HEPATOLOGY

HEPATOLOGY, VOL. 68, NO. 4, 2018

Metabolic Features of Nonalcoholic Fatty Liver (NAFL) in Obese Adolescents: Findings From a Multiethnic Cohort

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We conducted a prospective study in a large, multiethnic cohort of obese adolescents to characterize clinical and genetic features associated with pediatric nonalcoholic fatty liver (NAFL), the most common cause of chronic liver disease in youth. A total of 503 obese adolescents were enrolled, including 191 (38.0%) whites, 134 (26.6%) blacks, and 178 (35.4%) Hispanics. Participants underwent abdominal magnetic resonance imaging (MRI) to quantify hepatic fat fraction (HFF), an oral glucose tolerance test (OGTT) to assess glucose tolerance and insulin sensitivity, and the genotyping of three single-nucleotide polymorphisms (SNPs) associated with nonalcoholic fatty liver disease (NAFLD) (patatin-like phospholipase domain-containing protein 3 [*PNPLA3*] rs738409, glucokinase regulatory protein [*GCKR*] rs1260326, and transmembrane 6 superfamily member 2 [*TM6SF2*] rs58542926). Assessments were repeated in 133 subjects after a 2-year follow-up. Prevalence of nonalcoholic fatty liver (NAFL) was 41.6% (209 patients) and ranged widely among ethnicities, being 42.9% in whites, 15.7% in blacks, and 59.6% in Hispanics ($P < 0.0001$). Among adolescents with NAFL, blacks showed the highest prevalence of altered glucose homeostasis (66%; $P = 0.0003$). Risk factors for NAFL incidence were white or Hispanic ethnicity ($P = 0.021$), high fasting C-peptide levels ($P = 0.0006$), and weight gain ($P = 0.0006$), whereas baseline HFF ($P = 0.004$) and weight loss ($P = 0.032$) predicted resolution of NAFL at follow-up. Adding either gene variant to these variables improved significantly the model predictive performance. *Conclusion:* Black obese adolescents are relatively protected from liver steatosis, but are more susceptible to the deleterious effects of NAFL on glucose metabolism. The combination of ethnicity/race with markers of insulin resistance and genetic factors might help identify obese youth at risk for developing NAFL. (Hepatology 2018;68:1376-1390).

Paralleling the growing epidemic of childhood obesity, nonalcoholic fatty liver disease (NAFLD) has become the most common cause of chronic liver disease in pediatrics, with an estimate of 7 million children and adolescents affected in the United States.[1] NAFLD is characterized by excess fat accumulation in hepatocytes and encompasses a wide spectrum of disease severity, from simple nonalcoholic

Abbreviations: 2PD, 2-point Dixon; ALT, alanine aminotransferase; AST, aspartate transaminase; AUC, area under the curve; BMI, body mass index; BP, blood pressure; DBP, diastolic blood pressure; DI, disposition index; DNL, de novo lipogenesis; GCKR, glucokinase regulatory protein; GGT, γ-glutamyl transferase; GLM, general linear model; HbA1c, hemoglobin A1C; HDL, high-density lipoprotein; HFF, hepatic fat fraction; IGI, insulinogenic index; IQR, interquartile range; IR, insulin resistance; LDL, low-density lipoprotein; MRI, magnetic resonance imaging; NAFL, nonalcoholic fatty liver; NAFLD, nonalcoholic fatty liver disease; NAS, NAFLD activity score; OGTT, oral glucose tolerance test; PNPLA3, patatin-like phospholipase domain-containing protein 3; ROC, receiver operating characteristic; SBP, systolic blood pressure; SNPs, single-nucleotide polymorphisms; T2D, type 2 diabetes; TG, triglycerides; TM6SF2, transmembrane 6 superfamily member 2; WBISI, whole-body insulin sensitivity index.

Received January 17, 2018; accepted April 6, 2018.

Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep.30035/suppinfo.

Supported by American Heart Association (16IRG27390002, 13SDG14640038 and 11CRP5620013), Allen Foundation (DOI: 10.13039/100008796), National Institute of Diabetes and Digestive and Kidney Diseases (R01-HD-40787, R01-HD-28016, R01DK111038-01), R01DK114504, DK045735, and K24-HD01464), and National Center for Advancing Translational Sciences (UL1-RR-024139).

Trial Registration: NCT01966627.

View this article online at wileyonlinelibrary.com.

DOI 10.1002/hep.30035

Potential conflict of interest: Nothing to report.

fatty liver (NAFL) to nonalcoholic steatohepatitis (NASH),[2,3] which can progress to cirrhosis and end-stage liver disease (ESLD) even at a young age.[4-6] A landmark study by Feldstein et al.[4] has shown that adolescents with NAFLD have approximately 16 times higher risk of developing ESLD by the age of 20 compared to a group of American youth with similar age and sex. Moreover, studies assessing insulin resistance (IR) with state-of-the-art techniques (e.g., euglycemic clamp) have demonstrated that NAFL is a major risk factor for IR and type 2 diabetes (T2D), independent from visceral and intramyocellular lipid accumulation.[7]

Ethnic differences in the prevalence of NAFLD have been reported in adults[8,9] and children,[1,10] with Hispanics showing the highest prevalence and blacks showing a relative protection from hepatic fat accumulation, even in the presence of morbid obesity and severe IR.[11] So far, no data in pediatrics are available about putative differences among the three major ethnic groups in the United States in the metabolic profile associated with NAFL and about the predictors of changes in hepatic fat fraction (HFF) over time.[6]

To gain a deeper knowledge about pediatric NAFL and identify risk factors of changes in magnetic resonance imaging (MRI)-measured intrahepatic fat, we analyzed metabolic and imaging data obtained from a multiethnic cohort of 503 overweight and obese adolescents enrolled in an ongoing study on the pathogenesis of youth-onset NAFL. Subjects enrolled in the study were carefully phenotyped with respect to quantification of HFF and abdominal fat distribution using MRI. Glucose tolerance and markers of IR were derived from the oral glucose tolerance test (OGTT). Genotyping of patatin-like phospholipase domain-containing protein 3 (*PNPLA3*) rs738409, glucokinase regulatory protein (*GCKR*) rs1260326, and transmembrane 6 superfamily member 2 (*TM6SF2*) rs58542926 single-nucleotide polymorphisms (SNPs) previously associated with NAFL was also performed.[12-14] Moreover, after a median follow-up of 2 years, the abdominal MRI and all metabolic assessments were repeated in a subset of 133 obese adolescents.

Patients and Methods

THE YALE PEDIATRIC NAFLD COHORT

A total of 503 obese and overweight adolescents were recruited from the Yale Pediatric Obesity Clinic, including 191 (38.0%) whites, 134 (26.6%) blacks, and 178 (35.4%) Hispanics (Fig. 1). Ethnic distribution was comparable to the prevalence of the different races and ethnicities in the New Haven area.[15] A detailed medical and family history was obtained from all participants, and a physical examination was performed. Blood pressure (BP) was measured three times, and the last two measurements were averaged for analysis. Tanner stage was determined by a pediatrician and was based on breast stage and pubic hair development in girls[16] and genitalia development in boys.[17] Clinical and metabolic characteristics of the study cohort are shown in Supporting Table S1. Participants were 206 boys (41.0%) and 297 girls (59.0%), with an average age of 13.7 ± 2.8 years

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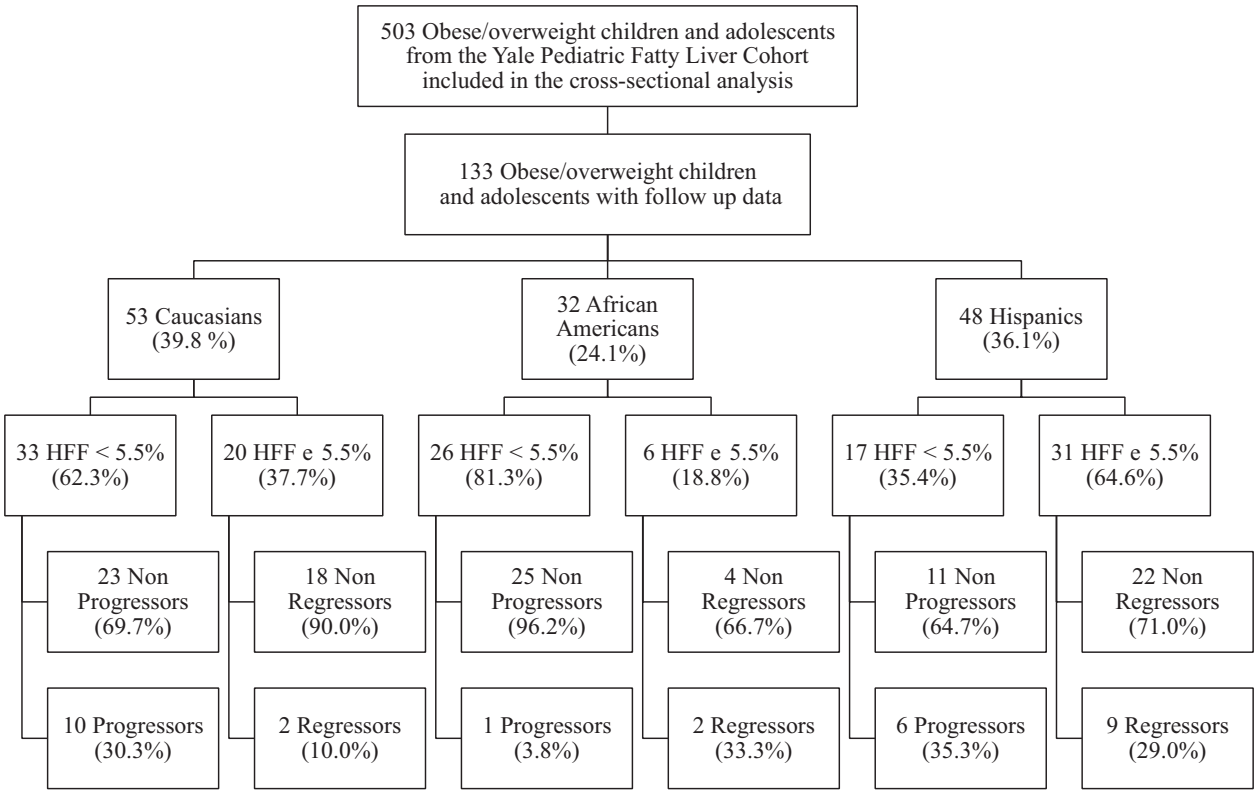

FIG. 1. Study flowchart. Study participants are stratified according to ethnicity, presence of NAFL at presentation (HFF >5.5%), and stability or progression/regression of NAFL at follow-up.

(median IQR [interquartile range], 13.7 [11.7-15.7]; age range, 8.2-18.9); Tanner stage 3.6 ± 1.4; *z*-score body mass index (BMI) 2.2 ± 0.4; systolic (SBP) and diastolic BP (DBP) 118.2 ± 11.1 and 67.8 ± 8.0 mm Hg, respectively; high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol 43.5 ± 10.2 and 89.8 ± 25.4 mg/dL, respectively; and fasting triglycerides (TG) 104.5 ± 62.7 mg/dL. Exclusion criteria were known hepatic diseases (except for NAFLD), alcohol consumption, and the use of medications that alter BP or glucose, lipid, or amino acid metabolism. At baseline, 24 boys and 14 girls (7.6% of study cohort) had alanine aminotransferase (ALT) levels 3 times higher than the normal upper limit (25 UI/L for boys and 22 UI/L for girls[18]. Subjects with persistent elevation of ALT levels for more than 6 months underwent appropriate blood tests to exclude autoimmune hepatitis, Wilson's disease, alpha-1-antitrypsin deficiency, hepatitis B and C, and iron overload.

Subjects were phenotyped with respect to their glucose tolerance by a standard OGTT (Supporting Methods) and to HFF and abdominal fat distribution by using fast MRI.[19-21] From OGTT data, insulin sensitivity and early-phase insulin secretion were assessed by the whole-body insulin sensitivity index (WBISI) and the insulinogenic index (IGI), respectively. The disposition index (DI) was calculated as the product of the WBISI and the IGI (Supporting Methods). Glucose tolerance was defined according to the American Diabetes Association criteria.[22] NAFL was defined as liver fat content (hepatic fat fraction [HFF]%) >5.5%.[8]

After the first visit, all participants received standard nutritional guidance as well as recommendations for physical activity and were scheduled to be followed up every 4-6 months. After a median follow-up of 2 years, metabolic and imaging studies were repeated in all available participants whose parents consented to repeat those assessments (133 subjects). Baseline demographic and anthropometric characteristics of participants with follow-up data were comparable to that of the original cohort (Supporting Table S1).

During the follow-up period, 12 subjects started new medications, including metformin (n = 7), omega-3-acid ethyl esters (n = 3), and angiotensin-converting enzyme inhibitors (n = 2). Metformin was suspended at least 7 days before metabolic evaluations.

The study was approved by the Yale University Human Investigation Committee. Written parental informed consent and written child assent were obtained from all participants before enrollment.

FAST MRI: ASSESSMENT OF LIVER FAT CONTENT AND ABDOMINAL FAT DISTRIBUTION

Abdominal and liver MRI studies were performed on a Siemens Sonata 1.5 Tesla system (Erlangen, Germany), as previously reported,[23-26] using an advanced magnitude-based liver fat quantification MRI technique, the 2-point Dixon (2PD) as modified by Fishbein et al.[27] Briefly, this method is based on phase-shift imaging where HFF is calculated from the signal difference between the vectors resulting from in-phase and out-of-phase signals. Five regions of interest were drawn on each image, and the mean pixel signal intensity level was recorded.[27] We validated the modified 2PD method against ^{1}H-NMR (proton nuclear magnetic resonance) in 34 lean and obese adolescents and found a very strong correlation between the two methods (r = 0.954; $P < 0.0001$).[23] To assess its repeatability, measurements were obtained within the same day on 12 subjects. The within-subject SD for HFF was 1.9%. This degree of reproducibility is well within the boundaries of that necessary to make this a viable method to assess the relation between HFF and metabolic outcomes. Kim et al.[25] demonstrated that a 2PD HFF cutoff of 3.6%, provided good sensitivity (80%) and specificity (87%) compared to an ^{1}H-NMR reference. In addition, we further validated the modified 2PD method against liver biopsy—the gold standard for diagnosing NAFLD—in 15 obese adolescents. We found a very strong correlation between percent of liver fat measured by the two methods (r = 0.836; $P = 0.0001$; Supporting Fig. S1). Fast MRI has also been found to be able to track longitudinal changes in liver fat content in obese adolescents with NAFL.[24]

Abdominal MRI was also used to quantify visceral and subcutaneous fat depots, as reported.[26] The pulse sequence was a T1-weighted fast low angle shot gradient echo. Slices were acquired using a 400-cm field of view (echo time 4.76, repetition time 100, 4 excitations, 90-degree flip angle, matrix 256 × 128, and bandwidth 140). Visceral and subcutaneous abdominal fat distribution was determined on a single slice obtained at the level of the L4/L5 disc space, using thresholding to discriminate fat from soft tissue.[26]

LIVER BIOPSY

Liver biopsy was performed in 15 subjects (Supporting Tables S1 and S2) with persistent elevation of ALT (156.2 ± 94.5 U/L; 95% confidence interval [CI], 101.7-210.8). Biopsies were formalin-fixed, paraffin-embedded and stained with hematoxylin and eosin, trichrome, and Gordon's reticulin techniques. All biopsies were 2 cm or more in length and were reviewed by a pathologist, who established the diagnosis of steatohepatitis.[28,29] Steatosis was assessed as the percentage of hepatocytes involved within a lobule (0%-100%, steatosis score). Staging and grading were performed according to Brunt et al.[28,29] The NAFLD activity score (NAS) was calculated according to Kleiner et al.[30] The score is defined as the sum of the scores for steatosis (0, <5%; 1, 5%-33%; 2, 33%-66%; 3, >66%), lobular inflammation (0, none; 1, <2 foci/×200 magnification field; 2, 2-4 foci/×200 magnification field; 3, >4 foci/×200 magnification field), and ballooning (0, none; 1, few; 2, many).

GENOTYPING

To test whether genetic variants previously associated with pediatric NAFLD might predict changes in HFF% over time, the *PNPLA3* rs738409, *GCKR* rs1260326, and *TM6SF2* rs58542926 variants were genotyped as previously reported.[12-14] Genomic DNA was extracted from peripheral blood leukocytes in 474 subjects, including 111 individuals who underwent a follow-up MRI (44 whites, 29 blacks, 38 Hispanics; 65 [58.6%] with HFF ≤5.5% and 46 [41.4%] with HFF >5.5%).

BIOCHEMICAL ANALYSES

Plasma glucose was determined using a glucose analyzer by the glucose oxidase method (Beckman Instruments, Brea, CA). Plasma insulin was measured by the Linco radioimmunoassay (St. Charles, MO).

Lipid levels were determined with an Auto-Analyzer (model 747-200, Roche Diagnostics, Indianapolis, IN). Liver enzymes were measured using standard automated kinetic enzymatic assays.

STATISTICAL ANALYSES

Categorical data are presented as counts and/or percentages and were analyzed with the chi-square test. Continuous variables are presented as means ± SDs. Distribution of continuous variables was examined for skewness using the Kolmogorov-Smirnov test, and non-normally distributed variables were log-transformed before data analysis to approximate univariate normality, except for HFF% for which a square root transformation was used. A general linear model (GLM) was used to test differences among groups. Post-hoc comparisons were performed by Tukey's honest significant difference (HSD) tests. A GLM was used also to evaluate the association between changes in HFF% over time and changes in clinical and metabolic variables, and age, sex, *z*-score BMI, and follow-up duration were used as covariates. Correlations between variables were tested using Spearman's rank correlations. To identify potential factors associated with changes in NAFL phenotype (progression or resolution) in adolescents without and with NAFL at baseline, a multivariable logistic regression was used. Variables included in the models were age, sex, ethnicity, *z*-score BMI, changes in *z*-score BMI, use of medications, and follow-up duration, in addition to the variables associated with progression or regression of NAFL in univariate analyses (namely fasting glucose and C-peptide for progression and baseline HFF% and subcutaneous fat for regression). The median number of visits during the follow-up time of 2 years was 1 (range, 0-4), and it was not different among groups at follow-up (all $P > 0.10$). During the follow-up, 12 subjects received new medications that could potentially influence the results. Hence, multivariable logistic analysis was first repeated by adding "medications" as a covariate, which did not show any significant effect in either the progression ($\beta = 0.848$; $P = 0.154$) or the regression ($\beta = 0.189$; $P = 0.698$) model. Then, a multivariable logistic analysis was repeated only in the group of subjects who did not receive any medications over time. Exclusion of individuals taking medications during the follow-up did not affect the results. Performance of different models was assessed by the area under the receiver operating characteristic (ROC) curve. A GLM was used to assess the association between the studied SNPs and the HFF%. Because genotype was available for 94% of the cross-sectional cohort and 83% of subjects from the initial longitudinal cohort, subjects who had not been genotyped were excluded from the genetic analysis. The subgroup excluded from the analysis did not differ from the tested cohort with respect to age, sex, and *z*-score BMI (all $P > 0.10$). Statistical tests were performed using SAS 9.4 and JMP Pro 11.2.0 (SAS Institute Inc., Cary, NC) using a two-sided alpha level of 0.05.

Results

METABOLIC PHENOTYPES OF OBESE ADOLESCENTS WITH NAFL ACCORDING TO ETHNICITY

Overall prevalence of NAFL was 41.6% (209 patients) and varied significantly by ethnicity, being 42.9% in whites, 15.7% in blacks, and 59.6% in Hispanics ($P < 0.0001$; Table 1). Subjects with NAFL were more likely to be boys (53.6%) than girls (46.4%; $P < 0.0001$; Table 1). In all three ethnic groups, subjects with NAFL had higher *z*-score BMI, fasting insulin, fasting C-peptide, 2-hour glucose, total cholesterol, TG, and visceral fat, and lower WBISI and DI, than those without NAFL (Table 1). Notably, although there was no difference in the overall HFF% (Fig. 2A) and visceral fat content (Fig. 2B) among the three ethnic groups with NAFL, stark differences emerged in their metabolic profiles, given that blacks with NAFL displayed a metabolic profile consistent with profound alterations in insulin (Fig. 2C) and glucose homeostasis (Fig. 2D). Compared to both whites and Hispanics with NAFL, black adolescents with liver steatosis (LS) had higher fasting glucose ($P = 0.020$ and $P = 0.028$, respectively), fasting insulin ($P = 0.0004$ and $P < 0.0001$), fasting C-peptide ($P = 0.038$ and $P = 0.002$), 2-hour glucose ($P = 0.043$ and $P = 0.003$), hemoglobin A1C (HbA1c; $P < 0.0001$ for both), IGI ($P = 0.0008$ and $P = 0.007$), and SBP ($P = 0.005$ and $P = 0.008$), as well as lower WBISI ($P = 0.031$ and $P = 0.001$), despite similar age ($P = 0.342$), sex ($P = 0.373$), and Tanner stage ($P = 0.575$) distribution (Table 1) and, more important, a similar amount of visceral fat depot ($P = 0.218$;

TABLE 1. Baseline characteristics of the study population stratified by ethnicity and by absence (HFF≤5.5%) or presence (HFF>5.5%) of NAFL

	Whites (*n* = 191; 38.0%)			Blacks (*n* = 134; 26.6%)			Hispanics (*n* = 178; 35.4%)		
	HFF ≤ 5.5% (*n* = 109)	HFF > 5.5% (*n* = 82)	*P*	HFF ≤ 5.5% (*n* = 113)	HFF > 5.5% (*n* = 21)	*P*	HFF ≤ 5.5% (*n* = 72)	HFF > 5.5% (*n* = 106)	*P*
Clinical features									
Age (years)	14.2 ± 2.7	13.2 ± 2.8	*0.154*	14.0 ± 3.1	14.1 ± 2.6	*0.529*	13.5 ± 2.6	13.1 ± 2.8	*0.711*
Sex (M/F) [%]	36/73 [33.0/67.0]	39/43 [47.6/52.4]	*0.208*	34/79 [30.1/69.9]	12/9 [57.1/42.9]	***0.049***	24/48 [33.3/66.7]	61/45 [57.5/42.5]	***0.003***
Body mass index *z*-score	2.09 ± 0.41	2.34 ± 0.34	***0.0002***	2.24 ± 0.42	2.52 ± 0.39	***0.011***	2.11 ± 0.43	2.26 ± 0.37	***0.036***
Body mass index (kg/m^2)	32.5 ± 6.5	33.9 ± 5.5	*0.108*	34.4 ± 6.6	38.5 ± 6.0	***0.009***	31.8 ± 6.3	32.7 ± 6.1	*0.341*
Body fat mass (%)	41.2 ± 7.4	45.4 ± 8.7	***0.001***	44.1 ± 7.5	48.4 ± 8.9	***0.046***	41.4 ± 7.2	43.9 ± 8.0	***0.033***
Tanner stage (1/2/3/4/5) [%]	[5.5/11.0/18.4/ 21.1/44.0]	[14.6/17.1/18.3/ 19.5/30.5]	*0.616*	[7.1/15.1/16.8/ 15.9/45.1]	[9.5/14.3/9.5/ 19.1/45.6]	*0.781*	[12.5/11.1/16.7/ 19.4/40.3]	[10.4/24.5/20.8/ 18.9 /25.4]	*0.127*
Systolic blood pressure (mmHg)	118.3 ± 11.6	117.5 ± 11.7	*0.173*	118.6 ± 9.9	126.2 ± 10.6	***0.010***	115.7 ± 11.3	118.3 ± 10.8	*0.345*
Diastolic blood pressure (mmHg)	68.0 ± 7.9	68.0 ± 8.8	*0.967*	67.6 ± 8.3	70.1 ± 6.9	*0.224*	66.7 ± 6.6	67.8 ± 8.1	*0.100*
Glucose metabolism									
Fasting glucose (mg/dl)	90.2 ± 7.4	91.9 ± 7.8	*0.347*	90.0 ± 8.7	98.1 ± 16.5	***0.022***	91.4 ± 7.3	92.3 ± 8.5	*0.969*
Fasting insulin (μU/ml)	27.5 ± 16.5	39.8 ± 16.6	***<0.0001***	30.8 ± 16.8	60.1 ± 30.4	***<0.0001***	32.3 ± 31.2	37.9 ± 22.5	*0.211*
Fasting C peptide (pmol/l)	1071.9 ± 430.1	1409.4 ± 69.4	***<0.0001***	971.4 ± 348.0	1747.3 ± 602.7	***<0.0001***	1171.9 ± 502.9	1274.0 ± 551.4	*0.209*
2 h glucose (mg/dl)	121.3 ± 31.3	133.0 ± 38.0	***0.022***	116.3 ± 28.5	152.9 ± 41.7	***<0.0001***	123.1 ± 25.0	126.2 ± 27.7	*0.938*
Hemoglobin A_{1c} (%)	5.39 ± 0.30	5.46 ± 0.30	*0.444*	5.56 ± 0.30	6.00 ± 0.64	***0.0001***	5.46 ± 0.27	5.56 ± 0.35	*0.109*
Whole Body Insulin Sensitivity Index (WBISI)	2.30 ± 1.44	1.36 ± 0.69	***<0.0001***	2.04 ± 1.19	0.89 ± 0.53	***<0.0001***	1.80 ± 0.89	1.54 ± 0.82	***0.042***
Insulinogenic index (IGI)	3.77 ± 3.09	4.21 ± 2.83	*0.949*	5.58 ± 4.18	7.21 ± 5.41	*0.086*	4.70 ± 3.48	4.81 ± 3.06	*0.883*
Disposition Index (DI)	6.83 ± 4.44	5.15 ± 3.35	***0.001***	9.99 ± 7.83	5.70 ± 4.47	***0.045***	7.30 ± 5.25	7.21 ± 6.65	*0.991*
Lipid profile									
Total cholesterol (mg/dL)	151.1 ± 30.5	161.8 ± 29.6	***0.007***	151.9 ± 26.3	158.0 ± 22.5	*0.205*	148.5 ± 29.4	156.7 ± 35.1	*0.183*
HDL cholesterol (mg/dL)	45.4 ± 10.5	42.4 ± 11.0	*0.470*	46.7 ± 9.7	39.0 ± 7.7	***0.006***	42.2 ± 9.9	40.7 ± 9.1	*0.745*
LDL cholesterol (mg/dL)	87.6 ± 25.2	91.0 ± 24.0	*0.365*	91.2 ± 22.7	97.1 ± 21.7	*0.193*	85.6 ± 24.8	90.8 ± 29.8	*0.414*
Triglycerides (mg/dL)	91.9 ± 44.4	144.6 ± 80.6	***<0.0001***	67.7 ± 36.3	109.2 ± 31.7	***<0.0001***	105.8 ± 72.8	124.5 ± 58.0	*0.079*
Body fat distribution									
Hepatic fat fraction (%)	1.5 ± 1.8	19.7 ± 11.6	***<0.0001***	0.9 ± 1.4	18.0 ± 10.6	***<0.0001***	1.5 ± 1.8	18.9 ± 10.9	***<0.0001***
Visceral fat (cm^2)	55.5 ± 25.8	75.1 ± 27.8	***0.001***	47.0 ± 24.5	67.4 ± 28.7	***0.043***	50.9 ± 19.9	66.4 ± 29.7	***0.006***
Subcutaneous fat (cm^2)	480.1 ± 203.9	511.2 ± 180.1	*0.374*	488.5 ± 214.5	641.6 ± 212.4	*0.064*	461.0 ± 177.3	484.1 ± 190.5	*0.603*
Liver enzymes									
Alanine Transaminase (U/L)	22.4 ± 17.5	42.3 ± 33.7	***<0.0001***	17.6 ± 11.4	35.1 ± 27.1	***0.0002***	18.6 ± 12.1	42.8 ± 29.8	***<0.0001***
Aspartate transaminase (U/L)	21.9 ± 9.7	31.0 ± 17.9	***0.0004***	21.4 ± 7.4	27.8 ± 14.7	***0.026***	20.6 ± 6.0	31.3 ± 15.1	***<0.0001***
γ-Glutamyl transferase (U/L)	21.0 ± 12.8	23.3 ± 15.0	*0.271*	20.9 ± 7.3	21.8 ± 8.6	*0.933*	17.1 ± 7.2	23.3 ± 9.0	***0.032***
Alkaline phosphatase (U/L)	154.9 ± 99.3	198.6 ± 93.9	***0.002***	181.9 ± 111.3	205.4 ± 104.8	*0.275*	178.9 ± 93.7	245.7 ± 108.2	***0.0002***

P values are adjusted for age, sex, and *z*-score BMI when appropriate. Statistically significant *P* values are indicated in bold.

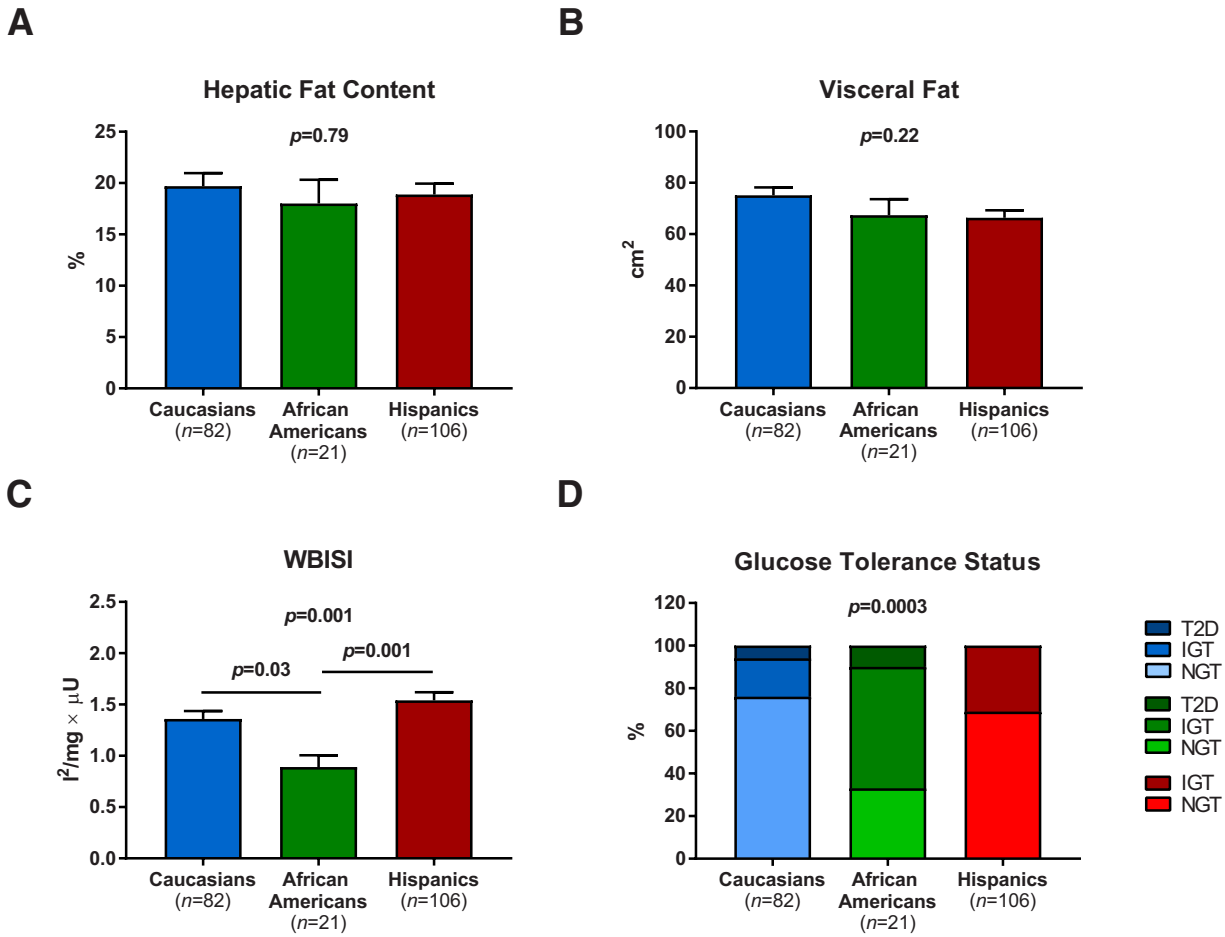

FIG. 2. (A) HFF, (B) visceral fat, (C) WBISI, and (D) glucose tolerance of white, black, and Hispanic obese youth with NAFL. Statistical comparisons among continuous variables were made using one-way ANOVA followed by post-hoc pair-wise comparisons by Tukey HSD tests. Differences in prevalence of impaired glucose control were assessed using Fisher's test. Abbreviations: ANOVA, analysis of variance; IGT, impaired glucose tolerance; NGT, normal glucose tolerance.

Fig. 2). Blacks with NAFL also had higher *z*-score BMI ($P = 0.007$) than Hispanics (Table 1). This pronounced reduction in insulin sensitivity observed in blacks with NAFL translated into a significantly higher prevalence (66.6%) of alterations of glucose tolerance compared to white (24.4%) and Hispanic (31.1%) obese adolescents with NAFL ($P = 0.0003$; Fig. 2).

ASSOCIATION BETWEEN GENE VARIANTS AND HFF% ACCORDING TO ETHNICITY

The allele frequency of the *PNPLA3* rs738409 minor allele (G) was 0.291 in whites, 0.158 in blacks, and 0.439 in the Hispanics. The *GCKR* rs1260326 minor allele (T) frequency was 0.404 in whites, 0.106 in blacks, and 0.352 in the Hispanics. The frequency of the *TM6SF2* rs58542926 T allele was 0.076 in whites, 0.013 in blacks, and 0.053 in Hispanics. The allele frequencies were consistent with those shown in similar ethnic groups in the Allele Frequency Database (ALFRED; https://alfred.med.yale.edu) as well as in HAPMAP (https://hapmap.ncbi.nlm.nih.gov/). Within each ethnic group, there was no evidence against the null hypothesis that the genotype distribution was in Hardy-Weinberg equilibrium for all the variants (all $P > 0.05$). The association of *PNPLA3* rs738409 with HFF% was statistically significant in all the ethnic groups ($P < 0.001$ in whites, $P = 0.0003$ in blacks, and $P = 0.02$ in Hispanics; Fig. 3A). The association between *GCKR* rs1260326 and HFF%

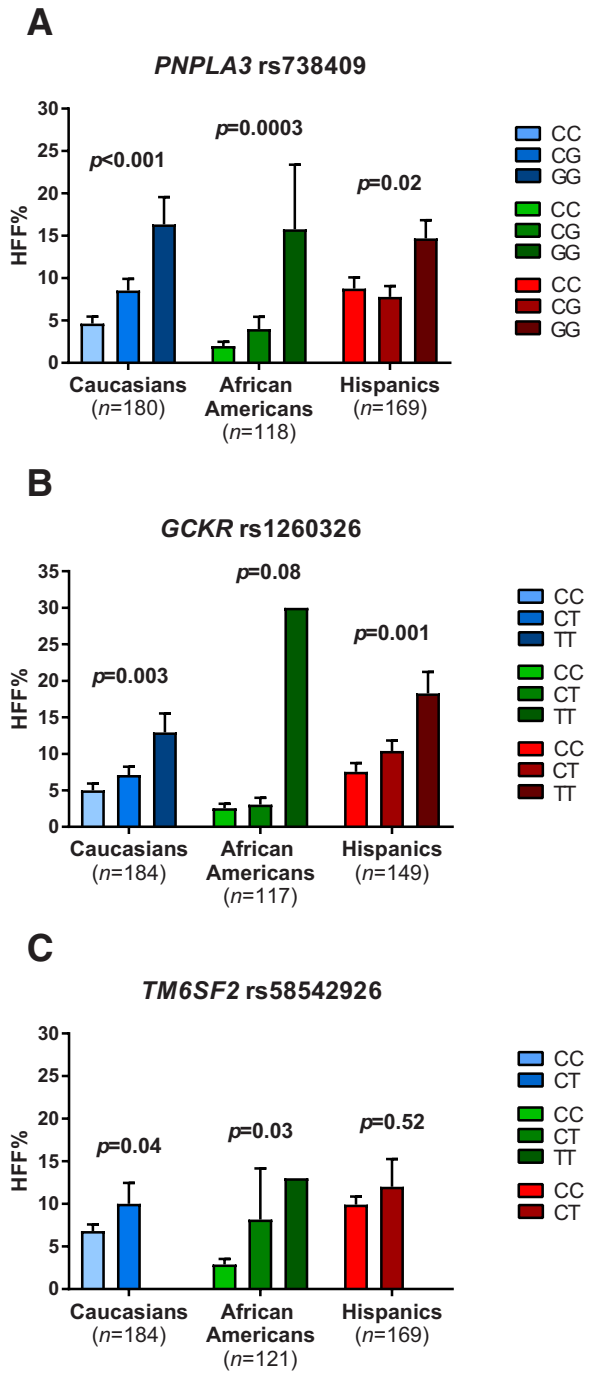

FIG. 3. Associations between (A) *PNPLA3* rs738409, (B) *GCKR* rs1260326, and (C) *TM6SF2* rs58542926 SNPs and HFF % in obese/overweight adolescents. Statistical comparisons between groups were made using one-way analysis of variance (ANOVA). Data are shown as mean ± SEM.

was statistically significant in whites ($P = 0.003$) and Hispanics ($P = 0.008$), and a similar trend was observed in blacks ($P = 0.08$; Fig. 3B). The association between the *TM6SF2* rs58542926 and HFF% was statistically significant in whites ($P = 0.04$) and blacks ($P = 0.03$), but not in Hispanics ($P = 0.52$; Fig. 3C). These data were partially shown in our previous studies.[12-14]

LONGITUDINAL ASSESSMENT OF THE YALE PEDIATRIC NAFLD COHORT

One hundred thirty-three subjects representative of the initial cross-sectional cohort (Supporting Table S1) were followed up for an average of 2.27 ± 1.44 years (median, 1.88; IQR, 1.27-2.80). During this period, which was similar among the three ethnicities/races (whites 2.21 ± 1.52 years, blacks 2.46 ± 1.51 years, and Hispanics 2.21 ± 1.31 years; $P = 0.691$), all subjects received nonpharmacological standard-of-care management of obesity, including nutritional consulting.

The main demographic and clinical features of these study subjects stratified by presence of NAFL are summarized in Table 2. Among them, 76 subjects (57.1%) did not have NAFL at baseline (33 whites, 26 blacks, and 17 Hispanics), whereas 57 subjects had NAFL (20 whites, 6 blacks, and 31 Hispanics; Table 2). Prevalence of NAFL in the longitudinal cohort varied largely among the three ethnic groups (37.7% in whites, 18.8% in blacks, and 64.6% in Hispanics; $P = 0.0002$), reflecting the difference observed in the cross-sectional analysis.

LONGITUDINAL DYNAMIC CHANGES IN HEPATIC FAT CONTENT

The absolute changes in HFF% ranged from –21.4 to +40 (median, 0; IQR, –2.25 to 3.55) and were associated with the individual changes in *z*-score BMI (r = 0.193; $P = 0.032$), fasting insulin (r = 0.256; $P = 0.005$), WBISI (r = –0.263; $P = 0.006$), visceral fat (r = 0.284; $P = 0.001$), subcutaneous fat (r = 0.397; $P < 0.0001$), ALT (r = 0.369; $P = 0.0001$), and aspartate transaminase (AST) levels (r = 0.262; $P = 0.009$; Table 3). After adjustment for age, sex, race, baseline *z*-score BMI, and follow-up duration, the association between changes in HFF% and in *z*-score BMI ($P = 0.006$), fasting insulin ($P = 0.004$), WBISI ($P = 0.015$), visceral fat ($P = 0.0002$), subcutaneous fat ($P < 0.0001$), ALT ($P = 0.0001$), and AST ($P = 0.041$) remained statistically significant.

TABLE 2. Characteristics of the study population stratified by absence (HFF≤5.5%) or presence (HFF>5.5%) of NAFL at baseline and by stability or progression/regression of NAFL at follow up

	Baseline HFF ≤ 5.5% (*n* = 76)			Baseline HFF > 5.5% (*n* = 57)		
	Non Progressors (*n* = 59)	Progressors (*n* = 17)	*p* Value	Non Regressors (*n* = 44)	Regressors (*n* = 13)	*p* Value
Clinical features						
Age (years)	14.2 ± 2.6	13.8 ± 3.1	*0.696*	13.3 ± 2.5	13.9 ± 2.3	*0.754*
Follow up duration (years)	2.59 ± 1.61	2.15 ± 1.46	*0.382*	2.39 ± 1.42	2.00 ± 1.19	*0.333*
Sex (M/F) [%]	19/40 [32.2/67.8]	4/13 [23.5/76.5]	*0.267*	26/18 [59.1/40.9]	9/4 [69.2/30.8]	*0.722*
Race (Caucasian/African American/Hispanic) [%]	23/25/11 [39.0/42.4/18.6]	10/1/6 [58.8/5.9/35.3]	***0.006***	18/4/22 [40.9/9.1/50.0]	2/2/9 [15.4/15.4/69.2]	*0.324*
z-score body mass index	2.12 ± 0.51	2.33 ± 2.21	*0.053*	2.40 ± 0.27	2.27 ± 0.40	*0.199*
Changes in body mass index *z*-score at follow up	-0.07 ± 0.36	0.13 ± 0.23	***0.012***	0.02 ± 0.22	-0.11 ± 0.23	***0.030***
Body mass index (kg/m^2)	33.4 ± 6.9	35.0 ± 5.8	*0.357*	34.8 ± 5.0	33.6 ± 5.9	*0.528*
Body fat mass (%)	41.3 ± 9.3	44.4 ± 5.6	*0.104*	46.9 ± 8.7	42.7 ± 7.5	*0.101*
Tanner stage (1/2/3/4/5) [%]	[5.1/8.5/20.3/22.0/44.1]	[11.8/5.9/29.4/17.6/35.3]	*0.538*	[9.1/15.9/15.9/20.5/38.6]	[7.7/7.7/38.5/0.0/46.1]	*0.140*
Changes in tanner stage at follow up (0/+1/+2) [%]	43/12/4 [72.9/20.3/6.8]	11/5/1 [64.7/29.4/5.9]	*0.795*	28/11/5 [63.6/25.0/11.4]	10/3/0 [76.9/23.1/0.0]	*0.618*
Systolic blood pressure (mmHg)	117.2 ± 9.7	119.3 ± 12.1	*0.281*	119.7 ± 8.9	121.5 ± 13.0	*0.609*
Diastolic blood pressure (mmHg)	68.8 ± 9.0	68.3 ± 8.8	*0.819*	69.3 ± 4.8	69.3 ± 6.9	*0.810*
Glucose metabolism						
Fasting glucose (mg/dl)	92.5 ± 8.9	98.7 ± 8.0	***0.028***	96.1 ± 8.5	92.4 ± 7.8	*0.186*
Fasting insulin (μU/ml)	30.3 ± 17.6	30.6 ± 9.8	*0.421*	43.2 ± 22.3	43.7 ± 24.3	*0.720*
Fasting C peptide (pmol/l)	1002.0 ± 349.7	1312.0 ± 345.4	***0.005***	1376.6 ± 427.0	1261.0 ± 359.7	*0.394*
2 h glucose (mg/dl)	125.3 ± 25.3	135.1 ± 23.2	*0.867*	133.3 ± 27.4	124.6 ± 24.0	*0.212*
Hemoglobin A_{1C} (%)	5.51 ± 0.40	5.55 ± 0.32	*0.308*	5.51 ± 0.38	5.57 ± 0.33	*0.873*
Whole Body Insulin Sensitivity Index (WBISI)	2.05 ± 1.06	1.63 ± 0.56	*0.497*	1.27 ± 0.67	1.20 ± 0.38	*0.638*
Insulinogenic index (IGI)	4.06 ± 3.19	3.07 ± 1.41	*0.178*	5.25 ± 3.70	7.00 ± 7.89	*0.219*
Disposition index (DI)	6.85 ± 4.39	4.76 ± 2.17	*0.170*	5.74 ± 5.48	8.00 ± 9.81	*0.387*
Lipid Profile						
Total cholesterol (mg/dL)	153.0 ± 29.7	150.2 ± 23.4	*0.961*	158.8 ± 36.9	162.7 ± 35.9	*0.674*
HDL cholesterol (mg/dL)	46.7 ± 12.9	42.7 ± 11.5	*0.606*	43.0 ± 10.4	37.7 ± 5.9	*0.138*
LDL cholesterol (mg/dL)	90.2 ± 24.1	89.4 ± 21.7	*0.849*	89.3 ± 33.5	96.3 ± 27.3	*0.457*
Triglycerides (mg/dL)	80.5 ± 41.7	91.7 ± 32.1	*0.893*	147.8 ± 111.2	143.5 ± 69.0	*0.875*
Body fat distribution						
Hepatic fat fraction (%)	0.98 ± 1.36	1.56 ± 1.63	*0.841*	21.27 ± 10.34	12.30 ± 6.18	***0.005***
Changes in hepatic fat fraction at follow up (%)	-0.11 ± 1.47	9.21 ± 9.01	***<0.001***	1.19 ± 11.13	-10.34 ± 6.55	***<0.001***
Visceral fat (cm^2)	49.9 ± 23.7	68.2 ± 26.4	*0.200*	79.1 ± 23.6	68.9 ± 30.1	*0.677*
Subcutaneous Fat (cm^2)	496.0 ± 196.7	537.7 ± 217.9	*0.360*	525.1 ± 145.0	564.4 ± 236.1	***0.011***

(*Continued*)

TABLE 2. (Continued)

	Baseline HFF ≤ 5.5% (*n* = 76)			Baseline HFF > 5.5% (*n* = 57)		
	Non Progressors (*n* = 59)	Progressors (*n* = 17)	*p* Value	Non Regressors (*n* = 44)	Regressors (*n* = 13)	*p* Value
Liver enzymes						
Alanine transaminase (U/L)	17.1 ± 13.6	25.0 ± 29.9	*0.849*	45.2 ± 34.2	32.4 ± 27.8	*0.369*
Aspartate transaminase (U/L)	20.0 ± 7.4	21.3 ± 12.9	*0.756*	32.5 ± 17.1	26.8 ± 10.3	*0.462*
γ-Glutamyl transferase (U/L)	21.7 ± 19.7	23.8 ± 15.4	*0.250*	26.3 ± 19.8	16.6 ± 6.1	*0.217*
Alkaline phosphatase (U/L)	166.5 ± 92.0	218.6 ± 128.9	*0.165*	182.1 ± 89.8	144.0 ± 105.7	*0.075*

P values adjusted for age, sex, ethnicity, and *z*-score BMI when appropriate. Statistically significant *p* values are indicated in bold.

RISK FACTORS OF PROGRESSION AND REGRESSION OF NAFL

Among subjects without NAFL at presentation, obese adolescents who developed NAFL over time were more likely whites (58.8%) and Hispanics (35.3%) than blacks (5.9%; $P = 0.006$) and had higher fasting glucose ($P = 0.028$) and C-peptide levels ($P = 0.005$; Table 2). Among subjects presenting with NAFL, a lower baseline HFF% ($P = 0.005$) and a higher subcutaneous fat mass, despite similar total body fat mass ($P = 0.101$), characterized obese adolescents whose HFF% decreased at follow-up reaching values below 5.5% (Table 2). Notably, small increments ($P = 0.012$) or decrements ($P = 0.030$) in *z*-score BMI over time were significantly associated with progression or resolution of NAFL at follow-up, respectively (Table 2).

Accordingly, ethnicity strongly predicted the onset of NAFL in obese adolescent who did not have NAFL at baseline ($P = 0.021$ at multivariate logistic regression analysis). Other significant predictors were changes in *z*-score BMI ($P = 0.0006$) and baseline fasting C-peptide levels ($P = 0.0006$). The area under the ROC curve for the prediction of NAFL by race, *z*-score BMI change and baseline fasting C peptide levels was 0.887 (Fig. 4A).

Adding either the *PNPLA3* rs738409, the *GCKR* rs1260326, or the *TM6SF2* rs58542926 variant to our model of progression to NAFL improved the area under the curve (AUC) of the ROC curve from 0.887 to 0.959, 0.978, and 0.976, respectively (Fig. 4A).

Among subjects with NAFL at baseline, the reduction of HFF% below 5.5% was more likely to follow a reduction of *z*-score BMI over time ($P = 0.032$) and to occur in obese subjects with a lower HFF at baseline ($P = 0.004$). The area under the ROC curve for the prediction of NAFL regression was 0.827, including *z*-score BMI change and baseline HFF as factors (Fig. 4B). Adding the *PNPLA3* rs738409, the *GCKR* rs1260326, or the *TM6SF2* rs58542926 variant to our predictive model of NAFL regression decreased or only mildly affected the AUC of the ROC curve (from 0.827 to 0.763, 0.828, and 0.814, respectively; Fig. 4B).

HISTOLOGICAL PHENOTYPES OF OBESE ADOLESCENTS WITH NAFLD

A total of 15 obese adolescents had biopsy-proven NAFLD, including 4 whites and 11 Hispanics. Their

TABLE 3. Correlations between changes in HFF and modifications in clinical and metabolic parameters at follow up

	HFF	BMIZ	SBP	DBP	Glu_0	Ins	CPEP	Glu_{120}	HBA_{1C}	WBISI	IGI	DI	CholT	HDL	LDL	TG	VFAT	SFAT	ALT	AST	GGT	ALP
HFF	1.000	0.193[a]	-0.149	-0.071	-0.055	0.256[b]	0.168	0.069	0.139	-0.263[b]	0.160	-0.165	0.126	-0.197	0.153	0.117	0.284[b]	0.397[d]	0.369[c]	0.262[b]	0.183	0.232[a]
BMIZ		1.000	0.115	0.120	0.342	0.325[c]	0.183	0.149	0.438	-0.399[d]	0.285[b]	-0.127	0.123	-0.147	0.186	0.028	0.210[a]	0.421[d]	0.230[a]	0.120	-0.094	0.145
SBP			1.000	0.563[d]	0.242[b]	-0.017	0.320[b]	0.180	0.255[a]	0.050	0.084	0.092	-0.001	-0.073	0.007	0.045	-0.052	-0.071	0.070	0.235[a]	0.190	0.021
DBP				1.000	0.172	0.106	0.391[c]	0.159	0.035	-0.048	0.108	-0.027	-0.048	-0.193	0.016	0.014	-0.042	-0.079	0.061	0.068	0.416	0.058
GLU_0					1.000	0.217[a]	0.213[a]	0.463[d]	0.178	-0.227[a]	-0.077	-0.343[c]	0.161	-0.068	0.147	0.147	0.255[b]	-0.052	0.046	0.094	0.525	0.018
INS						1.000	0.560[d]	0.367[d]	0.053	-0.811[d]	0.398[d]	-0.463[d]	0.171	-0.029	0.146	0.121	0.070	0.264[b]	0.248[a]	0.063	-0.265[a]	0.262[a]
CPEP							1.000	0.217[a]	0.059	-0.492[d]	0.391[c]	-0.261[a]	-0.044	-0.167	-0.074	0.104	-0.020	0.169	0.100	-0.022	-0.122	0.093
GLU_{120}								1.000	0.200	-0.435[d]	-0.052	-0.481[d]	0.098	-0.096	0.074	0.112	0.055	0.059	0.149	0.095	0.320	0.154
HBA1C									1.000	-0.036	0.091	-0.046	-0.070	-0.014	-0.104	0.064	-0.132	0.096	0.103	0.206	0.238	0.079
WBISI										1.000	-0.319[c]	0.566[d]	-0.266[b]	0.183	-0.272[b]	-0.229[a]	-0.208[a]	-0.377[c]	-0.066	0.056	0.088	-0.272[a]
IGI											1.000	0.448[d]	-0.094	-0.047	-0.003	-0.216[a]	-0.120	0.128	-0.104	-0.176	-0.193	0.168
DI												1.000	-0.281[b]	0.044	-0.184	-0.309[b]	-0.199[a]	-0.193	-0.165	-0.055	-0.260	-0.148
CHOLT													1.000	0.352[c]	0.851	0.251[a]	0.156	0.137	0.234[a]	0.275[b]	-0.004	0.057
HDL														1.000	0.135[d]	-0.201[a]	-0.343[c]	-0.179	0.044	0.134	0.303	-0.062
LDL															1.000	-0.037	0.229[a]	0.255[a]	0.174	0.229[a]	0.374	0.035
TG																1.000	0.210[a]	-0.093	0.139	0.152	0.028	0.099
VFAT																	1.000	0.432[d]	0.071	0.108	-0.171	0.110
SFAT																		1.000	0.219[a]	0.165	-0.141	0.097
ALT																			1.000	0.760[d]	0.000	-0.038
AST																				1.000	-0.018	0.017[a]
GGT																					1.000	-0.284
ALP																						1.000

Correlations between variables were assessed by Spearman correlations. [a]$p<0.05$; [b]$p<0.01$; [c]$p<0.001$; [d]$p\leq0.0001$.
HFF%, hepatic fat fraction; BMIZ, body mass index z-score; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; GLU0, plasma glucose at fasting; INS, plasma insulin at fasting; CPEP, plasma C peptide at fasting; GLU120, plasma glucose at 120 min (end of OGTT); HBA1C, hemoglobin A1c; WBISI, whole-body insulin sensitivity index; IGI, insulinogenic index; DI, disposition index; CHOLT, total cholesterol; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; TG, triglycerides; VFAT, visceral fat; SFAT, subcutaneous fat; ALT, alanine transaminase; AST, aspartate transaminase; GGT, gamma-glutamyl transferase; ALP, alkaline phosphatase.

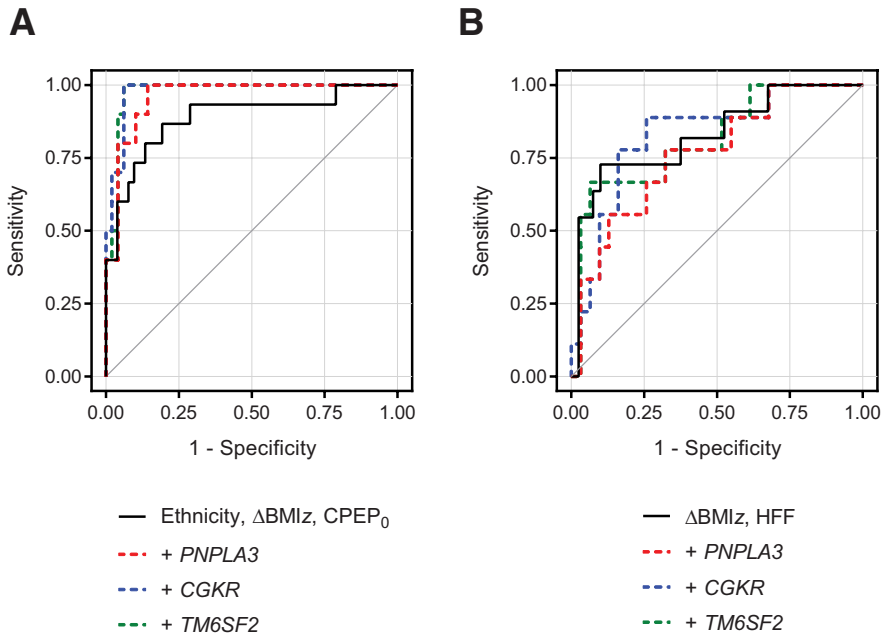

FIG. 4. ROC curves for predicting (A) NAFL progression (n = 76) or (B) regression (n = 57) at follow-up in obese/overweight adolescents. In (A), the AUC was 0.887 when using ethnicity, *z*-score BMI change (ΔBMI*z*), and fasting C peptide ($CPEP_0$) at baseline as predictors and increased to 0.959, 0.978, and 0.976 when adding either the *PNPLA3* rs738409, *GCKR* rs1260326, or *TM6SF2* rs58542926 variant to the model, respectively. In (B), the AUC was 0.827 when using ΔBMI*z* and HFF% at baseline as predictors and did not improve by adding either the *PNPLA3* rs738409, *GCKR* rs1260326, or *TM6SF2* rs58542926 variant (0.763, 0.828, and 0.814, respectively).

clinical and metabolic characteristics are shown in Supporting Table S1. The two ethnicity groups showed similar sex distribution ($P = 0.28$), age ($P = 0.61$), Tanner stage ($P = 0.57$), adiposity (*z*-score BMI, $P = 0.28$), and IR (WBISI, $P = 0.19$). Compared to the overall study cohort, adolescents with liver biopsy showed significantly higher HFF%, fasting insulin, fasting C-peptide, 2-hour glucose, fasting TG, visceral fat, ALT, AST, and γ-glutamyl transferase (GGT), as well as lower WBISI, DI, and HDL cholesterol level (Supporting Table S1).

The percentage of intrahepatic fat content assessed by liver biopsy was 49.7 ± 21.6% (median, 50%; 95% CI, 37.7-61.6; Supporting Table S2). In most subjects, biopsies showed the presence of fibrosis (stage 1-2 in 13 subjects and stage 3 in 1 subject) and an overall NAS indicative for NASH (NAS ≥5 in 12 subjects), without significant differences between ethnicities ($P > 0.50$). Intrahepatic fat content assessed by liver biopsy at baseline showed a strong correlation with HFF assessed by MRI either at baseline (r = 0.836; $P = 0.0001$; n = 15) or after a 2.5- ± 1.2-year follow-up (r = 0.768; $p = 0.016$; n = 9; Supporting Fig. S1).

Discussion

This study provides insights into the relative importance of ethnicity, clinical risk factors, and gene variants in the development of NAFL in adolescents. Consistent with previous cross-sectional studies,[1,8,10,31] we observed that black youth have a lower prevalence of NAFL and a lower tendency to accumulate intrahepatic fat over time as compared to whites and Hispanics. However, when NAFL is present, black obese adolescents show a more severely impaired metabolic phenotype than whites and Hispanics, with profound alterations in insulin and glucose homeostasis that translate into a 2-fold higher prevalence of prediabetes and T2D (Fig. 2). We used a multiple logistic regression analysis to determine which clinical factors might help predict changes in HFF in youth. This analysis revealed that ethnicity strongly predicted the onset of NAFL in obese adolescents who did not have NAFL at baseline ($P = 0.021$). Other significant predictors were changes in *z*-score BMI ($P = 0.0006$) and baseline fasting C-peptide levels ($P = 0.0006$). On the other hand, basal HFF and weight loss were the major factors associated with reduction of intrahepatic fat content at follow-up. In addition, we observed that adding the three major SNPs associated with NAFLD, such as the rs738409 in the *PNPLA3* gene,[13,32] the rs1260326 in the *GCKR* gene,[12,33] and the rs58542926 in *TM6SF2* gene,[14] significantly increased the likelihood to predict changes in HFF at follow-up.

The lower propensity of black patients to develop NAFL is certainly independent of degree of IR, given

that black obese youth have a similar or even higher degree of IR than whites and Hispanics.[10] Therefore, such a diversity is more likely to reflect biological and genetic differences in lipid metabolism rather than differences in IR or degree of obesity.[11] Notably, the minor allele frequency of the SNP rs738409 in the *PNPLA3* and rs1260326 in the *GCKR* varies among races, being the highest in Hispanics and the lowest in blacks.[10,34,35] thus reflecting the different prevalence of NAFL among these ethnic groups. Fat distribution likely also plays a role in the lower propensity of blacks to develop NAFL. In fact, visceral adipose tissue is the main source of free fatty acids (FFAs) for hepatic TG synthesis (~65%)[36] and is typically less represented in blacks than in whites and Hispanics. Therefore, one could speculate that differences in the degree of visceral fat accretion among races might lead to a lower flux of FFA in the liver explaining the lower HFF even in the presence of greater IR.[8,11] Finally, although hepatic *de novo* lipogenesis (DNL) is a major contributor of intrahepatic fat accumulation,[37] whether differences in DNL exist among ethnic groups remains unexplored.

It is interesting to note that in our group of adolescents with NAFL, the volume of the visceral fat depot was similar across ethnicities/races, even in the blacks in whom the volume visceral depot is known to be very small.[38] This suggests that black adolescents with NAFL may carry a genetic predisposition to accumulate fat into the visceral depot. Therefore, it would be important to explore whether genetic differences between black adolescents with low and high visceral depot exist and how they might contribute to the metabolic derangements observed in this group.

Ethnic differences were also observed in the longitudinal data. In fact, we show in a longitudinal setting that blacks were the least prone to develop NAFL over time, as compared to whites and Hispanics. Furthermore, we found that among metabolic features associated with NAFL in obese youth, high fasting glucose and C-peptide levels at baseline were associated with development of NAFL in subjects without evidence of intrahepatic fat. This probably reflects the fact that IR in obese patients is selective for glucose metabolism, whereas the high concentration of insulin available in plasma can still enhance the synthetic rate of hepatic DNL, which, in such an environment, is already increased as a consequence of the high availability of substrates (glucose). Interestingly, the degree of IR seemed to be a strong risk factor for developing NAFL also among blacks, who overall carry the lowest risk to accumulate intrahepatic fat. This observation further supports the role of IR as a major trigger of NAFL in obese adolescents.

Although the molecular bases of the relationship between IR and NAFLD are not entirely clear, puberty *per se* seems to play an important role. In fact, puberty is characterized by considerable metabolic and hormonal changes.[39] In particular, adolescents experience a decline in insulin sensitivity during puberty, with a nadir in midpuberty and a complete recover at the end of it, when the Tanner stage 5 is achieved.[40] It is conceivable that the IR of puberty might fuel the accumulation of intrahepatic TG and lead to hepatic IR, but this hypothesis remains to be studied. Pubertal IR is more severe in girls than in boys,[40] and this might be partially explained by differences in adiposity distribution. In fact, for a given BMI, girls tend to have greater triceps and subscapular skinfold thickness than boys.[40]

In addition, consistent with previous cross-sectional studies,[1,8,10,31] we showed that the prevalence of NAFL is higher in boys than girls and associated with the degree of obesity and IR in our multiethnic cohort of adolescents. This difference might be attributed to the different levels of estrogens during puberty. In fact, it has been shown in ovariectomized female mice that estradiol protects against intrahepatic lipid accumulation.[41]

Along with these metabolic features, genetic biomarkers were associated with intrahepatic fat accumulation. Common gene variants previously associated with NAFLD, particularly the rs1260326 in the *GCKR* gene, further strengthen the association between our model factors and progression of NAFL.

Although our longitudinal analyses involved a relatively small sample, they clearly demonstrate that taking into account the ethnic background, tracking some simple clinical information (such as fasting glucose and C-peptide and changes in BMI) and knowing the genotype of one of the major genetic risk factors for NAFLD might represent a strong tool for the clinician in the follow-up of obese adolescents.

STRENGTHS AND LIMITATIONS

Although some cross-sectional studies assessing racial and ethnic variation of NAFL have focused

primarily on the prevalence of the disorder,[8,10] there are no studies that have delved further into metabolic associations of NAFL in different racial and ethnic groups over time. Similarly, no studies have explored whether the metabolic phenotype is different among youth with NAFL in different ethnic groups in the United States. In the present study, using an advanced magnitude-based liver fat quantification MRI technique in a large cohort of obese adolescents that have undergone a detailed characterization of abdominal fat patterning, extensive metabolic phenotyping, and genotyping of common SNPs, we performed a detailed analysis of the associations of ethnicity with clinical and metabolic features of NAFL early in its development in a pediatric population. Limitations of our study are the lack of liver biopsy for most subjects, the lack of a larger sample size at follow-up and of a replication cohort to validate longitudinal data, and the lack of a more detailed genetic assessment of the global ancestry markers. The lack of a larger number of liver biopsies is certainly a major limitation of the study. In fact, collecting liver biopsies in all study subjects would have provided useful additional information about the real prevalence of NASH[42] and the natural history of NAFLD in youth, ethnic differences in NAFLD, and the relationship between histological changes occurring in the liver of subjects with NAFLD and glucose metabolism.

In conclusion, black adolescents are protected from NAFL, even in the presence of severe obesity. On the other hand, when NAFL is present in blacks, they show a more severe metabolic profile than whites and Hispanics, characterized by a higher prevalence of prediabetes and T2D. Moreover, taking into account ethnicity, degree of IR, and genetic risk factors allows to identify obese adolescents at highest risk for development of NAFL.

Acknowledgments: The authors are grateful to the patients and their families as well as to the Yale Center for Genome Analysis, the Yale Center for Clinical Investigation and Hospital Research Unit.

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