Genetic and Clinical Markers of Elevated Liver Fat Content in Overweight and Obese Hispanic Children

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Genetic and Clinical Markers of Elevated Liver Fat Content in Overweight and Obese Hispanic Children

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What is already known about this subject?

-The prevalence of nonalcoholic fatty liver disease (NAFLD) continues to rise in both children and adults.

-Hispanics are disproportionately affected by NAFLD, due in part to high obesity rates in this population.

-Variants in the PNPLA3 gene (rs738409) explain some of the elevated risk in Hispanics, with effects seen in children as young as 8 years old.

What does this study add?

-Liver fat significantly increased as a function of PNPLA3 (rs738409) risk allele number and subjects with higher genetic risk score (GRS) had significantly higher liver fat.

-Current clinical reference ranges for ALT produce high false negative diagnoses of elevated liver fat (ELF) in Hispanic children.

-A GRS significantly added to the ability of common clinical risk factors to predict ELF, and potential NAFLD, in obese Hispanic children.

ABSTRACT

Objective: Genetic variation in six genes has been associated with elevated liver fat and nonalcoholic fatty liver disease in adults. We sought to determine the influence of these genes on liver fat and whether a genetic risk score (GRS) would improve upon the ability of common clinical risk factors to predict elevated liver fat content (ELF) in Hispanic children.

Design and Methods: 223 obese Hispanic children were genotyped for six SNPs. MRI was used to measure liver fat. A GRS was tested for association with ELF using multivariate linear regression. Predictors were assessed via ROC curves and pair-wise analysis was used to determine significance alone and combined with clinical markers.

Results: Only variants in *PNPLA3* and *APOC3* genes were associated with liver fat ($p<0.001$, $p=0.01$, respectively). Subjects with a GRS=4 had ~3-fold higher liver fat content than subjects with GRS of 0 (15.1±12.7% vs. 5.1±3.7%, $p=0.03$). Addition of the GRS to a model containing BMI and liver enzymes increased ROC AUC from 0.83 to 0.85 [95% CI, 0.79-0.89], ($p=0.01$) and improved detection of ELF.

Conclusions: Only PNPLA3 and APOC3 were related to ELF and a GRS comprised of these susceptibility alleles added incrementally to the discriminatory power of traditional markers for clinical assessment of liver fat.

INTRODUCTION

As national rates of overweight and obesity continue to increase, so does the prevalence of nonalcoholic fatty liver disease (NAFLD). This trajectory, in terms of both increasing obesity and NAFLD, is also present in the pediatric population (1, 2). NAFLD, a condition characterized by an elevated liver fat content (ELF) of greater than 5.5 percent, which can be determined by imaging and/or histology (3), represents the proximal end of a broad spectrum of liver disease including nonalcoholic steatohepatitis (NASH), cirrhosis, and ultimately hepatocellular carcinoma. NAFLD represents the leading cause of chronic liver disease amongst all children in the United States (4, 5) and Hispanics are at an elevated risk of developing NAFLD, due in part to high obesity rates and potentially genetic factors.

Early clinical detection of ELF can be difficult, as the condition is usually asymptomatic. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST), as well as obesity status are currently used in the clinical evaluation of ELF and NAFLD (6, 7). MRI and magnetic resonance spectroscopy (MRS) can quantitatively assess, with a high degree of accuracy, hepatic fat content but are expensive clinical tests and not used routinely. By comparison, ultrasound is commonly used to assess liver fat content for clinical purposes and has limited utility due to the qualitative nature of the measure. While these three imaging techniques are commonly used, the “gold standard” remains liver biopsy, which can present an added risk for complications, particularly in the pediatric population. These current assessment methods represent a significant added burden and expense when compared to anthropometric and plasma assay measures in combination with genetic testing.

Recent genome-wide association studies (GWAS) and candidate gene approaches in adults have implicated six genes in the development of NAFLD and/or NASH, including patatin-like phospholipase 3 (*PNPLA3*), glycogen binding subunit of protein phosphatase1 (*PPP1R3B*), neurocan (*NCAN*), glucokinase regulatory protein (*GCKR*), lysophospholipase-like 1 (*LYPLAL1*), and apolipoprotein C3 (*APOC3*) (8-10). Previously, we validated the effect of *PNPLA3* on hepatic triglyceride levels in 188

Hispanic children and demonstrated that this effect manifests as early as 8 years of age. However, the independent and combined effects of the five other variants on hepatic fat content in this pediatric population are not known. Therefore we sought to evaluate the association of these six genetic variants on hepatic fat content in obese Hispanic children and determine whether cumulative genetic burden improves the prediction of ELF beyond traditional clinical risk factors.

METHODS

Participants

This study was a cross-sectional analysis of 223 overweight and obese (BMI of 25-45 kg/m^2), Hispanic children (41% male) between the ages of 8-17 years old who were recruited from several Districts in Los Angeles County. Participants were defined as Hispanic if they reported both parents and all four grandparents as Hispanic. All participants had medical and family history screening to ensure eligibility criteria were met (11). Patients were not eligible for the study if the following conditions were indicated: *i*) met any diagnostic criteria for diabetes; *ii*) the use of medications or supplements or the past or present diagnosis of other syndromes or diseases known to influence liver function, insulin action or lipid levels; *iii*) previous diagnosis of any major illness since birth; or *iv*) smoking (currently smoked or had smoked greater than 100 cigarettes in their lifetime) or drinking alcohol on a regular basis (in excess of 2 drinks per week as determined by questionnaire. Participants were not eligible for the study if they had current or past involvement with any weight loss/exercise/sports program in the six months prior to participation. Informed written child assent and parental consent were obtained from all patients. The Institutional Review Board of the USC Keck School of Medicine approved the study.

Anthropometry and Fasting Glucose

Height and weight were measured in triplicate for all patients, and BMI was expressed as kg body weight per m^2 height. Fasting glucose was screened at the first outpatient visit, as previously described (12). If fasting blood glucose measured ≥126 mg/dl, an additional sample was immediately drawn to confirm the value and an additional visit

was scheduled to confirm the diagnosis of diabetes. Diagnosis of diabetes resulted in exclusion from the study and referral to an endocrinologist.

Body Composition and Liver Fat

Whole body fat and soft lean tissue mass was estimated by dual energy x-ray absorptiometry (DEXA) using a Hologic QDR 4500W (Hologic, Bedford, MA). MRI was carried out on a General Electric 1.5-Tesla magnet (GE Healthcare, Waukesha, WI) to assess subcutaneous adipose tissue (SAT), visceral adipose tissue (VAT) and hepatic fat fraction using a modification of the Dixon 3-point technique as previously described (13). ELF was defined as >5.5% hepatic fat fraction as determined by MRI, a value commonly attributed to likely NAFLD (13) and used in previous genetic studies with adults.

Alanine aminotransferase (ALT) and aspartate aminotransferase (ALT) measurements

ALT and AST levels were measured in plasma samples from 208 participants via the kinetic rate method using a Synchron CX analyzer (Beckman Coulter Inc. Fullerton, CA) (14, 15). All samples were prepared in accordance with manufacturer specifications and analyzed in duplicate. The analytical range for both ALT and AST was 5-400 IU/L. ALT levels were considered abnormal when they exceeded recently established population-based upper limits of normal (ULN) values (16). An ALT normal reference range of 5-23.5 U/L was utilized in this study, with 23.5 representing the mean of the following ULN cutoff values established from NHANES data in boys (>22 U/L) and girls (>25 U/L) (16).

Molecular Genetic Analyses

Genomic DNA was extracted from whole blood. SNPs were selected based upon previously identified associations with NAFLD and/or hepatic steatosis from GWAS (9) and other association studies. The following 8 SNPs were genotyped in the study population: *PNPLA3* (rs738409), *APOC3* (rs2854116 and rs2854117), *GCKR* (rs780094 and rs1260326), *NCAN* (rs2228603), *LYPLAL1* (rs12137855) and *PPP1R3B* (rs4240624). Initial genotyping for the two variants in *APOC3* (rs2854116 and

rs2854117) and *GCKR* (rs780094 and rs1260326) showed these SNPs to be in high linkage disequilibrium (r^2>0.8, data not shown). Therefore, subsequent analyses were only conducted with rs2854117 for *APOC3* and rs780094 for *GCKR*. Genotyping was performed with the TaqMan Allelic Discrimination System. Each 96-well DNA plate contained the same four control DNA samples, two from International HAPMAP Project and two randomly chosen DNAs from our Hispanic study cohort. Replicate quality control samples yielded 99% concordance, and the overall call rate was 95%.

Calculations and Statistical Analyses

All variants were first tested for Hardy-Weinberg equilibrium (HWE) using a χ^2 test prior to analysis. General linear models were used to compare mean values of quantitative traits across groups, with adjustment for covariates. Liver fat values were natural log-transformed for analyses. A genetic risk score (GRS) was constructed for each participant based upon the sum of the *PNPLA3* and *APOC3* risk alleles that individually showed a significant association with elevated liver fat fraction. A weighted GRS (wGRS) was also calculated by multiplying the effect estimate (beta) on liver fat, obtained from separate linear regression analyses of each included SNP, adjusting for age and gender, by the number of risk alleles for that corresponding variant (0,1, or 2), and summing these values. GRS groups were defined as 1 (0-1 risk alleles), 2 (2-3 risk alleles) and 3 (4 risk alleles). Analysis of variance (ANOVA), controlling for age and gender, was used to determine between group effects with post hoc Bonferroni adjustment for multiple tests. Logistic regression was used to determine the impact of a composite score comprised of BMI percent and ALT (model A) or BMI percent, ALT and GRS (model B) on the prediction of ELF, controlling for age and gender. Resultant classification tables were used to calculate model performance. Receiver Operating Characteristic (ROC) curves were constructed for the models that included BMI and ALT/AST as conventional predictors with and without the GRS. The state variable for the test was defined by ELF status (liver fat ≥ 5.5%). The resultant area under the curve (AUC) for each ROC was obtained (17) and pair wise AUC comparisons were conducted (18). Data analysis was carried out using SAS software (version 9.2 of the

SAS System, Cary, NC. USA) and SPSS software (version 18, SPSS Inc., Chicago, IL. USA).

RESULTS

Clinical Characteristics of the Study Population

The clinical characteristics of the participants are shown in Table 1. Subjects with ELF, as defined by greater than 5.5% liver fat, were more likely to be male and had nearly four-fold higher liver fat content compared to normal liver fat participants (15.2 ±9.4 vs. 3.7±1.1, $p=5.4x10^{-32}$). Similarly, measures of adiposity were elevated in ELF subjects, most notably BMI percentile (97.9±3.6 vs. 92.3±10.8, $p=1.5x10^{-6}$) (Table 1). The association between BMI percentile and ELF is further illustrated in Figure 1A where 90% of subjects with liver fat over 5.5% met the criteria for obesity, as defined by BMI ≥95th percentile, compared to 64% of the normal liver fat content participants. Subjects with ELF also had significantly higher serum ALT and AST levels than normal liver fat content subjects (ALT: 20.7±12.3 vs. 10.8±5.4 IU/L, $p=2.2x10^{-13}$; AST: 24.8±10.3 vs. 17.9±4.4 IU/L, $p=4.4x10^{-10}$) (Table 1). However, 69% and 77% of ELF subjects had ALT and AST levels, respectively, that were within the accepted normal reference ranges for these analytes (Figure 1B and C). When standard ALT cutoffs (5-35 U/L) for ULN were utilized, 84% of ELF subjects fell within the normal reference range. Taken together, these data indicate that while BMI percentile-defined obesity accurately reflects the presence of ELF, a cutoff using the clinical definition of elevated liver enzymes does not provide similar discrimination.

Genetic Effects on Liver Fat Content

To explore the genetic determinants of liver fat content in this pediatric population, we genotyped six previously reported variants in *PNPLA3*, *PPP1R3B*, *NCAN*, *GCKR*, *LYPLAL1*, and *APOC3*. All SNPs were in HWE and, with the exception of *PNPLA3*, *LYPLAL1*, and *PPP1R3B,* the effect allele frequencies (EAF) of the SNPs in *APOC3*,

GCKR, and *NCAN* in this Hispanic cohort were similar to those previously reported in Caucasians (Table 2).

Of the variants tested, those in *PNPLA3* and *APOC3* demonstrated significant dose-dependent associations with liver fat content even after adjustment for age, sex, and VAT (Table 2). For example, each copy of the risk allele for *PNPLA3* rs738409 and *APOC3* rs2854117 increased liver fat by ~40-50% ($p_{trend}=4.2x10^{-6}$) and 20-30% ($p_{trend}=0.01$), respectively. In addition, the association of *APOC3* with liver fat remained significant (p=0.05) after conditioning on the effect of *PNPLA3*, but there was no was no evidence for an interaction between the two genes ($p_{interaction}=0.40$).

We next evaluated the combined effects of *PNPLA3* and *APOC3* on liver fat content. For each subject, an unweighted GRS was calculated for rs738409 and rs2854117. As shown in Figure 2, the GRS was normally distributed in this study population and there was a significant stepwise increase in liver fat content as a function of the number of risk alleles carried ($p_{trend}=3.12x10^{-5}$). Participants with 4 risk alleles had 3-fold higher liver fat than those with a GRS of 0 (15.1±12.7% vs. 5.1±3.7%, p=0.03). These associations remained significant when controlling for age, sex, and VAT. An analysis using the wGRS yielded similar results ($p_{trend}=1.3x10^{-4}$; data not shown).

Discriminatory Analysis

We next determined whether cumulative genetic burden could improve upon the discriminatory ability of common clinical risk factors to detect ELF. Based on our observed clinical associations described above, and high correlation between ALT and AST levels (r=0.72, p<0.001), BMI percentile and ALT were chosen as representative conventional risk factors in these analyses. The model including GRS had higher discriminatory ability than BMI and ALT alone, ($\chi^2=80.6$, p<0.001 with df=2 vs. $\chi^2=72.7$, p<0.001 with df=1, respectively) and overall prediction success was marginally, but significantly, improved in the full model (0.76 vs. 0.75,; p<0.01). For every 1-unit increase in GRS Hispanic children were three times more likely to have ELF.

We next carried out ROC curves for the predictors independently and in combination (Figure 3). Compared to BMI percentile alone, the AUC for the model with BMI + ALT was significantly higher (0.77, 95% CI, 0.70-0.82 vs. 0.84, 95% CI, 0.78-0.89; p=0.03), indicating that ALT levels significantly improve discrimination of ELF. A model that included BMI percentile and the GRS also significantly improved discrimination (0.80 95% CI 0.73-0.86) compared to BMI percentile alone, although not to the same extent as the addition of ALT. *PNPLA3* alone demonstrated a similar ability to discriminate ELF (0.64 95% CI, 0.56-0.72) compared to the GRS, however addition of *APOC3* to the model did not improve AUC values over *PNPLA3* alone. Finally, inclusion of all three risk factors (BMI percentile, ALT, and GRS) slightly increased the discriminatory power to detect ELF to 0.85 (95% CI, 0.79-0.89; p=0.01). The performance of the wGRS for ELF discriminatory ability was nearly identical to that of the GRS (data not shown). As shown in Table 3, the addition of GRS to BMI percentile and ALT improved sensitivity and specificity parameters. Taken together, these data indicate that the addition of GRS marginally, but significantly, improves overall ELF discrimination (19).

To test the ability of ALT, BMI percentile and GRS to predict liver fat on a continuous scale, we conducted linear regression analyses. All three variables were significant, independent predictors of liver fat percent, with ALT explaining the most variance in liver fat (39%). A per-unit increase in ALT, BMI and GRS corresponded to a significant increase in liver fat of 0.39, 0.81 and 2.1%, respectively. In multiple regression analyses, ALT, BMI percentile and GRS were tested as predictors of liver fat percent in a stepwise model of regression. The full prediction model explained the most variance in liver fat percent (48%), with an overall model significance of p=0.001(Table 4).

DISCUSSION

In the present study, we evaluated the association of commonly used clinical measures with ELF and examined the contribution of genetic variants to hepatic lipid content in a Hispanic pediatric population. We further assessed whether the combination of BMI, plasma liver enzyme levels, and cumulative genetic burden could serve as potential clinical tool for re-classification of ELF.

Our data show that almost 90% of children with MRI-defined ELF fell above the 95th percentile for BMI, illustrating the strong effect of obesity on hepatic fat content. However, 64% of the subjects with normal liver fat content were also above the 95th percentile for BMI, suggesting that this measure alone is not sufficient for indicating ELF. Similarly, ALT levels correlated significantly with liver fat, but 69% of ELF subjects fell within the recently established pediatric clinical reference range considered to be normal (5-23.5 IU/L). This high false negative rate could be problematic for diagnosing NAFLD in larger populations, as ALT is a commonly used primary indicator of potential NAFLD in children (20). It is established that the upper limits of normal levels for ALT vary between populations (21, 22) and abnormal ALT levels have been shown to occur in about 36% of obese Hispanics (23). In this regard, Ruhl et al. suggest that ALT upper limit ranges should be both ethnic specific and adjusted to reflect the growing prevalence of NAFLD in the adult population (24). Our data support this recommendation. Reducing the current normal reference range for ALT could also potentially decrease false negative diagnoses and improve the sensitivity of ALT as a predictor of ELF in obese Hispanic children.

Of the genes tested, only *PNPLA3* and *APOC3* demonstrated significant associations with liver fat content. Since being identified (8), *PNPLA3* has been reproducibly associated with NAFLD and related phenotypes in numerous studies with both adults and children (5, 25-29), including one by our group where the effect of *PNPLA3* was observed in Hispanic children as young as 8 years of age (5). Although the underlying molecular mechanism for how *PNPLA3* promotes triglyceride accumulation in the liver is not entirely known, it is clear that *PNPLA3* rs738409 represents the strongest validated genetic risk factor for NAFLD identified thus far. By comparison, we did not observe any evidence for association of *GCKR*, *NCAN*, *PPP1R3B* and *LYPLAL1* with ELF in this study sample. Given that these genes were identified through a GWAS of over 7000 Caucasian adults (9), it is likely that our study was underpowered to detect their smaller effects on liver fat. However, *GCKR* was associated with liver fat fraction in Chinese adults (30) as well as in a recent study of Caucasian, African American, and Hispanic

children, in which an effect was observed in all three ethnicities (31). Thus, it remains to be determined whether the effect of this gene is stronger in adults than in children.

Our study also demonstrated an association of *APOC3* with liver fat content. *APOC3* is transported on circulating triglyceride-rich lipoproteins and inhibits both lipoprotein lipase and hepatic lipase, thus delaying the catabolism of triglyceride-rich particles (32, 33). Interestingly, over-expression of *APOC3* in mice leads to diet-induced ELF and hepatic insulin resistance (34). Petersen et al. identified two tightly linked promoter polymorphisms in *APOC3* that were associated with NAFLD in a relatively small candidate gene study of Asian-Indian men (10). Of note, the association of *APOC3* with liver fat content in our Hispanic pediatric population was observed even after controlling for the strong effect of *PNPLA3*. However, other studies in different ethnicities have failed to detect an association with *APOC3* (31, 35-38), including two in children (31, 38). Such discrepancies could be due to ethnic and age-specific genetic effects (39), differences in the study populations, and/or the methods used to quantitate liver fat. Thus, additional studies, particularly in larger samples sizes and multiple ethnicities, will be needed in order to clarify the genetic contribution of *APOC3* to hepatic lipid content.

Using a GRS comprised of *PNPLA3* and *APOC3* susceptibility alleles, we showed that individuals carrying all 4 risk alleles have almost 3-fold higher hepatic fat content than non-variant carriers, illustrating the joint effects of *PNPLA3* and *APOC3* on liver fat content. Together, the additive effects of *PNPLA3* rs738409 and *APOC3* rs2854117 account for 12% of the variance in liver fat in our study population, although we did not obtain evidence for a genetic interaction between these variants. However, the GRS only resulted in a modest improvement in ELF discrimination beyond BMI percentile and ALT levels. A model containing ALT, BMI percentile and the GRS was able to explain 47% of the variability in liver fat, whereas BMI percentile and ALT alone explained 41%. Other studies have used similar approaches to predict NAFLD by using biomarkers and anthropometric measures, which have been extended to the prediction of NASH and fibrosis (40, 41).

Hispanic children are vulnerable to the current obesogenic environment and poised to benefit the most from early ELF detection. However, clinically useful predictors of ELF or NAFLD, other than liver enzymes and BMI, are lacking in this population. One study showed that ALT and BMI-z score were both independent predictors of NAFLD and together they accounted for most of NAFLD prediction in obese Italian children (42). However this study also noted that ALT alone should not be used as a surrogate marker of NAFLD in obese children (42), supporting existing literature in adults (43) as well as our current findings. Maffeis et al. were able to develop a highly discriminatory NAFLD predictive model in obese Italian children using waist-to-height ratio, ALT, adiponectin, and insulin resistance (44). While encouraging, this would require administration of oral glucose tolerance tests and additional biomarker assays.

Our approach with genetic factors, which in previous studies have been shown to explain up to 25% of the variability in hepatic fat content (31), was statistically significant but did not result in a substantial improvement in clinical utility over two common predictors of ELF. Additionally, *PNPLA3* appears to be driving the utility of the GRS in this population, which is not surprising given such a strong association of the gene variant with both NAFLD and NASH in other studies (5, 25-29). Thus, it is not clear whether such an incremental increase in ELF discrimination merits the clinical implementation of a *PNPLA3* and *APOC3* GRS in the detection of ELF at the present time. Furthermore, the increased discrimination we observe is specific to Hispanic children and it remains to be determined whether a GRS similarly improves risk classification in adults and/or other ethnicities. We did not detect associations of liver fat content with the four other validated NAFLD variants in *PPP1R3B*, *NCAN*, *GCKR*, and *LYPLAL1*, presumably due to our relatively small sample size. Thus, it is possible that a more comprehensive GRS could still provide additional prognostic value for the early detection of ELF, which could be addressed in the future through prospective study designs with larger numbers of subjects.

While potentially relevant from a clinical perspective, our study has limitations, including defining ELF and/or NAFLD as liver fat content greater than 5.5%. This cut-point is

generally accepted in the literature but is an arbitrary level based on previously published work. Although the use of MRI to detect percent liver fat fraction correlates highly with values derived through histology, liver biopsy remains the gold standard in steatosis quantification. However, we were not able to assess the association of a GRS with the level of histological damage (i.e. NASH) in this study, which is an important consideration since steatosis often does not progress to fibrotic conditions. Therefore, further studies in populations where biopsy samples are available will be necessary to assess the utility of a GRS in predicting the degree of liver inflammation and/or fibrosis in addition to liver fat percent. In this regard, the development of an accurate and inexpensive GRS test for predicting abnormal liver fat content may still represent an alternative to costly and invasive biopsies or imaging techniques.

In conclusion, we have shown that variants of *PNPLA3* and *APOC3* independently and in an additive manner contribute to elevated hepatic triglyceride content in Hispanic children. Although BMI percentile and ALT were useful predictors of ELF in this population, they lack sensitivity when used alone. Moreover, risk prediction models with BMI percentile that incorporated a GRS with *PNPLA3* and *APOC3*, either alone or in combination with ALT, marginally improved upon the ability to discriminate ELF. Larger study populations may allow a more comprehensive evaluation of the clinical utility of a GRS, which could lead to early detection and treatment of ELF or NAFLD in at-risk subjects.

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CONFLICTS

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FIGURE LEGENDS

Fig. 1: Distribution of BMI percentile, ALT and AST by liver fat content.
90% of subjects with ELF had a BMI ≥95th percentile (dark shaded box), compared to 64% of participants with normal liver fat content (A). 69% and 77% of ELF subjects had ALT (B) and AST (C) levels, respectively, that were within the normal reference ranges (ALT = 5-23.5 U/L, AST = 5-35 U/L) indicated by the light shaded box. Normal = normal liver fat content, Yes= ELF. ELF = elevated liver fat content defined as ≥ 5.5%.

Fig. 2: **Combined genetic effects on liver fat content**.
GRS category (0-4) is shown along the x-axis and mean liver fat ± 95% CI (open circles with bars) is plotted on the left y-axis. The right y-axis denotes the number of subjects in each GRS category, represented by the non-shaded bars. The distribution of GRS in the population was normal. Mean liver fat significantly increased as a function of GRS (p_{trend}=3.12 x 10^{-5}).

Fig. 3: **Utility of a GRS to predict Elevated Liver Fat Content.**
The ROC curves for the different models are shown where the state variable was defined by ELF. Test variables were BMI percentile, ALT, GRS and combinations thereof (listed next to line legend with AUC [CI]). BMI+ALT+GRS (solid red line) was a significantly better discriminator of ELF than BMI+ALT (AUC=0.85; 95% CI, 0.79-0.89 vs. AUC=0.84; 95% CI, 0.78-0.89, respectively [p=0.01]). The reference line (AUC=0.5) represents no predictive ability. ELF = elevated liver fat content ≥ 5.5%.

Table 1. The Clinical Characteristics of the Study Population

Trait	All Participants (n=223)	Normal Liver fat (n=126)	ELF (n=97)	p-value
Age (year)	13.5 ± 2.9	13.5 ± 3.1	13.5 ± 2.8	NS
Male/Female (n)	93/130	43/83	50/47	0.005
Height (cm)	157.3 ± 16.6	157.4 ± 18.2	157.2 ± 11.6	NS
Weight (kg)	77.9 ± 28.2	72.1 ± 27.4	85.4 ± 27.6	<0.001
BMI (kg/m^2)	30.5 ± 7.6	28.2 ± 7.1	33.4 ± 7.4	<0.001
BMI percentile	94.5 ± 10.3	92.3 ± 10.8	97.9 ± 3.6	<0.001
SAT (L)	12.1 ± 7.1	10.4 ± 6.6	14.2 ± 7.2	<0.001
VAT (L)	1.8 ± 1.3	1.4 ± 0.9	2.2 ± 1.4	<0.001
Total Fat (kg)	29.1 ± 12.1	26.1 ± 11.9	32.9 ± 11.5	<0.001
Liver fat (%)	8.8 ± 8.5	3.7 ± 1.1	15.2 ± 9.4	<0.001
ALT (IU/L)‡	14.9 ± 10.2	10.8 ± 5.4	20.7 ± 12.3	<0.001
AST (IU/L)‡	20.8 ± 8.2	17.9 ± 4.4	24.8 ± 10.3	<0.001
TAG (mg/dL)†	107.6 ± 52.7	96.6 ± 45.5	121.8 ± 58.1	0.001
Total Cholesterol (mg/dL)†	140.9 ± 29.6	138.4 ± 28.6	143.9 ± 30.7	NS
HDL (mg/dL)†	37.7 ± 9.4	39.0 ± 10.1	35.9 ± 8.3	0.024
LDL (mg/dL)†	85.2 ± 28.4	84.4 ± 28.8	86.2 ± 28.1	NS

Data are shown as mean ± SD. ELF was defined as liver fat content greater than 5.5% by MRI. NS=not significant. p-values are given for comparison between ELF and normal liver fat content groups and were obtained via independent t-tests. SAT = subcutaneous adipose tissue, VAT = visceral adipose tissue. ELF = elevated liver fat content, TAG = triacylglycerol. ‡n=208, †n=183.

Table 2. Individual Effects of SNPs on Liver Fat Content.

					Effect Allele Copy Number			
Gene	**SNP**	**Alleles[a]**	**EAF[b]**	**Reported EAF[c]**	**0**	**1**	**2**	**p-value[d]**
PNPLA3	rs738409	G/C	0.52	0.239	5.6±4.3 (n=53)	8.0±8.3 (n=109)	12.3±9.8 (n=60)	$4.2x10^{-6}$
APOC3	rs2854117	A/G	0.35	0.302	7.1±7.6 (n=91)	9.4±8.4 (n=103)	11.3±10.5 (n=26)	0.01
GCKR	rs780094	A/G	0.32	0.391	8.6±9.0 (n=101)	8.5±7.4 (n=102)	10.7±9.9 (n=22)	0.44
NCAN	rs2228603	C/T	0.97	0.901	NA	9.2±7.8 (n=11)	8.7±8.5 (n=212)	0.96
LYPLAL1	rs12137855	T/C	0.08	0.201	8.9±8.5 (n=184)	7.8±8.4 (n=36)	9.7±0 (n=1)	0.50
PPP1R3B	rs4240624	G/A	0.33	0.081	8.7±7.9 (n=101)	8.8±9.6 (n=88)	8.5±7.8 (n=28)	0.38

Data are shown as mean liver fat content (%) ± SD as a function of carrying 0, 1, or 2 copies of the effect alleles for selected SNPs. [a]Effect/Other allele. [b]EAF, effect allele frequency in Hispanics. [c]Reported effect allele frequency in Caucasians (HapMap-CEU).[d]p-values are obtained from multiple linear regression using natural log-transformed values, adjusted for age, sex and VAT.

Table 3. Performance Parameters of Clinical and Genetic Risk Factors on ELF Prediction.

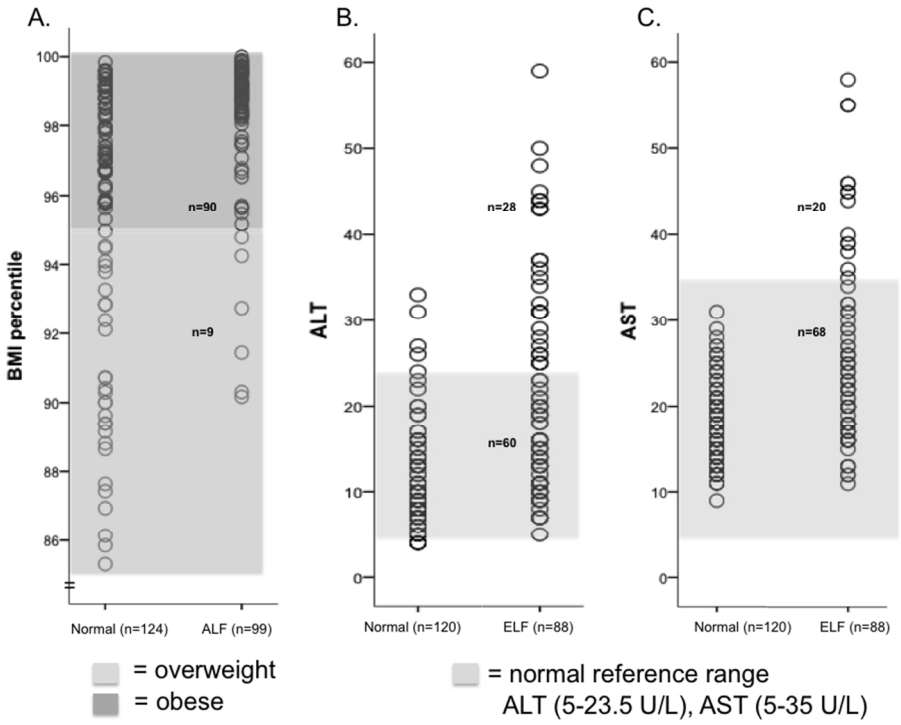
Parameter	GRS	ALT	BMI	BMI+ALT	BMI+GRS	BMI+ALT+GRS
Sens	0.69	0.76	0.65	0.75	0.66	0.76
Spec	0.60	0.68	0.78	0.75	0.73	0.75
Prev	0.23	0.30	0.58	0.40	0.52	0.40
PPV	0.34	0.51	0.80	0.67	0.73	0.68
NPV	0.86	0.87	0.61	0.81	0.67	0.82
DLR+	1.71	2.35	2.91	2.94	2.48	3.11
DLR-	0.52	0.36	0.46	0.34	0.46	0.31

The estimated sensitivity (Sens), specificity (Spec), prevalence (Prev), positive and negative predictive values (PPV and NPV, respectively) and diagnostic likelihood ratio (DLR+ and DLR-) values for the predicted probability of ELF are listed by different models (columns). ELF = elevated liver fat content, GRS = genetic risk score, ALT = alanine aminotransferase, BMI = body mass index.

Table 4. ALT, BMI Percentile and GRS as Predictors of Liver Fat Content.

Model	Predictor(s)	r^2	SEE	β	p
1	ALT	0.39	6.9	0.39	<0.001
2	GRS	0.1	8.3	2.07	<0.001
3	BMI	0.09	8.2	0.81	<0.001
4	ALT + GRS	0.44	6.7	-	<0.001
5	ALT + GRS + BMI	0.48	6.5	-	0.001

Output values from multiple regression analyses. ALT, GRS and BMI percentile were tested as predictors of liver fat content (expressed as a percentile) independently and in stepwise models of regression. All variables were tested for collinearity and outliers. The full prediction model explained the most variance in liver fat content (48%), with an overall model significance of p=0.001. GRS = genetic risk score, ALT = alanine aminotransferase, BMI = body mass index, SEE = standard error of the estimate, β = beta regression coefficient, p = model significance.



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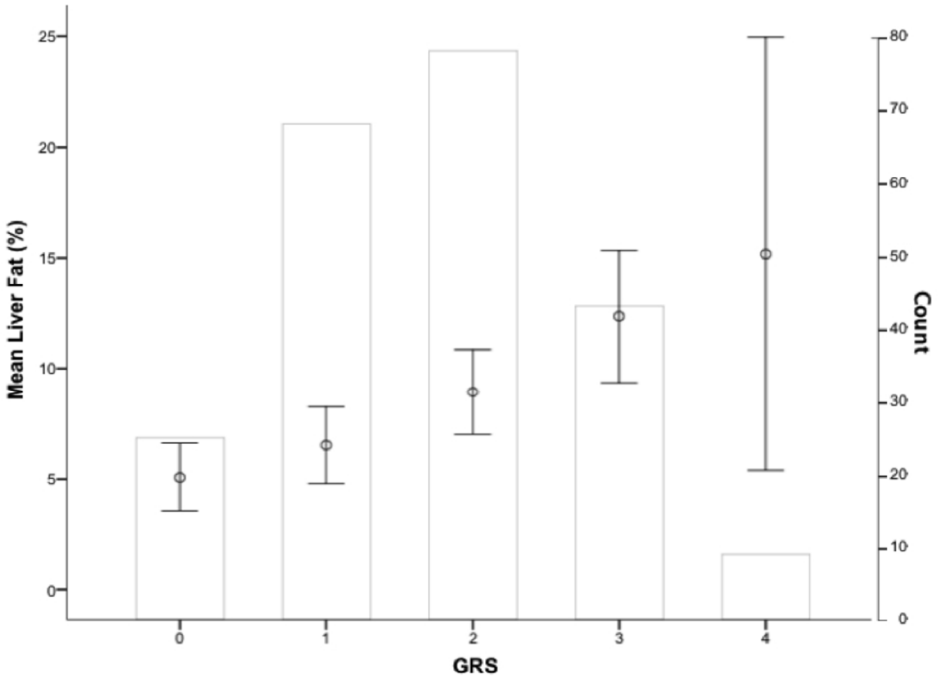

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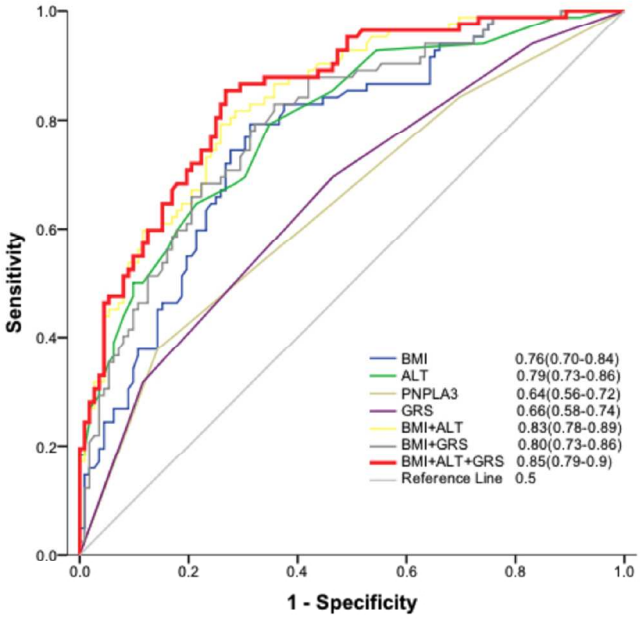

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