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Interactions of physical activity, muscular fitness, adiposity, and genetic risk for NAFLD

Theresia M. Schnurr[1,2] | Sophia Figueroa Katz[1,3] | Johanne M. Justesen[1,2] |
Jack W. O'Sullivan[1] | Peter Saliba-Gustafsson[1,4] | Themistocles L. Assimes[1,5] |
Ivan Carcamo-Orive[1,6] | Aijaz Ahmed[7] | Euan A. Ashley[1,8,9] | Torben Hansen[2] |
Joshua W. Knowles[1,6,10]

[1]Department of Medicine, Division of Cardiovascular Medicine and Cardiovascular Institute, Stanford University, Stanford, California, USA

[2]Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen, Kobenhavn, Denmark

[3]Cleveland Clinic Lerner College of Medicine of Case Western Reserve University, Cleveland, Ohio, USA

[4]Cardiovascular Medicine Unit, Department of Medicine, Center for Molecular Medicine at BioClinicum, Karolinska University Hospital, Karolinska Institutet, Stockholm, Sweden

[5]VA Palo Alto Health Care System, Palo Alto, California, USA

[6]Stanford Diabetes Research Center, Stanford, California, USA

[7]Division of Gastroenterology and Hepatology, Stanford University School of Medicine, Stanford, California, USA

[8]Department of Genetics, Stanford University, Stanford, California, USA

[9]Department of Biomedical Data Science, Stanford University, Stanford, California, USA

[10]Stanford Prevention Research Center, Stanford, California, USA

Correspondence
Theresia M. Schnurr, Falk Cardiovascular Research Center, 300 Pasteur Drive, Stanford, CA 94305, USA.
Email: schnurr@stanford.edu

Abstract

Genetic predisposition and unhealthy lifestyle are risk factors for nonalcoholic fatty liver disease (NAFLD). We investigated whether the genetic risk of NAFLD is modified by physical activity, muscular fitness, and/or adiposity. In up to 242,524 UK Biobank participants without excessive alcohol intake or known liver disease, we examined cross-sectional interactions and joint associations of physical activity, muscular fitness, body mass index (BMI), and a genetic risk score (GRS) with alanine aminotransferase (ALT) levels and the proxy definition for suspected NAFLD of ALT levels > 30 U/L in women and >40 U/L in men. Genetic predisposition to NAFLD was quantified using a GRS consisting of 68 loci known to be associated with chronically elevated ALT. Physical activity was assessed using accelerometry, and muscular fitness was estimated by measuring handgrip strength. We found that increased physical activity and grip strength modestly attenuate genetic predisposition to elevation in ALT levels, whereas higher BMI markedly amplifies it (all *p* values < 0.001). Among those with normal weight and high level of physical activity, the odds of suspected NAFLD were 1.6-fold higher in those with high

Theresia M. Schnurr and Sophia Figueroa Katz contributed equally to this work.

versus low genetic risk (reference group). In those with high genetic risk, the odds of suspected NAFLD were 12-fold higher in obese participants with low physical activity versus those with normal weight and high physical activity (odds ratio for NAFLD = 19.2 and 1.6, respectively, vs. reference group). *Conclusion:* In individuals with high genetic predisposition for NAFLD, maintaining a normal body weight and increased physical activity may reduce the risk of NAFLD.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is an ever-growing public health concern affecting one billion individuals worldwide.[1,2] NAFLD is characterized by excess accumulation of triglycerides in the liver (defined as hepatic fat content >5%) not due to increased alcohol intake, medications, infections, autoimmune processes, or other etiologies of chronic liver disease.[1] Numerous studies have demonstrated a deleterious role of NAFLD in the development of some types of cardiovascular disease,[3,4] type 2 diabetes,[2,5] end-stage liver disease, and hepatocellular carcinoma.[1]

Lifestyle modification for weight loss is the cornerstone intervention in the treatment of NAFLD in the absence of approved pharmacologic agents.[6] Observational studies have shown an inverse association among physical activity,[7–9] grip strength (indicator of muscular fitness), and NAFLD.[10–12] Importantly, randomized controlled trials have shown the benefits of exercise to reduce liver fat.[13,14] However, the effects of physical activity, muscular fitness, and weight loss regimes on NAFLD risk may differ between individuals due to genetic variation. To understand this, it is important to elucidate the interactions between genetic predisposition and lifestyle modifications such as physical activity, muscular fitness, and adiposity.

Previous studies have suggested that lifestyle factors, such as diet, alcohol and obesity, modify the association of genetic predisposition with cirrhosis and increased alanine aminotransferase (ALT) levels.[15–19] In these studies, genetic risk was estimated using single nucleotide polymorphisms (SNPs)[15] or a calculated genetic risk score (GRS) based on a small number of genetic loci.[16–18] However, these studies did not strictly exclude individuals with excessive alcohol consumption, and therefore did not specifically study NAFLD.[16,17] Recently, a large multi-ancestry genome-wide association study (GWAS) in the Million Veteran Program used a validated noninvasive proxy phenotype for NAFLD based on chronic elevation of liver enzyme ALT and by excluding other known causes of liver disease or significant alcohol use.[20,21] This study identified 77 genetic loci associated with NAFLD (including 68 loci not known to be associated with adiposity),

and thus facilitated the development of a larger NAFLD GRS to estimate overall genetic risk for NAFLD.

Here we examined the interactions between genetic risk (using a 68-SNP GRS that excludes known loci associated with adiposity), lifestyle factors (defined as objectively assessed physical activity and muscular fitness), or adiposity, with ALT levels. We also examined the joint associations of genetic risk, lifestyle factors, and obesity with suspected NAFLD defined as ALT levels >30 U/L in women and >40 U/L in men. We applied a cross-sectional study design and performed analyses on up to 242,524 participants from the UK Biobank after excluding individuals with excessive alcohol consumption above recommended weekly limits[22] and known liver disease.

PARTICIPANTS AND METHODS

Study population

The UK Biobank is a cohort of over 500,000 adults that has tracked health behaviors, anthropometric measurements, medical history, and biological samples longitudinally since their enrollment in 2006–2010. We used UK Biobank baseline data and Hospital Episode Statistics (HES) data linked by unique identifiers. HES contains inpatient records from the National Health Service, a health care system that covers most of the UK population. As outlined in Figure S1, we excluded individuals who withdrew consent (n = 167) and those who reported excessive alcohol consumption (n = 128,477). Excessive alcohol consumption was defined as weekly alcohol consumption of ≥ 140 g for women and ≥ 210 g for men based on clinical practice guidelines for the management of NAFLD by the joint European Associations for the Study of Liver, Diabetes, and Obesity.[22] In addition, we excluded those with other known liver diseases, alcohol use disorder, and human immunodeficiency virus infection based on International Classification of Diseases, Ninth Revision (ICD-9) and Tenth Revision (ICD-10) codes (n = 3022; Table S1). Furthermore, we excluded individuals with short-term poor prognosis, including diagnosis of metastatic cancer within 1 year of the baseline visit and

palliative care or hospice status based on ICD-9 and ICD-10 codes (n = 4497; Table S1). After additional exclusion of individuals with missing genotype information, non-White British ancestry, and sex mismatch, 242,524 individuals remained (Figure S1). The UK Biobank study was approved by the North West Multi-Center Research Ethics Committee, and all participants provided written informed consent to participate. All data assessment was performed in accordance with relevant guidelines and regulations. The UK Biobank study protocol is available online.[23] The presented analyses were conducted as part of UK Biobank study application 13721.

ALT and suspected NAFLD

Our primary outcome of interest was continuous ALT, which can discriminate between patients with and without steatosis, as measured by liver H–magnetic resonance spectroscopy[24] and is comparable with other well-known surrogate measures of NAFLD, such as the fatty liver index and the hepatic steatosis index.[24] We also examined ALT as a dichotomous variable and defined suspected NAFLD as elevated ALT > 30 U/L for women and >40 U/L for men, based on the upper limit of normal values used in previous studies.[20,25]

Genetic risk score construction

Genotyping and genotype quality control procedures for participants in the UK Biobank have previously been described.[26] To create the GRS, we selected SNPs from the largest (external from the UK Biobank) and recently published trans-ancestry GWAS of NAFLD in the Million Veteran Program, including 90,408 NAFLD cases and 128,187 controls.[21] Overall, 77 SNPs reached the genome-wide significance threshold ($p < 5 \times 10^{-8}$) in the GWAS discovery study, and a GRS of these 77 SNPs was predictive of NAFLD in 7397 liver biopsy–based histologically characterized NAFLD cases and 56,785 population controls from various clinical studies ($p = 3.7 \times 10^{-28}$).[21] This supports the clinical relevance of a GRS based on SNPs derived from the proxy NAFLD phenotype based on elevated ALT levels.[21] Of these 77 SNPs, we included 73 loci with minor allele frequency >1% and good imputation quality (INFO > 0.7) in UK Biobank. We excluded five additional genetic variants that were in strong linkage disequilibrium ($r^2 > 0.8$ in the 1000 Genomes European panel) with a body mass index (BMI)–associated locus in a large published GWAS study for BMI.[27] Using the remaining 68 loci, we calculated the 68-SNP GRS (hereafter referred to as "GRS" unless specified differently) as the weighted sum of the risk alleles by using effect sizes from the reference GWAS as weights.[21] Of

these 68 loci, 15 loci were externally validated to associate with histologically or imaging-defined NAFLD[21]; therefore, we also created a 15-SNP GRS to test whether this smaller GRS (restricted to externally validated SNPs) replicates the results of the larger 68-SNP GRS. The 15-SNP GRS was correlated with the 68-SNP GRS (Pearson's product moment correlation rho = 0.74; $p < 2 \times 10^{-16}$; Figure S2). Information on the SNPs included in the 68-SNP GRS and the 15-SNP GRS, the risk alleles, risk-allele frequencies, imputation INFO scores, and respective effect sizes that were used as weights for the calculation of both GRSs are presented in Table S2. The GRS was stratified into low (quintile 1), intermediate (quintiles 2–4), and high risk (quintile 5).

Assessment of physical activity

Physical activity was measured objectively by an Axivity AX3 wrist-worn triaxial accelerometer. The device was used to measure physical activity intensity every 5 s over 7 days as previously described.[28] By necessity due to study logistics, participants wore the monitor sometime after the baseline visit.[28] Exercise behavior is moderately to highly stable across the life span, particularly in adulthood[29]; hence, we assumed that the different timepoints for ALT and physical activity assessment should not influence our results. The expert working group calibrated data, removed gravity and sensor noise, identified wear/nonwear periods, imputed nonwear time, and finally calculated overall physical activity by averaging the vector magnitude of worn and imputed values of acceleration recorded in milligravity (mg) units.[28] We stratified individuals into low (quintile 1), intermediate (quintile 2–4), or high (quintile 5) physical activity. For sensitivity analyses, we used a subjective measure of physical activity in metabolic equivalent of task (MET) hours/week (n = 241,842) based on the international physical activity questionnaire.

Assessment of handgrip strength

A Jamar J00105 hydraulic hand dynamometer was used to measure grip strength bilaterally in the sitting position. Participants were instructed to squeeze the device as hard as possible for 3 s. The highest value reached was recorded in whole kilogram force units. Due to the high correlation between absolute grip strength and body size, relative grip strength (which is adjusted for body size) has been deemed more suitable to reflect muscular fitness.[30–32] Thus, we calculated relative grip strength as the average of the left and right hand (in kilograms) divided by whole-body fat-free mass (FFM) (in kilograms). For sensitivity analysis, we also calculated an alternative estimate of relative

grip strength defined as the average of the left and right hand (in kilograms) divided by body mass (in kilograms).[33,34] We stratified individuals into low (quintile 1), intermediate (quintile 2–4), or high (quintile 5) grip strength based on sex-specific and age-specific subsets, in line with previous work.[30]

Measures of adiposity

BMI was calculated as weight in kilograms divided by height in meters squared. We classified individuals as normal weight (BMI < 25 kg/m^2), overweight (25 ≤ BMI < 30 kg/m^2), or obese (BMI ≥ 30 kg/m^2).

Statistical analyses

All analyses were conducted using R (version 3.5.1). We used linear regression models to confirm that the GRS, physical activity, grip strength, and BMI were associated with continuous ALT levels. Quantitative traits were rank-inverse normally transformed to approximate normal distribution with a mean of zero and SD of 1, and to facilitate comparison between effect sizes of different measures. We then tested for interactions between the GRS and physical activity, grip strength, and BMI, using gene-environment interaction models with ALT levels as the outcome, with main effect terms for GRS and physical activity, grip strength, or BMI, and with an additional effect term for the interaction between GRS and physical activity, grip strength, or BMI, respectively. We reported the interaction effects and p values from analyses of continuous inverse normally transformed traits. We performed tests for interaction using continuous variables to both increase power and prevent selecting quintiles of the underlying variable, thus influencing the results. Logistic regression was used to examine the combined associations of the GRSs, obesity, and physical activity or grip strength, with the odds of suspected NAFLD based on elevation in ALT levels. All analyses were adjusted for age, sex, region of the UK Biobank assessment center, and Townsend index reflecting socioeconomic status. Analyses including the GRS were additionally adjusted for genotyping array and the first 10 genome-wide principal components to correct for population stratification.

We performed the following sensitivity analyses: (1) stratification by sex, (2) analyses with self-reported physical activity, and (3) grip strength divided by weight to confirm the direction of effect from analyses of accelerometer-assessed physical activity and grip strength scaled by FFM, respectively; as well as (4) analyses using the 15-SNP GRS that was computed based on loci that were externally validated to associate with histologically or imaging-defined NAFLD status.[21]

RESULTS

Population characteristics

The baseline characteristics of the 242,524 UK Biobank participants and the 25,716 individuals with suspected NAFLD included in the presented analyses are provided in Table 1. The mean age of all participants was 57.0 years (SD, 8.0 years), and 59% were women. Median ALT levels of all participants were 18 U/L in women and 23 U/L in men, with 11% of participants meeting ALT criteria for suspected NAFLD, as defined in the Methods section. Mean BMI was 27.4 kg/m² (SD, 4.9), with 35% of all participants classified as having normal weight, 41% as overweight, and 24% as obese.

Observational associations of the GRS, physical activity, grip strength, and BMI with ALT levels

Higher GRS was associated with higher ALT levels ([main effect of GRS] = 0.13 SD; $p < 2 \times 10^{-16}$; n = 230,747). We also found that a higher BMI was associated with higher ALT levels (β [main effect of BMI] = 0.28 SD; $p < 2 \times 10^{-16}$; n = 229,969), whereas higher physical activity and higher grip strength were associated with lower ALT levels (β [main effect of physical activity] = −0.071 SD; $p < 2 \times 10^{-16}$; n = 50,714; β [main effect of grip strength] = −0.09 SD; $p < 2 \times 10^{-16}$; n = 225,648, respectively). In sensitivity analysis, we found that the associations among GRS, physical activity, grip strength, and BMI on ALT levels were similar in women and men (data not shown). We confirmed the inverse association between physical activity and grip strength on ALT levels for self-reported physical activity (MET hours/week) and grip strength scaled by weight (data not shown). When applying the 15-SNP GRS, the association between higher GRS and increased ALT did not change materially (Table S3).

Interactions of the GRS with physical activity, grip strength, and BMI on ALT levels

We found that the association of GRS with increased ALT was attenuated with increasing levels of physical activity ([GRS physical activity interaction] = −0.28 SD; $p = 1.5 \times 10^{-7}$; n = 50,714) and grip strength (β [GRS grip strength interaction] = −0.0067 SD; $p = 0.00061$; n = 225,648; Figures 1 and 2). Conversely, the association between GRS and increased ALT was amplified as BMI increased ([GRS–BMI interaction] = 0.037 SD; $p < 2 \times 10^{-16}$; n = 229,969; Figures 1 and 2). When stratifying by gender, these interactions remained statistically significant (Figures S3A,B). Importantly, we confirmed

TABLE 1 Baseline characteristics of the overall study population included in our analysis of the UK Biobank

Variable	Overall study population (n = 242,524)	Individuals with suspected NAFLD[b] (n = 25,716)
Age (years)	57.0 ± 8.0	56 ± 7.7
Sex		
Women (%)	143,976 (59.4)	15,051 (58.5)
ALT (n = 231,032[a])		
Women (U/L)	17.5 [13.9–22.9]	30.0 [32.9–46.2]
Male (U/L)	23.3 [18.1–30.9]	49.1 [43.7–55.1]
Overall (U/L)	19.6 [15.1, 26.4]	43.3 [35.8–52.8]
Suspected NAFLD[b] (%)	25,716 (11.1)	
GRS (n = 242,524[a])		
Low (Q1)	47,442 (20.0)	3,289 (12.8)
Intermediate (Q2–4)	142,236 (60.0)	14,898 (57.9)
High (Q5)	48,505 (20.0)	7529 (29.3)
Mean ± SD[c]	50.9 ± 4.8	52.3 ± 4.9
Physical activity (n = 53,332[a])		
Low (Q1)	10,695 (20.1)	719 (14.0)
Intermediate (Q2–4)	31,965 (59.9)	3035 (59.2)
High (Q5)	10,672 (20.0)	1373 (26.8)
Median [IQR] (mg units)	26.7 [21.8, 32.2]	25.0 [20.3, 30.1]
Muscular fitness (n = 200,350[a])		
Low (Q1)	47,442 (20.0)	3949 (15.8)
Intermediate (Q2–4)	142,236 (60.0)	14,696 (58.6)
High (Q5)	10,672 (20.0)	6421 (25.6)
Median [IQR]	0.6 [0.5, 0.7]	0.5 [0.5, 0.6]
BMI (n = 241,688[a])		
Normal weight (<25 kg/m^2)	83,487 (34.5)	3915 (15.4)
Overweight (25–30 kg/m^2)	100,069 (41.4)	10,236 (40.0)
Obese (>30 kg/m^2)	58,132 (24.1)	11,418 (44.6)
Mean ± SD	27.4 ± 4.9	30.0 ± 5.3

Note: Data are presented as n (%), mean ± SD, or median [interquartile range [IQR]].

Abbreviations: mg, milligravity; NAFLD, nonalcoholic fatty liver disease; Q, quintile.

[a]Number of individuals (from overall study population) with available trait information.

[b]Individuals with suspected NAFLD were defined as >30 U/L in women and >40 U/L in men.

[c]Number of ALT-increasing alleles for 68-SNP GRS.

the magnitude and direction of the interaction effect for interactions between the GRS and self-reported physical activity measured as MET hours/week on ALT levels, and between the GRS and grip strength scaled by weight on ALT levels (Figure S4). When applying the smaller 15-SNP GRS, the interactions between the GRS with physical activity, grip strength, and BMI on ALT levels did not change materially (Table S3).

We translated the observed effect sizes into clinically meaningful effects. For example, among women with high physical activity, median plasma ALT was 16 U/L in those with low GRS and 17 U/L in those with a high GRS (absolute difference, 1 U/L; relative difference, 6%), whereas among women with low physical activity, the corresponding values were 17 U/L for those with low GRS and 20 U/L

for those with high GRS (absolute difference, 3 U/L; relative difference, 15%; Figure S3A and Table S4A). Similar observations in terms of absolute and relative differences were made among men, as well as for grip strength and adiposity (Figure S3A,B and Table S4A,B).

Combined associations of the GRS, physical activity, grip strength, and obesity with odds for suspected NAFLD

Individuals who ranked high for all three risk factors (obesity, high GRS, and low physical activity) had higher odds ratios (ORs) of suspected NAFLD compared to individuals with normal weight, low GRS, and
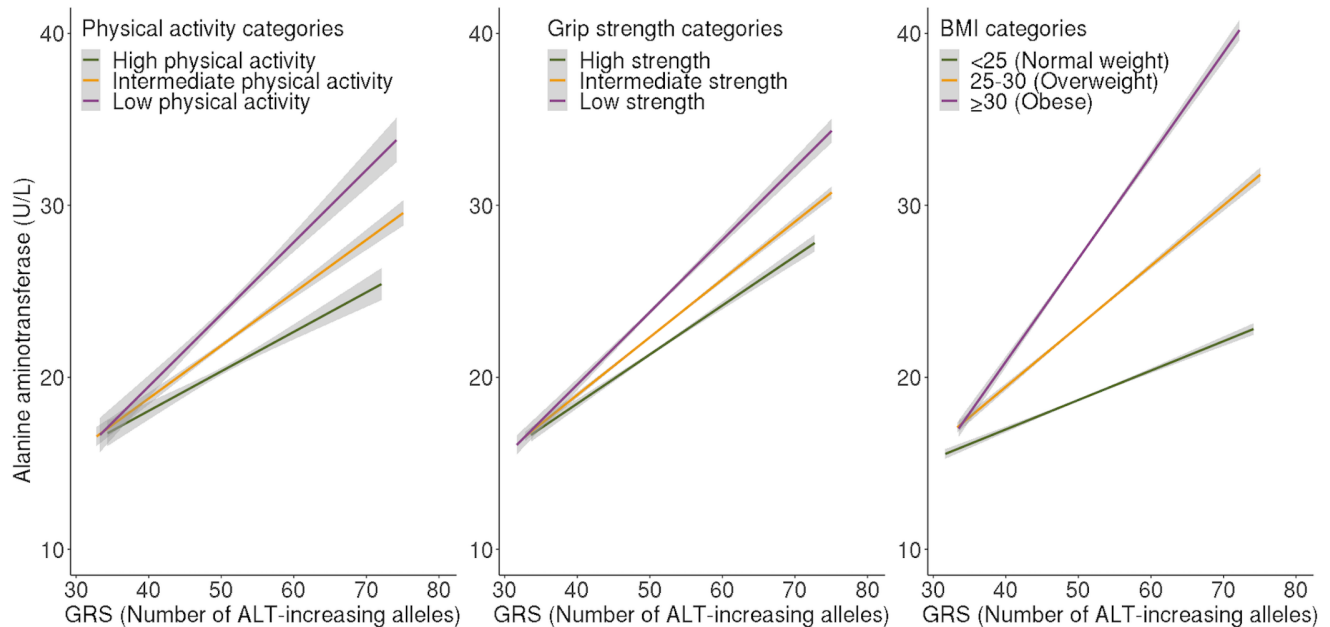
FIGURE 1 Plasma alanine aminotransferase (ALT) levels (in U/L) as a function of genetic risk score (GRS; number of ALT-increasing risk alleles) stratified by levels of physical activity, grip strength, and body weight status. The lines in the panels depict regression lines, and the light shading shows the 95% confidence intervals. The ALT-increasing effect of a higher GRS was attenuated by increasing levels of physical activity and grip strength, whereas it was amplified by increasing adiposity (all p values < 0.001). BMI, body mass index
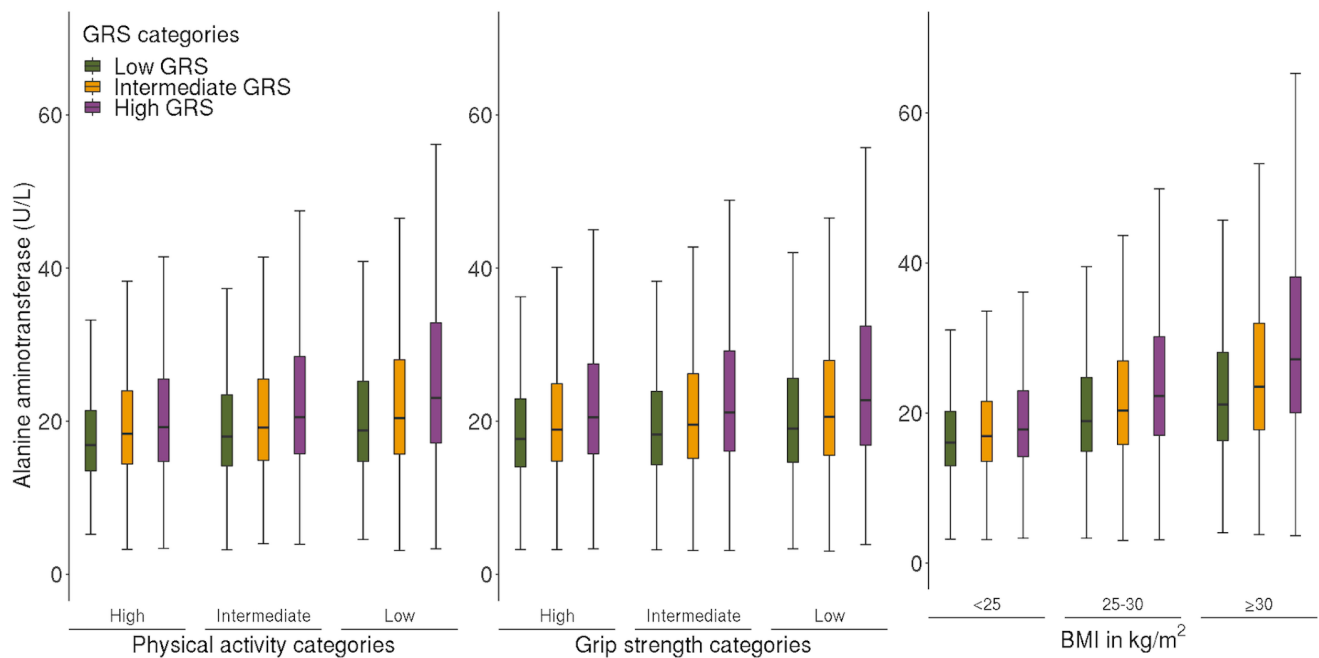

FIGURE 2 The observed interaction effects between the GRS and physical activity, grip strength, and adiposity translated into median ALT levels. The box plots depict medians and interquartile ranges, and the whiskers extend to the fifth and 95th percentiles of ALT levels (in U/L). The NAFLD-increasing effect of a higher GRS on ALT levels was attenuated by increasing levels of physical activity and grip strength, whereas it was amplified by increasing adiposity (all p values < 0.001). Sex-stratified plots are shown in Figure S3

high physical activity (OR, 19.2; 95% confidence interval [CI], 13.3–28.4). Notably, among individuals with low GRS and high physical activity, obesity was strongly associated with higher odds of suspected NAFLD (OR, 5.9; 95% CI, 3.5–10.0) compared with individuals with

normal weight in the same GRS and physical activity stratum (Table 2 and Figure S5). Individuals with high GRS, obesity, and low physical activity levels had a 12-fold higher estimated OR for NAFLD than those with high GRS who maintained normal body weight and

high physical activity levels (OR, 19.2 vs. 1.6, respectively; Table 2 and Figure S5). The absolute prevalence of NAFLD in the reference group was 3% versus 35% in the high-GRS, obese, and low physical activity subgroup. The joint effects of these risk factors followed a dose-response pattern across the BMI, GRS, and physical activity strata. Importantly, physical activity had an independent impact on suspected NAFLD within each weight category (Table 2 and Figure S5).

Individuals with obesity, high GRS, and low grip strength had higher odds of suspected NAFLD (OR, 14.9; 95% CI, 12.5–17.9) compared with individuals with normal weight with low GRS and high grip strength. Even among individuals with low GRS and high grip strength, obesity was strongly associated with higher odds for suspected NAFLD (OR, 3.7; 95% CI, 2.8–4.9) compared with individuals with normal weight in the same GRS and grip strength stratum (Table 3 and Figure S6). We also observed that individuals with high GRS, obesity, and low grip strength demonstrated an 8-fold higher estimated OR for NAFLD than those with high GRS who maintained normal body weight and high grip strength levels (OR, 14.9 vs. 1.9, respectively; Table 3 and Figure S6). Overall, grip strength showed a weaker independent impact on suspected NAFLD compared with the joint associations observed for physical activity (Figure S5 vs. Figure S6).

DISCUSSION

In this cross-sectional study including up to 242,524 UK Biobank participants including 25,716 participants with suspected NAFLD and without evidence of co-existing excessive alcohol intake or other known causes of liver disease, we found that increased physical activity and muscular fitness moderately attenuate the genetic risk

of suspected NAFLD, whereas adiposity characterized by higher BMI markedly amplifies the genetic risk of suspected NAFLD. The associations between physical activity and muscular fitness underscore the importance of weight management to prevent NAFLD.

The results from the joint associations further suggest that the protective effects of physical activity on suspected NAFLD are most pronounced in intermediate to high GRS subgroups with obesity, illustrating a beneficial effect of higher levels of physical activity by attenuating the genetic risk for suspected NAFLD. These findings support the hypothesis that gene–lifestyle interactions have a role in optimizing the management of NAFLD.[18,19] We translated the observed interactions into absolute and relative differences of median ALT levels and found a considerable increase in median ALT levels in individuals with high genetic risk and low physical activity compared with high physical activity levels. A protective effect of similar magnitude was observed for the interaction with muscular fitness. We also demonstrated that adiposity, as measured by BMI, amplifies genetic risk for NAFLD. This is in line with other studies that found that genetic variants predisposing to nonalcoholic and alcoholic fatty liver disease, have greater effects on liver fat as BMI increases.[15–17] These findings are more pronounced versus analyses of gene–lifestyle interaction studies in other complex diseases, such as coronary artery disease, cardiovascular disease, and type 2 diabetes, which suggest that a healthy lifestyle offsets genetic risk, but relative risk reductions are similar among those with low versus high genetic risk.[35–37]

Collectively, our study suggests that individuals at elevated genetic risk of NAFLD might be able to offset this risk by maintaining normal body weight, but also by increasing physical activity independently of body

TABLE 2 Combined associations of GRS, physical activity, and BMI with odds of suspected NAFLD

GRS categories stratified by BMI		Physical activity categories					
		High		Intermediate		Low	
		OR (95% CI)	N_cases (%)	OR (95% CI)	N_cases (%)	OR (95% CI)	N_cases (%)
Low GRS	Normal weight	1.0 (Reference)	35 (3.0)	1.1 (0.7–1.7)	76 (3.2)	1.8 (1.0–3.0)	24 (4.9)
	Overweight	1.9 (1.2–3.1)	36 (5.5)	2.6 (1.8–3.8)	175 (6.8)	2.3 (1.5–3.7)	47 (6.1)
	Obese	5.9 (3.5–10.0)	28 (15.5)	4.7 (3.2–6.9)	136 (12.0)	4.9 (3.3–7.5)	85 (12.1)
Intermediate GRS	Normal weight	1.6 (1.1–2.4)	164 (4.7)	1.7 (1.2–2.5)	355 (4.8)	2.0 (1.4–3.0)	86 (5.4)
	Overweight	3.1 (2.1–4.5)	173 (8.4)	3.7 (2.7–5.4)	735 (9.6)	4.4 (3.1–6.5)	274 (10.9)
	Obese	6.8 (4.6–10.3)	91 (17.3)	8.8 (6.3–12.7)	680 (20.5)	9.8 (7.0–14.2)	427 (21.5)
High GRS	Normal weight	1.6 (1.0–2.4)	52 (4.5)	2.2 (1.6–3.3)	149 (6.1)	3.1 (2.0–5.0)	44 (8.2)
	Overweight	5.3 (3.6–8.0)	92 (13.5)	6.5 (4.6–9.4)	388 (15.6)	8.1 (5.6–12.0)	162 (18.0)
	Obese	11.5 (7.2–18.5)	47 (26.1)	15.9 (11.3–23.2)	337 (31.6)	19.2 (13.3–28.4)	216 (35.1)

Note: Analyses were adjusted by sex, age, socioeconomic status, assessment center, genotyping array, and the first 10 principal components.

Abbreviations: OR (95% CI), odds ratio and 95% confidence interval; N_cases (%), number and percent of cases in each of the subgroups defined by 68–single nucleotide polymorphism (SNP) GRS, BMI, and physical activity categories.

TABLE 3 Combined associations of GRS, muscular fitness, and BMI with odds of suspected NAFLD

GRS categories stratified by BMI		Grip strength categories					
		High		**Intermediate**		**Low**	
		OR (95% CI)	N_{cases} (%)	OR (95% CI)	N_{cases} (%)	OR (95% CI)	N_{cases} (%)
Low GRS	Normal weight	**1.0 (Reference)**	148 (3.3)	1.1 (0.9–1.3)	326 (3.5)	1.3 (0.9–1.7)	73 (4.0)
	Overweight	2.2 (1.8–2.7)	251 (6.9)	2.2 (1.8–2.6)	775 (6.6)	2.4 (2.0–3.0)	248 (7.3)
	Obese	3.7 (2.8–4.9)	93 (11.1)	4.2 (3.5–5.1)	772 (12.3)	4.7 (3.9–5.7)	516 (13.4)
Intermediate GRS	Normal weight	1.5 (1.3–1.8)	667 (4.9)	1.5 (1.3–1.8)	1328 (4.8)	1.7 (1.4–2.1)	301 (5.3)
	Overweight	3.5 (3.0–4.2)	1136 (10.5)	3.4 (2.9–4.1)	3571 (10.1)	3.6 (3.0–4.3)	1052 (10.5)
	Obese	7.0 (5.8–8.5)	491 (19.0)	7.5 (6.4–8.9)	3682 (19.8)	7.8 (6.6–9.3)	2296 (20.4)
High GRS	Normal weight	1.9 (1.5–2.3)	289 (6.1)	2.0 (1.7–2.5)	585 (6.4)	2.2 (1.7–2.8)	128 (6.9)
	Overweight	5.8 (4.8–7.0)	584 (16.1)	5.6 (4.7–6.7)	1818 (15.5)	6.7 (5.6–8.1)	612 (17.8)
	Obese	14.3 (11.5–17.7)	288 (32.2)	13.3 (11.2–15.8)	1833 (30.4)	14.9 (12.5–17.9)	1191 (32.9)

Note: Analyses were adjusted by sex, age, socioeconomic status, assessment center, genotyping array, and the first 10 genetic principal components.

weight. Individuals at high genetic risk who were physically inactive and obese had 12-fold higher OR for suspected NAFLD than those who maintained normal body weight and high physical activity levels (translating to a prevalence of about 5% vs. 35% in these subgroups). The independent impact of muscular fitness was weaker. Nevertheless, we observed an 8-fold increase in the odds for suspected NAFLD due to combined effects of low grip strength in the setting of obesity among those who were genetically susceptible to NAFLD. A recent randomized weight loss trial investigated the effect of exercise, liraglutide, and both treatments combined for healthy weight-loss maintenance and found that the combined strategy of pharmacotherapy and exercise reduced body weight and body-fat percentage approximately twice as much as the single-treatment strategies.[38] Importantly, the combined strategy was associated with additional health benefits, such as improvements in insulin sensitivity, cardiorespiratory fitness, and physical functioning.[38] Thus, interventions to promote weight loss, including lifestyle, pharmacotherapy, weight-loss surgery, and possibly a combination thereof, might have increased efficacy among individuals at high genetic risk of NAFLD.[15,17] An important question to address in future studies is whether a GRS individually and/or compounded with lifestyle factors can add prognostic value and play a role in precision management of NAFLD.

Strengths of the present study include the large number of individuals with genetic and objectively assessed physical activity and grip strength, collected as part of the UK Biobank, in which the same protocol was used for all participants.[39] Applying a GRS based on a larger, more comprehensive set of NAFLD associated genetic variants may have a greater statistical power to detect gene–lifestyle interactions[39–41] compared with single SNPs and smaller GRSs used in the previous studies.[15–17] We used external weights, the gold standard, for the construction of the GRS[42] by computing the weighted GRS based on weights for each genetic marker as derived by GWAS in the Million Veteran Program.[21] This limits potential bias from overestimating the true genetic effect size as a consequence of the winner's curse.[43] In sensitivity analysis, we validated our observation of gene–lifestyle interactions in NAFLD by applying a smaller 15-SNP GRS, restricted to loci that were validated to associate with external histologically and/or radiologically defined NAFLD status[21] and found that the results were materially the same as compared with applying the larger 68-SNP GRS. This can be explained by the large correlation between the 15-SNP GRS and the 68-SNP GRS. Furthermore, we speculate that the 15 loci that were externally validated to associate with NAFLD status are more specific to NAFLD, while some of the remaining 53 SNPs that were included in the 68-SNP GRS (but not in the 15-SNP GRS) may be involved more directly in ALT biology rather than NAFLD. Importantly, to avoid spurious interactions between the GRS and adiposity due to gene-environment dependence, we excluded known BMI-associated loci when constructing the NAFLD GRS.[44] Additionally, we used relative grip strength to diminish confounding by body size and better reflect muscular fitness.[30–32]

As a limitation, we used ALT levels as a surrogate measure of NAFLD, and our findings warrant replication in a large data set with accurate assessment of NAFLD. Although liver biopsy and magnetic resonance imaging are the gold standards for diagnosing NAFLD, these invasive and expensive technologies are not yet feasible for population-based identification of NAFLD in clinical practice and research.[20] To mitigate this limitation, we excluded individuals with co-existing excessive alcohol use and other known causes of liver diseases that could lead to elevated ALT levels, making our findings more specific to NAFLD. We defined suspected

NAFLD based on cutoffs used in recent studies, including the Million Veteran Program GWAS from which our GRS was derived. However, other studies have suggested an upper limit of 19 U/L and 30 U/L for ALT in women and men, respectively.[45] Participants of the UK Biobank are generally healthier compared with the general population,[46] and the "healthy volunteer" bias may explain the low prevalence of 11% of suspected NAFLD in our study. Another limitation is that analyses were performed in a population of European genetic ancestry and cannot be generalized to other ancestry groups. As with secondary database analysis, there are limitations such as risk of classification bias with ICD codes and missing data, although this may be partly overcome by the very large sample size. Furthermore, alcohol intake was estimated based on self-reported information. We applied a cross-sectional study design, and future studies using a longitudinal study design or formal Mendelian randomization analyses will be needed to address causality. Moreover, we did not have data on total skeletal muscle mass or fat-to-muscle ratio, which would better characterize body composition compared with BMI (i.e., a person of athletic build may have a high amount of skeletal muscle mass, leading to a BMI in the overweight or obese category). It has been shown that intense muscular training (i.e., weightlifting) increases liver enzyme levels, including ALT levels, up to 7 days after the bout of exercise.[47,48] Although this could introduce bias if a participant was engaged in strenuous exercise training shortly before the baseline visit and ALT assessment, we believe that risk of bias is small, as we focused on daily physical activity levels and overall grip strength as part of our study, as compared with acute exercise training. Moreover, we demonstrated in the present study that higher physical activity and higher grip strength were associated with lower ALT levels. Recent studies support the idea that NAFLD is a part of a broader multisystem disease that also includes other cardiometabolic conditions such as obesity, type 2 diabetes, high blood pressure, and high cholesterol.[49] Classifying individuals into suspected NAFLD with or without other cardiometabolic conditions in future studies may provide new opportunities for gaining insight into gene–lifestyle interactions.

In conclusion, in this cross-sectional study, physical activity and muscular fitness attenuated, while adiposity amplified, genetic risk for elevated ALT levels. We demonstrated that the effect of obesity on suspected NAFLD risk is dominant over genetic risk, physical activity, and muscular fitness. Taken together, our findings support current health guidelines and indicate that lifestyle guidance to increase physical activity, muscular fitness, and evidently maintain a normal weight should be universally recommended for the prevention of NAFLD, especially for individuals with a high genetic predisposition.

CONFLICT OF INTEREST

E.A.A. reports advisory board fees from Apple, DeepCell, AstraZeneca and Personalis, outside the submitted work. The other authors have nothing to report.

AUTHOR CONTRIBUTIONS

Study concept: Theresia M. Schnurr, Sophia Figueroa Katz, Johanne M. Justesen, and Joshua W. Knowles. *Data analysis:* Theresia M. Schnurr, Sophia Figueroa Katz, Johanne M. Justesen, and Joshua W. Knowles. *Manuscript draft:* Theresia M. Schnurr, Sophia Figueroa Katz, Joshua W. Knowles, and Ivan Carcamo-Orive. *GRS expertise:* Jack W. O'Sullivan. *Computational analyses:* Themistocles L. Assimes. *Discussion:* Johanne M. Justesen, Jack W. O'Sullivan, Peter Saliba-Gustafsson, Themistocles L. Assimes, Euan A. Ashley, and Torben Hansen. *Data interpretation, manuscript revisions, and final approval of the manuscript:* All authors. Theresia M. Schnurr, Sophia Figueroa Katz, and Joshua W. Knowles had full access to all of the data and were responsible for the decision to submit for publication.

ORCID

Theresia M. Schnurr https://orcid.org/0000-0002-6573-4959
Sophia Figueroa Katz https://orcid.org/0000-0003-3495-4681

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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