demographic characteristics by race/ethnicity, using one-way ANOVA for continuous variables and chi-squared or Pearson exact tests for categorical variables. We defined NAFLD as an HFF of 5% or greater.[37]

We used linear regression models with inverse-normalized HFF as a function of each genetic risk factor of interest (SNPs or the GRS), controlling for age, age-squared, sex, and the first 3 genetic principal components (as discussed elsewhere in this article) to account for potential confounding by genetic ancestry, experimental batch effects and any residual relatedness among participants. This is generally in line with the approach used in published analyses of HFF, with the exception that we chose not to control for alcohol intake because this was a pediatric cohort, and the reported alcohol intake estimated from FFQs was negligible (maximum of 0.35 g; a standard drink contains 14 g of alcohol).[5] We then checked for an interaction between non-Hispanic white race/ethnicity and each genetic risk factor in the subset of individuals who were either non-Hispanic white or Hispanic (n = 308). For any genetic risk factor with an interaction *P* of .05 or less, we ran stratified models in non-Hispanic whites and Hispanics. We considered *P* of .01 as significant according to a Bonferroni threshold to account for multiple comparisons (5 SNPs), and nominally significant *P* values are also noted. All analyses were performed using R v3.5.0.[38]

To evaluate predictors of HFF and the added predictive value of SNPs, we performed sequential regression models. The first model included the control variables from the model described elsewhere in this article: age, age-squared, and sex; then BMI z-score and HOMA-IR were added; and finally dietary patterns previously associated with HFF in this cohort (a prudent pattern characterized by high intake of fruits and vegetables, and a Western pattern characterized by high intake of fried foods and refined carbohydrates; **Table I**) were added. We then added the genetic risk factors significantly associated with HFF(rs738409 or the weighted GRS) to the final regression model. We estimated the changes in model fit using the adjusted R^2 values. In these regression models, we also considered the inclusion of exposure to diabetes in utero, maternal prepregnancy weight, physical activity measures, and acanthosis nigricans as predictor variables, but these factors did not improve model fit.[39] We imputed missing covariates for BMI z-score (n = 1) and HOMA-IR (n = 7) using the R *mice* package and age, sex, race/ethnicity, and nonmissing BMI z-score and HOMA-IR as predictors.[40] We ran these regressions stratified by ethnicity among all non-Hispanic whites and Hispanics first in the overall cohort and then in the subset of normal weight individuals.

Results

The cohort of 347 individuals was predominantly of non-Hispanic white ethnicity (**Table II**; n = 190 [54.8%]); about one-third were of Hispanic ethnicity (n = 118 [34.0%]), and the remaining individuals were African American (n = 32 [9.2%]) or other races (mostly Asian, n = 7 [2.0%]). We saw significant differences in adiposity measures by race/ethnicity; African Americans had the highest BMI z-score, obesity prevalence, and subcutaneous fat, followed by Hispanics and then non-Hispanic whites.[41] Waist circumference and visceral fat showed a slightly different pattern with nonsignificant differences by race/ethnicity, but the values were highest among Hispanics, then non-Hispanic whites, and African

Table II. Demographic characteristics and risk factors among the EPOCH population by race/ethnicity

Characteristics	Non-Hispanic white	Hispanic	African American	Other	*P* value
No. (%)	190 (54.8%)	118 (34.0%)	32 (9.2%)	7 (2.0%)	
Age (years)	16.9 (1.1)	16.5 (1.3)	16.2 (1.1)	16.8 (0.4)	.003
Male sex	92 (48.4)	65 (55.1)	15 (46.9)	3 (42.9)	.648
Risk factors					
GDM	44 (23.2)	17 (14.4)	4 (12.5)	0 (0.0)	.097
Smoker	14 (7.4)	4 (3.4)	0 (0.0)	0 (0.0)	.191
BMI z-score	0.23 (1.03)	0.50 (1.15)	0.74 (1.21)	−0.16 (0.71)	.016
BMI percentile	56.9 (29.2)	62.1 (30.4)	67.7 (30.7)	45.2 (24.8)	.096
BMI category					
Normal	149 (78.4)	79 (66.9)	18 (56.2)	7 (100.0)	.004
Overweight	25 (13.2)	14 (11.9)	4 (12.5)	0 (0.0)	
Obese	16 (8.4)	25 (21.2)	10 (31.2)	0 (0.0)	
Waist circumference	80.0 (10.7)	81.7 (15.5)	79.4 (11.7)	70.2 (5.5)	.097
Visceral fat	32.4 (18.9)	34.4 (23.5)	26.9 (16.7)	17.8 (8.0)	.071
Subcutaneous fat	176.2 (116.9)	220.0 (166.9)	248.9 (185.4)	114.1 (71.8)	.004
HOMA-IR	3.5 (2.8)	3.8 (3.5)	4.0 (3.0)	2.8 (1.5)	.649
Cholesterol	144.6 (28.3)	145.0 (26.5)	144.6 (29.4)	139.1 (28.8)	.962
HDL	45.9 (8.7)	46.6 (11.5)	46.9 (9.1)	50.7 (6.4)	.560
Dietary pattern factor scores					
Prudent	0.18 (1.10)	−0.17 (0.92)	−0.17 (0.82)	−0.13 (0.39)	.025
Western	−0.02 (1.01)	−0.01 (1.03)	−0.04 (0.88)	−0.25 (0.47)	.964
Hepatic outcomes					
NAFLD	8 (4.2)	16 (13.6)	1 (3.1)	0 (0.0)	.012
HFF	2.0 (1.4)	3.2 (4.8)	2.1 (1.2)	2.1 (1.2)	.010

GDM, gestational diabetes mellitus; *HDL*, high-density lipoprotein cholesterol.

Americans. NAFLD prevalence and HFF were highest in Hispanics. Genetic risk for NAFLD also varied with race/ethnicity, with African Americans and other races having lower GRSs than Hispanics and non-Hispanic whites. The frequency of the HFF risk alleles by race/ethnicity in EPOCH is comparable with that of prior studies (**Table III**). Almost all individuals in the cohort had at least 1 risk allele for the SNPs examined (distribution of SNPs shown in **Table IV** [available at www.jpeds.com]).

Levels of HFF differed substantially by race/ethnicity (**Figure 1**; available at www.jpeds.com). There were 5 Hispanics who had HFF values of greater than 10%, with a maximum of 38.2%; no other ethnic groups had an individual with an HFF of greater than 8.6%. We ran regression models of HFF, and the effect estimates were generally in line with prior studies, except for PPP1 R3B rs4240624, which had a much weaker association with HFF in EPOCH compared with prior studies (**Figure 2**, A). The only SNP showing a nominally significant association with HFF was PNPLA3 rs738409 ($P = .028$). We evaluated the interaction between the SNPs of interest and non-Hispanic white ethnicity among the subset of non-Hispanic white or Hispanic individuals (n = 308), and there were significant interactions for PNPLA3 rs738409 and the GRS. These genetic risk factors showed substantially stronger effects among Hispanics than non-Hispanic whites (**Figure 2**, B). PNPLA3 rs738409 had negligible effect estimates among non-Hispanic whites, whereas both rs738409 and the weighted GRS met the Bonferroni threshold of a *P* value of .01 or less for significance ($P \leq .0035$) in Hispanics.

Table III. Allele frequency in EPOCH by race/ethnicity for SNPs previously associated with HFF

		EPOCH			
Genes	SNP	Non-Hispanic white	Hispanic	African American	Other
PNPLA3	rs738409 (G)	0.23	0.41	0.11	0.21
NCAN	rs2228603 (T)	0.08	0.03	0.02	0
GCKR	rs1260326 (T)	0.42	0.41	0.2	0.35
PPP1 R3B	rs4240624 (A)	0.92	0.85	0.86	0.93
LYPLAL1	rs12137855 (C)	0.79	0.85	0.9	0.79

We ran sequential linear regression models stratified by ethnicity (**Figure 3**) to investigate the incremental proportion of variation in HFF explained by adding, in order, demographics, cardiometabolic markers, dietary information, and genetic predictors (PNPLA3 rs738409 or the weighted GRS). The adjusted R^2 values (amount of variation explained in HFF) for the baseline demographic models improved substantially with the addition of cardiometabolic markers (increases of 27.2% for Hispanics and 7.0% for non-Hispanic whites), and showed subsequent improvement with the further addition of dietary information only in non-Hispanic whites (change of −1.0% for Hispanics and 5.2% for non-Hispanic whites). The inclusion of genetic risk factors only improved the model among Hispanics (increase of 6.4% for rs738409; 4.3% for the GRS); among non-Hispanic whites, the adjusted R^2 values decreased with the addition of genetic information.

We were particularly interested in elevated HFF among normal weight individuals, 5 of whom met the diagnostic criteria for NAFLD. All 5 of these individuals had at least 1 risk allele in PNPLA3 rs738409. We ran the same sequential linear regressions of HFF among the normal weight individuals in the cohort (n = 253), and the baseline models had very poor R^2 values that showed little or no improvement with the

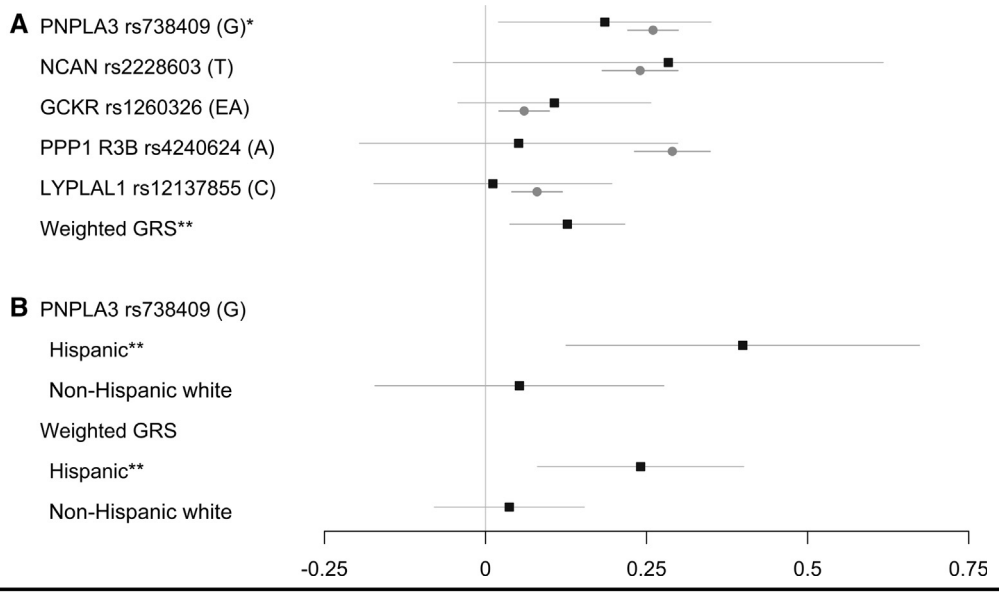

Figure 2. Forest plot of estimates and 95% CIs from linear regressions of HFF as a function of SNPs previously associated with HFF in adults (prior reported estimates shown with grey circles for comparison) and a weighted GRS composed of these SNPs, **A**.[34] PNPLA3 rs738409 and the GRS showed a significant interaction with ethnicity; effect estimates stratified by Hispanic/non-Hispanic white ethnicity are shown in **B**. (Estimates for African Americans and other races not estimated owing to small sample size.) Both rs738409 and the GRS were significantly associated with HFF among Hispanics but not non-Hispanic whites in EPOCH. Control variables include age, age squared, sex, and the first 3 principal components for models not stratified by ethnicity. *Nominally significant *P* values (≤.05); ***P* values meeting the Bonferroni threshold for significance (≤.01).

inclusion of cardiometabolic markers or diet (**Figure 3**). The addition of genetic information showed the same pattern as in the overall cohort by ethnicity: it greatly improved the R^2 of models among Hispanics, even more so than in the overall cohort with increases of 12.6% and 4.4% for rs738409 and the GRS, respectively. Adjusted R^2 values decreased among non-Hispanic whites with the addition of genetic information. Although BMI z-scores were a statistically significant predictor of HFF in lean Hispanics, HOMA-IR was not. Neither genetic risk factors nor cardiometabolic markers were significantly associated with HFF in lean non-Hispanic whites.

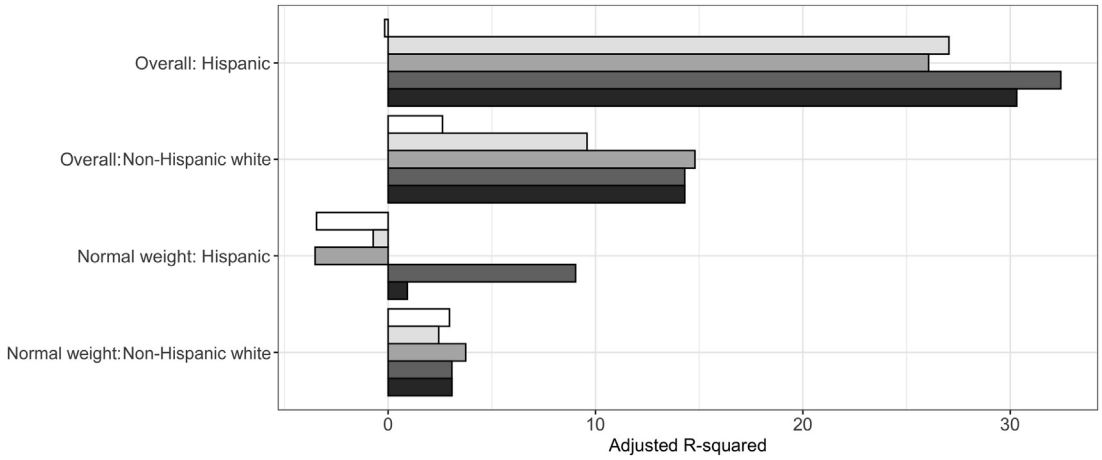

Figure 3. Plots of the adjusted R^2 values from sequential linear regressions of HFF among Hispanics and non-Hispanic whites in EPOCH, stratified by ethnicity, in the overall cohort and then among normal weight individuals. Predictors included (shown in shades from white to black) (1) demographics (age, age squared, and sex), (2) demographics and cardiometabolic markers (BMI z-score and HOMA-IR), (3) demographics, cardiometabolic markers, and diet, (4) demographics, cardiometabolic markers, diet, and PNPLA3 rs738409, and (5) demographics, cardiometabolic markers, diet, and weighted GRS. Cardiometabolic markers explain substantially more variation in HFF than other risk factors in the overall cohort. Genetic risk (particularly rs738409) only improves the models among Hispanic individuals, and particularly among normal weight Hispanics.

Discussion

In this multiethnic cohort of youth, we found that the SNP PNPLA3 rs738409 and a weighted GRS including 5 SNPs were significantly associated with HFF among Hispanic but not non-Hispanic white individuals. Cardiometabolic risk factors explained substantially more variation in HFF than genetic risk factors in both Hispanics and non-Hispanic whites. Many of the prior pediatric studies of hepatic fat have focused exclusively on obese individuals.[10] However, NAFLD affects normal weight individuals as well, in whom the condition is less likely to be diagnosed and may have clinically distinct features.[42] Our results suggest that genetic risk, particularly the rs738409G allele, may be a particularly important risk factor for high HFF among normal weight individuals.

There are differences in NAFLD prevalence by race/ethnicity, as well as in the environmental and genetic risk factors for NAFLD.[1,13,16] Hispanic ethnicity is not precisely defined and encompasses individuals of diverse cultures with an admixture of genetic ancestry of Native American, European, and African Ancestry, with the proportion of each varying regionally.[43] Studies have also shown that there are regional differences in the prevalence of suspected NAFLD among Hispanics.[44,45] One study found that the metabolic syndrome was more strongly associated with HFF in Mexican Americans and Non-Hispanic blacks than in non-Hispanic whites.[46] Although we did not examine the metabolic syndrome, which is not straightforward to define in adolescents, we likewise saw that HOMA-IR was significantly associated with HFF in Hispanics but not non-Hispanic whites, though BMI z-score was associated with HFF in both ethnic groups.[47] In contrast, another study found that HOMA-IR was a risk factor for NASH among non-Hispanic white but not Hispanic adults with biopsy-proven NAFLD.[48]

In this study, we examined 5 SNPs, and only 1 of them, PPP1 R3B rs4240624, showed a notably lower effect estimate relative to a prior study of HFF in adults.[31] The relationship between this SNP and HFF and liver damage is controversial.[31,49,50] It was initially associated with HFF from computed tomography scans, but not with histologic NAFLD or NASH, and in recent work, it was associated with protection against HFF and fibrosis in individuals at high risk of NAFLD.[50] It is possible that this SNP has different effects in different subpopulations, masking the association in this cohort, or that it may promote hepatic fat accumulation at older ages than represented in this cohort.[49]

Early-onset NAFLD may differ from NAFLD in adults in that it is characterized by different patterns of steatosis, inflammation, and cellular injury, and it may be more prone to progress toward more serious conditions, such as NASH, which is among the leading indications for liver transplants.[51,52] One study found that almost one-half of children who are diagnosed with NAFLD have NASH at the time of diagnosis.[51] This finding could reflect the higher threshold needed for physicians to suspect NAFLD in youth, or it may reflect differences in disease presentation and progression in younger populations. Profiling genetic risk may help to target early prevention efforts for NAFLD. However, it is important to understand whether genetic risk substantially improves our ability to identify children with high levels of HFF. Our results suggest that genetic risk factors may show a stronger association with HFF among Hispanic than non-Hispanic white youth. Cardiometabolic risk factors that are more routinely captured in routine clinical care, including BMI z-scores and HOMA-IR, show a stronger association with HFF in this cohort. However, genetic risk factors may be an important tool to identify risk for elevated HFF among normal weight youth.

There are major gaps in our understanding of NAFLD among lean individuals, including the contribution of genetic and other predisposing risk factors.[53] NAFLD in lean individuals is suspected to occur in metabolically unhealthy lean individuals.[42] Although this may be the case, HOMA-IR was not significantly associated with HFF among normal weight individuals in our cohort. We also found that, in lean Hispanics, albeit a small sample, genetic risk factors explained a greater proportion of variation in HFF than cardiometabolic markers. Although it is possible that there are different genetic risk factors unique to NAFLD in lean individuals, we confirmed that in lean Hispanic youth, the primary established genetic risk factor for NAFLD, rs738409, was strongly associated with HFF.[53]

This study has limitations. The cohort is relatively small, limiting its power, particularly for less common SNPs. The Hispanics in this cohort had much higher prevalence of NAFLD than other racial/ethnic groups and much greater variation in HFF, which may have limited our relative ability to detect associations with HFF in non-Hispanic whites. The weighted GRS was based on regression estimates from the Genetics of Obesity-related Liver Disease (GOLD) study, which includes individuals of European descent, and we applied this GRS to all of the individuals in this multiethnic cohort.[31] Although factors like epigenetics and socioeconomic status may also contribute toward hepatic fat accumulation, we were unable to explore these influences because this information was not available for most of the cohort.[16] A major strength of this study is that HFF was measured by MRI, which is the most precise noninvasive method to capture HFF.[54] Furthermore, this study included children across the spectrum of weight classes, in contrast with many of the prior studies of genetics in pediatric NAFLD, which focused exclusively on obese children.[10]

Although there is a substantial body of literature to support that genes play a role in NAFLD, our results suggest that poor cardiometabolic health may be more important to consider when predicting HFF in young populations.[3] In this multiethnic cohort of youth, genetic risk factors for HFF had the highest prevalence among those with Hispanic ethnicity, and these genetic risk factors were only associated with HFF among Hispanics. PNPLA3 rs738409 was not associated with HFF among non-Hispanic whites, despite a frequency of 0.23 for the risk allele. Further studies are necessary to elucidate whether genetic risk is an important predictor of HFF among young populations of non-

Hispanic whites who have more severe levels of HFF, and whether genetic factors are particularly useful for HFF risk profiling among lean individuals. ■

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Data Statement

Data sharing statement available at www.jpeds.com.

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50 Years Ago in *The Journal of Pediatrics*

Perception of the Severity of the Effects of the Bite of a Venomous Snake

Sadan M, Soroker B. Observations on the effects of the bite of a venomous snake on children and adults. J Pediatr 1970;76:711-5.

In the early 1970s, it was believed that the effects of snakebite from a venomous species were more severe in children than in adults. This was due to the assumption that children would receive a proportionately larger amount of venom when bitten than the adult. During the past decades, several countries have reported their statistics over the severity and mortality from snakebites. During multiple retrospective studies, the medical community began to notice that the severity of illness and the mortality induced from venomous snakebite were greater in the adult population, noticing that both populations had minimal venenation, with severe reactions and deaths involving mostly adults.

The classification for the grade of venenation may vary, but it differentiates the bites from mild, moderate, and severe cases. The early classification and identification of symptoms has remained until this day the most important outpatient and inpatient basis to determine the morbidity and mortality of the patients. Sadan and Soroker show that most patients receive medical attention during the first stage of the snakebite, granting discharge of the patient at early stages, and avoiding complications or progression of the venenation. It is also evident that most patients who have fatal outcomes presented a delay in medical attention (delayed arrival at hospital, attention in an under qualified unit, etc).[1]

It has been established that the species of venomous snakes vary from region and climate. Most venomous species are identified and most common bites have specific antidotes. This is extremely important because most mortal victims had a delay in medical attention. Most fatal victims presented with coagulopathies such as bleeding from bite site, hematuria, and melena during the first 24 hours, eventually progressing to shock and or acute renal failure.[2]

The understanding of the effects of snakebites have evolved during this past 50 years, allowing the medical community to develop, improve, and offer adequate medical attention. It must remain a priority in endemic regions, and we have to avoid serious complications.

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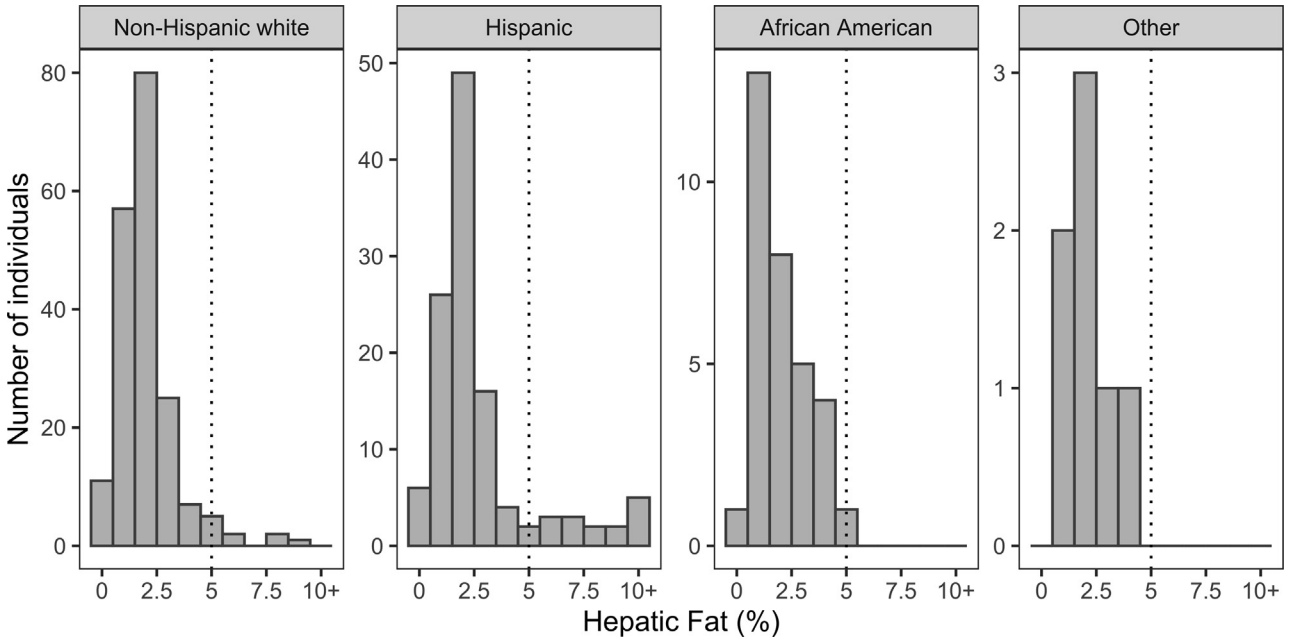

Figure 1. HFF distribution by race/ethnicity. A common cutoff for the diagnosis of NAFLD is 5% HFF, which is indicated by the *dotted line*. Hispanics showed much greater variation in HFF than other racial/ethnic groups in EPOCH, and they had the highest prevalence of NAFLD.

Table I. Composition of the 2 dietary patterns at age 12-19 years associated with HFF among youth in EPOCH

Food groups	Variance	Factor loading
Factor 1 (prudent)	8.80%	
Leafy greens		0.68
Vegetables		0.68
Fruit		0.58
Cruciferous vegetables		0.46
Nuts and seeds		0.46
Yogurt		0.44
Stir-fried vegetables		0.4
Salad dressing		0.38
Sugar-sweetened beverages		−0.35
Fast food		−0.35
Beef		−0.36
Factor 2 (Western)	5.80%	
Fried potatoes		0.58
Ketchup		0.52
Beef		0.44
Fast food		0.42
Salad dressing		0.41
Fried packaged snacks		0.36
Cereal		−0.48

Table IV. Distribution of HFF risk SNPs among the EPOCH population by race/ethnicity

No. of risk SNPs	Combinations of risk SNPs	All	African American	Hispanic	Non-Hispanic white	Other
1	rs4240624 (A)	1	0	1	0	0
2		78	17	15	45	1
2	rs4240624 (A), rs12137855 (C)	69	17	14	38	0
2	rs1260326 (T), rs4240624 (A)	6	0	1	5	0
2	rs738409 (G), rs4240624 (A)	3	0	0	2	1
3		147	11	54	76	6
3	rs1260326 (T), rs4240624 (A), rs12137855 (C)	90	8	26	51	5
3	rs738409 (G), rs4240624 (A), rs12137855 (C)	44	3	25	15	1
3	rs2228603 (T), rs4240624 (A), rs12137855 (C)	6	0	0	6	0
3	rs738409 (G), rs1260326 (T), rs4240624 (A)	3	0	2	1	0
3	rs738409 (G), rs1260326 (T), rs12137855 (C)	1	0	1	0	0
3	rs738409 (G), rs2228603 (T), rs12137855 (C)	1	0	0	1	0
3	rs2228603 (T), rs1260326 (T), rs4240624 (A)	1	0	0	1	0
3	rs738409 (G), rs2228603 (T), rs4240624 (A)	1	0	0	1	0
4		110	3	45	62	0
4	rs738409 (G), rs1260326 (T), rs4240624 (A), rs12137855 (C)	92	3	41	48	0
4	rs2228603 (T), rs1260326 (T), rs4240624 (A), rs12137855 (C)	12	0	1	11	0
4	rs738409 (G), rs2228603 (T), rs4240624 (A), rs12137855 (C)	6	0	3	3	0
5	rs738409 (G), rs2228603 (T), rs1260326 (T), rs4240624 (A), rs12137855 (C)	11	1	3	7	0

The table shows the number of individuals having at least 1 risk allele for each of the SNPs examined, and the distribution of the combination of SNPs.