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The body mass index increases the genetic risk scores' ability to predict risk of hepatic damage in European adolescents: The HELENA study

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Abstract

Background: Hepatic disorders are often complex and multifactorial, modulated by genetic and environmental determinants. During the last years, the hepatic disease has been progressively established from early stages in life. The use of genetic risk scores (GRS) to predict the genetic susceptibility to a particular phenotype among youth has gained interest in recent years. Moreover, the alanine aminotransferase (ALT) blood biomarker is often considered as hepatic screening tool, in combination with imaging techniques. The aim of the present study was to develop an ALT-specific GRS to help in the evaluation of hepatic damage risk in European adolescents.

Methods: A total of 972 adolescents (51.3% females), aged 12.5–17.5 years, from the Healthy Lifestyle in Europe by Nutrition in Adolescence study were included in the analyses. The sample incorporated adolescents in all body mass index (BMI) categories and was divided considering healthy/unhealthy ALT levels, using sex-specific cut-off points. From 1212 a priori ALT-related single nucleotide polymorphisms (SNPs) extracted from candidate gene selection, a first screening of 234 SNPs univariately associated was established, selecting seven significant SNPs ($p < .05$) in the multivariate model. An unweighted GRS (uGRS)

on behalf of the HELENA study group are detailed in Appendix S1.

was developed by summing the number of reference alleles, and a weighted GRS (wGRS), by multiplying each allele to its estimated coefficient.

Results: The uGRS and wGRS were significantly associated with ALT ($p < .001$). The area under curve was obtained integrating BMI as clinical factor, improving the predictive ability for uGRS (.7039) and wGRS (.7035), using 10-fold internal cross-validation.

Conclusions: Considering BMI status, both GRSs could contribute as complementary tools to help in the early diagnosis of hepatic damage risk in European adolescents.

KEYWORDS

ALT levels, BMI and adolescents, GRS, hepatic disorders, single nucleotide polymorphism

1 | INTRODUCTION

Some hepatic disorders are generally characterized by the presence of intrahepatic fat, already affecting children and adolescents.[1] In addition to imaging techniques, the risk of hepatic damage could be identified through abnormal levels in certain blood biomarkers, such as the alanine aminotransferase (ALT). Recommendations from the European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) and the North American Society of Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN), suggest ALT as one of the screening tools for children with or at risk of hepatic steatosis, combined with ultrasonography and the presence of other related comorbidities.[2,3]

The role of genetic determinants in the aetiology of hepatic diseases is of increasing interest.[4] The patatin-like phospholipase domain-containing 3 (*PNPLA3*) seems to be strongly associated to liver damage across different ethnic groups in early age populations.[5] This is observed in populations with diverse ancestry,[6] showing genetic variability in the pathogenesis involved in hepatic alterations. In this sense, the highest prevalence of hepatic damage was observed in Hispanic populations, showing a worse disease progression due to their genetic predisposition compared to other ethnicities.[7]

The association between the *PNPLA3* gene I148M variant (rs738409) and liver damage was first identified in adult population.[8] Then, the same genetic susceptibility related to hepatic fat content was observed in children of European origins.[9,10] Confirming this genetic susceptibility observed would be of great interest in other cohorts of European adolescents. However, the ability of single nucleotide polymorphisms (SNPs) to predict genetic variance by themselves could be limited as these polymorphisms represent a small fraction of the hepatic heritability.[11]

Combining a series of SNPs, either by summing the number of included alleles or by multiplying each estimated coefficient by the number of included alleles, would contribute to create a genetic risk score (GRS) to help in the prediction of higher ALT levels from early stages in life.[12] Scarce evidence has been found in the literature considering the development of a GRS aiming to predict predisposition to abnormal ALT levels in young populations. A cohort of children of different ethnic groups showed an association between a specific five SNP weighted GRS and hepatic fat content, being the association stronger among Hispanic individuals.[13] Moreover, in a cohort of European adolescents with obesity, another 11 SNP-GRS was developed, being significantly associated with the risk of hepatic damage assessed by ultrasonography.[10] However, studies conducted in adolescents of European ancestry with heterogeneous weight status remain scarce.

In combination of other clinical markers, the genetic background of individuals could partly explain the early predisposition to hepatic dysfunction from young age. The presence of other comorbidities such as obesity has been associated with elevated ALT serum levels, triggering the risk of hepatic alterations in adolescents of all ethnic groups.[14] Therefore, the aim of the present study was to develop an ALT-specific GRS including genome wide significant variants and the body mass index (BMI) status, to help in the evaluation of hepatic damage risk in European adolescents. Additionally, we intended to explore potential associations between new ALT-related genetic variants from candidate genes and ALT levels.

2 | METHODS

2.1 | Study design

The Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) study is a European, multi-centric and cross-sectional study carried out in 3528 adolescents,

aged 12.5–17.5 years across in 10 European cities.[15] The study sample criteria, recruitment process, data collection and other related procedures and methodology have been previously described.[16] The HELENA study design aimed to obtain reliable and comparable information on adolescents' nutritional status, life course exposures and health-related aspects to assess the underlying predisposition and prevention of chronic diseases in adulthood. Adolescents were recruited from randomly selected schools in each participating city. The HELENA study protocol complied with the ethical guidelines of the Declaration of Helsinki 1964 (revision of 2000), the Good Clinical Practice and the legislation about clinical research in humans. In addition, the study was approved by the local Research Ethics Committees from all participating countries in the present study.[17] A written consent was read and signed by all parents or guardians of the participating adolescents within the HELENA study. In terms of sample size, one third of the total participants were randomly selected for blood sampling ($n = 980$). Those adolescents with complete genomic (after performing a quality control check of the genotyping process for the present analysis) and hepatic related biomarkers data met the inclusion criteria ($n = 972$, 51.3% females). Having other hepatic disease or/and any other disease accompanied with elevated blood transaminase levels, such as viral hepatitis, toxic hepatitis or autoimmune diseases, were considered as exclusion criteria. The diagram flow chart of the inclusion criteria to obtain the final sample is displayed in Figure S1.

2.2 | Parental education level

Parental educational level data were collected using a specific questionnaire.[18] Parental educational level was adapted from the International Standard Classification of Education (ISCED) (http://uis.unesco.org/sites/default/files/documents/international-standard-classification-of-education-1997-en_0.pdf) in each participating country.

2.3 | Body composition measurements

Qualified professionals performed the anthropometric measurements according to the standard procedures and protocols.[19] Body height was obtained in participants in barefoot to the nearest .1 cm using a telescopic stadiometer (SECA 225). Body weight was measured in underwear and barefoot conditions to the nearest .1 kg. using an electronic scale (SECA 861). All measurements were recorded in triplicate. Continuous BMI was derived following the equation weight in kilograms divided by the square of height in metres. On the contrary, BMI status by categories was divided into normal weight versus overweight, including obesity (OW/OB), according to sex and age specific BMI international cut-off points suggested by the World Obesity Federation.[20] Finally, pubertal status was assessed during a medical examination carried out by a paediatrician following the standardized methodology proposed by *Tanner* and *Whitehouse*.[21] Pubertal status was categorized as Tanner stages ranging from no sexual maturation (stage I) to complete sexual maturation (stage V).

2.4 | Blood sampling procedure and hepatic biomarkers

The blood collection, transportation and sample analysis were performed according to standard procedures and were certified in an authorized laboratory.[22] Blood for DNA isolation was collected in ethylene diamine tetraacetate K3 (EDTA K3) tubes, stored at the Analytical Laboratory at the University of Bonn (Germany). Samples were sent to the Genomic Analysis Laboratory at the Institut Pasteur de Lille (France). The ALT liver enzyme was selected to detect the risk of developing hepatic alterations. The blood sample was extracted by venipuncture after an overnight fast. The ALT levels were measured in serum using standard protocols with the clinical chemistry system RxL (Dade Behring). Biology-based thresholds for diagnosis of hepatic diseases in children were used, considering sex-specific cut-off values of ALT: 25.8 U/L in males and 22.1 U/L in females, respectively, in healthy weight, metabolically normal and liver disease-free conditions.[23]

2.5 | Genomic information

DNA was extracted from white blood cells with the Puregene kit (QIAGEN) and stored in at −20°C by the Laboratoire d'Analyse Genomique Centre de Ressources Biologiques (LAG-CRB, BB- 0033-00071 Institut Pasteur de Lille) The whole genome genotyping was performed with the GSA chip of the Illumina system (San Diego).

After quality control, 515,612 genotyped SNPs were available. Additionally, around 7 million SNPs were obtained with imputation using the Haplotype Reference Consortium reference panel (variants were excluded if the imputation information <.3 and the MAF <.01).

2.6 | Genetic risk score development

For the purpose of the present analysis, a candidate gene approach was the procedure to select relevant genes

previously related to hepatic damage from the literature. A total of 12 genes related with hepatic damage (*PNPLA3*, *TM6SF2*, *GCKR*, *MBOAT7*, *HSD17B13*, *ENPP1*, *PPP1R3B*, *PLAGL1*, *PPARG*, *HFE*, *LEPR* and *LEP*) were investigated and extracted from the HELENA GWAS dataset, obtaining 1212 eligible SNPs. SNPs in high linkage disequilibrium (LD) ($r^2 > .8$) were removed, leading to a final number of 448 SNPs available for the present analysis: 52 SNPs were genotyped on the GSA chip and 396 SNPs were imputed. For the purpose of identifying potential genetic variability across geographical location and ethnic background between individuals, the first two principal components (PC) were calculated and subsequently considered in the analyses. Genotype distributions were tested for the Hardy–Weinberg equilibrium (HWE) ($p < .05$). Minimum allele frequency (MAF) was estimated, and SNPs not meeting criteria ($p < .10$) were discarded. HWE and MAF calculations were performed using the SNPassoc R package.[24] Based on the above, a total of 234 SNPs related to hepatic disorders available in the HELENA GWAS dataset were used to develop the GRS. In order to build the GRS, plasma ALT levels were selected as the variable to predict. Then, SNPs were recoded as 0, 1 or 2 depending on the number of risk alleles defined by the HELENA GWAS dataset. A further SNP selection was performed using univariate generalized linear models (GLM) to establish an initial cut-off point ($p < .50$) to filter the eligible SNPs. A total of 137 SNPs were used in a step by step algorithm to select the significant SNPs under the $p < .05$ threshold in a multivariate model. The final shortlist was formed by seven SNPs significantly associated with the ALT enzyme. The unweighted GRS (uGRS) was calculated by summing the number of risk alleles from the seven SNP variants with a rescaling, considering the SNPs that appear as protector factors. The weighted GRS (wGRS) was obtained as a result of multiplying the number of risk alleles at each locus (0, 1, 2) for each estimated beta coefficient of the multivariate model. Both uGRs and wGRS were derived using the predictABEL R package.[25] Multilinear models adjusted by sex, centre, Tanner, origins (PCs) and parental education level were developed trying to predict abnormal ALT levels by the combination of generic factors (GRS) and clinical factors (BMI). Therefore, individuals with no information from adjusting variables were removed, obtaining a final sample size of 819 adolescents for the purpose of building the final adjusted generalized model. Receiver operating characteristics (ROC) curve analysis[26] was applied to test the diagnostic accuracy of the GRS to classify potential individuals for ALT disturbances.[27] The area under curve (AUC) was calculated in uGRS and wGRS considering ALT levels as binary variable, using sex-specific cut-off points.[23] The Delong test was used to detect the higher value of the area under the curve (AUC) compared on uGRS and wGRS to proceed with the design of the final model. A 10-fold cross-validation analysis was performed to internally validate the model. The maximization of the Youden index[28] was performed to provide the best cut-off point for the use of the GRS as a dichotomic variable. Additionally, the concordance between predicted probabilities by uGRS and wGRS and the real occurrence of the event was analysed by means of calibration curves.

2.7 | Statistical analysis

The sex-specific descriptive characteristics are displayed as median and interquartile range (IQR) for continuous variables and as absolute and relative frequencies for categorical variables. Pearson's chi-square test was used for categorical variables and Mann–Whitney–Wilcoxon test for continuous variables to compare differences by sex. HWE was performed using the Pearson's chi-square statistic test. Shapiro–Wilk nonparametric test was used to check the variables' normality. The statistical part involving the development of the GRSs was previously described. RStudio Version 1.2.5001 [RStudio Team (2015). RStudio: Integrated Development for R. RStudio, Inc. URL http://www.rstudio.com/] was the software used to perform the analysis considering $p < .05$ significance level.

3 | RESULTS

3.1 | Demographics of the study sample

Main characteristics of the participants ($n = 972$, 460 males and 512 females) are shown in Table 1. The median age of the participants did not differ between males (14.7 yo, IQR 13.6–15.7) and females (14.6 yo, IQR 13.5–15.7) ($p = .659$). Although not significant, the prevalence of elevated ALT levels was higher in males (27.8%) than in females (23.2%) ($p = .117$).

In all subjects ($n = 972$), the subgroup analysis elevated ALT concentrations ($n = 245$) showed differences between adolescents within the normal weight categories ($n = 158$, 64.5%) versus adolescents with overweight or obesity (OW/OB) ($n = 87$, 35.5%) ($p = .042$). Moreover, subgroup analysis by OW/OB ($n = 217$) showed no differences between adolescents with elevated ALT ($n = 87$, 40.1%) concentrations versus normal ALT concentrations ($n = 130$, 59.9%) ($p = .373$). Adolescents with OW/OB and elevated ALT levels represented the 8.9% of the total studied sample ($n = 87$) (Table 1).

In the general model sample ($n = 819$), significant differences were observed in terms of weight ($p < .001$), height ($p < .001$), BMI (z) total sample ($p = .021$) and

TABLE 1 Main characteristics of the HELENA sample.

Variable name	All *n* = 972	Males *n* = 460	Females *n* = 512	*p*
Age (years)	14.6 (13.5–15.7)	14.7 (13.6–15.7)	14.6 (13.5–15. 7)	.751
ALT categories [*n* (%)]				.117
Normal ALT levels	727 (74.7)	332 (72.2)	395 (76.8)	
Elevated ALT levels	245 (25.3)	128 (27.8)	117 (23.4)	
Elevated ALT levels [*n* (%)]	*n* = 245	*n* = 128	*n* = 117	.042
Normal weight	158 (64.5)	75 (58.6)	83 (70.9)	
OW/OB	87 (35.5)	53 (41.4)	34 (29.1)	
OW/OB status [*n* (%)]	*n* = 217	*n* = 123	*n* = 94	.373
Normal ALT levels	130 (59.9)	70 (56.9)	60 (63.8)	
Elevated ALT levels	87 (40.1)	53 (43.1)	34 (36.2)	
HDL-c (mmol/L)	55.0 (49.0–63.0)	53.0 (47.0–61.0)	57.0 (50.0–64.0)	**<.001**
TG (mmol/L)	60.0 (46.0–81.0)	57.0 (42.0–77.0)	63.0 (49.0–85.0)	**<.001**
HOMA index	*n* = 938	*n* = 446	*n* = 492	
	1.92 (1.36–2.71)	1.84 (1.30–2.69)	2.01 (1.38–2.76)	.087
FMI (kg/m^2)	*n* = 920	*n* = 418	*n* = 502	
	4.68 (3.1–6.7)	3.47 (2.3–6.1)	5.3 (4.0–7.0)	**<.001**
WC (cm)	*n* = 883	*n* = 416	*n* = 467	
	71.0 (66.6–76.35)	72.9 (68.0–78.9)	69.5 (65.2–74.5)	**<.001**
General model	**All *n* = 819**	**Males *n* = 378**	**Females *n* = 411**	***p***
Weight (kg)	58.6 (49.9–65.0)	61.8 (52.1–69.7)	55.7 (48.8–60.6)	**<.001**
Height (cm)	165.3 (158.9–171.9)	169.2 (161.9–176.8)	161.8 (156.8–166.8)	**<.001**
BMI (kg/m^2)	21.1 (18.6–22.8)	21.1 (18.5–22.8)	21.0 (18.8–22.7)	.507
BMI (*z*)				
Total sample	.35 (−.38–1.15)	.41 (−.28–1.29)	.25 (−.44–1.03)	**.021**
Normal ALT	*n* = 610	*n* = 275	*n* = 335	.094
	.27 (−.44–.99)	.38 (−.33–1.15)	.22 (−.48–.95)	
Elevated ALT	*n* = 209	*n* = 103	*n* = 106	.165
	.56 (−.23–1.58)	.83 (−.03–1.71)	.44 (−.31–1.52)	
Pubertal stage [*n* (%)]				.039
I	8 (1.0)	7 (1.8)	1 (.2)	
II	67 (8.2)	38 (10.0)	29 (6.5)	
III	177 (21.6)	74 (19.5)	103 (23.3)	
IV	333 (40.7)	150 (39.6)	183 (41.5)	
V	234 (28.6)	109 (28.8)	125 (28.3)	
Mother's education [*n* (%)]				.788
Lower education	53 (6.5)	21 (5.6)	32 (7.3)	
Lower secondary education	196 (24.0)	90 (23.8)	106 (24.0)	
Higher secondary education	284 (34.7)	132 (34.9)	152 (34.5)	
Higher education	286 (35.0)	135 (35.7)	151 (34.2)	

Note: Table values are presented as median (p25–75). Mann–Whitney-Wilcoxon test was performed tested sex differences in age, weight, height, HDL-c, TG, HOMA index, FMI, WC, body mass index (BMI) and BMI z-score in the general model. Chi-square tested sex differences between ALT categories [cut-off values: 25.8 U/L (male) and 22.1 U/L (female)], elevated ALT levels and OW/OB status; pubertal stages and mother's education in the general model. Significant *p*-value (<.05) displayed in bold format.

Abbreviations: ALT, alanine aminotransferase; BMI, body mass index; FMI, fat mass index; HDL-c, high density lipoprotein cholesterol; HOMA, homeostatic model assessment; OW/OB, overweight/obesity; TG, triglycerides; WC, waist circumference.

pubertal stage ($p = .039$) (Table 1). Finally, differences were observed in terms of GRS for adolescents with elevated ALT levels (uGRS: 6, IQR 5–7; wGRS: .871, IQR .539–1.173) versus normal ALT levels (uGRS: 5, IQR-4-6; wGRS: .574, IQR .238–.918) ($p < .001$).

3.2 | Associations between SNPs and hepatic biomarkers

A final number of seven SNPs were significantly associated with ALT levels within the HELENA participants (Table 2). The univariate and multivariate model's odds ratio (OR) of each of the selected SNPs in the GRS development, according to normal versus elevated ALT levels as binary variable, is shown in Table 3. The direction (OR), either protective or risk factor, for each SNP is presented in a forest plot (Figure 1).

Thus, *ENPP1* rs12209268 and *PNPLA3* rs1883350 were inversely considered in terms of ALT susceptibility, whereas *PNPLA*3 rs738409, *LEPR* rs11208659 and rs9436299, *PLAGL1* rs2064495 and rs17073227 were positively considered to increase the ALT levels. A multivariate logistic regression model to observe risk of higher ALT levels was used to compute a wGRS. The predictive ability of both GRS models, using the ROC curve, AUCs and Youden Index is displayed in Figure 2.

The AUC results for both GRSs, also considering BMI and adjusting covariates, indicated sufficient ability for clinical discrimination in terms of presence of potential hepatic disorders (uGRS: .7039 vs. wGRS: .7035). AUC's comparisons did not show statistically significant differences between GRSs to predict the presence of potential hepatic disorders ($p = .505$). In order to assess the discrimination ability of both GRSs, internal validation was carried out using 10-fold cross-validation analysis, indicating robust predictions according to the AUC results (uGRS = .7039 vs. wGRS = .7035). Distribution of uGRS and wGRS values for the groups risk versus no risk of elevated ALT levels is shown in a boxplot (Figure 3).

Both GRSs could discern between groups although there is not a threshold which could differentiate

TABLE 2 Main characteristics of the seven single nucleotide polymorphisms (SNPs) forming the alanine aminotransferase (ALT) genetic risk score (GRS).

rs number	Nearest gene	Alleles (major/minor)	MAF	Imputation score	HWE
rs9436299	*LEPR*	A/C	.707	.923	.089
rs11208659	*LEPR*	T/C	.106	.989	.235
rs17073227	*PLAGL1*	C/T	.343	.971	.394
rs12209268	*ENPP1*	A/G	.212	.995	.213
rs2064495	*PLAGL1*	T/C	.792	.892	.284
rs1883350	*PNPLA3*	T/C	.311	.995	.709
rs738409	*PNPLA3*	C/G	.245	.995	.225

Note: SNPs ordered by chromosome number. Association of SNPs in relation to the alanine aminotransferase [enzyme displayed in *p* values (*p*)].

Abbreviations: *ENPP1*, ectonucleotide pyrophosphatase/phosphodiesterase 1; HWE, Hardy–Weinberg Equilibrium; *LEPR*, leptin receptor; MAF, minimum allele frequency; *PLAGL1*, *PLAG1* like Zinc finger 1; *PNPLA3*, patatin-like phospholipase domain-containing protein 3.

TABLE 3 Selection of the seven single nucleotide polymorphisms forming the genetic risk score and its association with alanine aminotransferase (ALT) levels (normal vs. elevated levels).

		Univariate		Multivariate	
rs number	Chromosome	OR (95% CI)	*p*	OR (95% CI)	*p*
rs9436299	1	1.416 (1.143–1.753)	.001	1.562 (1.248–1.994)	<.001
rs11208659	1	1.145 (.817–1.588)	.421	1.428 (1.001–2.021)	.046
rs17073227	6	1.336 (1.076–1.658)	.008	1.569 (1.248–1.994)	<.001
rs12209268	6	.753 (.573–.980)	.037	.712 (.540–.932)	.014
rs2064495	6	1.259 (.978–1.616)	.070	1.501 (1.139–1.976)	.003
rs1883350	22	.884 (.705–1.105)	.284	.769 (.590–.997)	.049
rs738409	22	1.154 (.915–1.451)	.22	1.380 (1.052–1.810)	.019

Note: Univariate (individual model of SNP-hepatic risk association) and multivariate model (multiple model SNP-hepatic risk association) shown with odds ratios (OR) and 95% confidence intervals (CI). SNPs ordered by chromosome number. Association of SNPs in relation to the alanine aminotransferase [enzyme displayed in *p* values (*p*)].

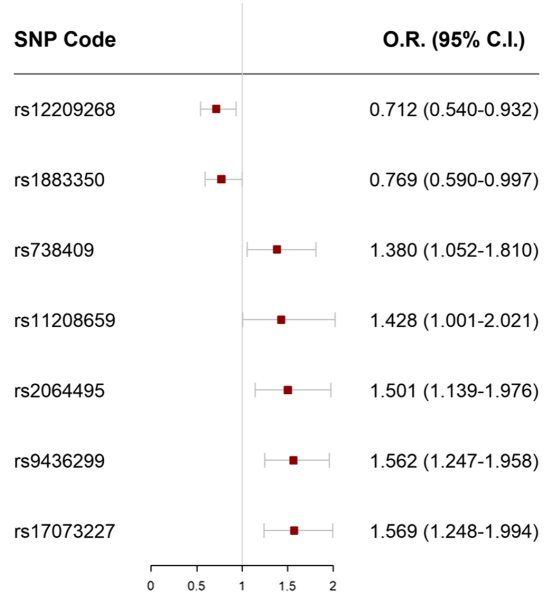

FIGURE 1 Forest plot of single nucleotide polymorphisms (SNPs) with negative (odds ratio < 1) and positive (odds ratio > 1) association with alanine aminotransferase (ALT) levels (normal vs. elevated levels). SNPs ordered by odds ratio (OR) values. Protective SNPs against ALT levels are shown in the upper part of the forest plot; deleterious SNPs for ALT levels are shown in the bottom part. Multivariate model OR and 95% confidence intervals displayed.

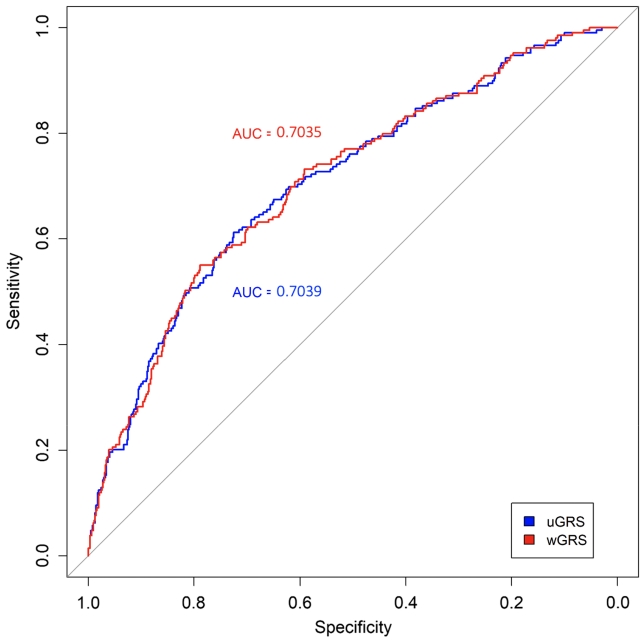

FIGURE 2 Receiver operating characteristics (ROC) curves of the unweighted genetic risk score (uGRS) and the weighted genetic risk score (wGRS). Areas under curves (AUC) are indicated. The straight line represents the ROC expected by chance only.

between healthy or unhealthy ALT levels. Lastly, the Youden Index was calculated: uGRS: 5.5 (53.2% specificity, 56.8% sensitivity) versus wGRS: .62 (51.5% specificity, 69.6% sensitivity). An in-depth analysis of sensitivity, specificity, positive and negative predictive value, and accuracy is displayed in Table S1. The calibration curves displayed in Figure 4 (uGRS in left panel and wGRS in right panel) show a good agreement between actual and predicted probabilities, with a minimum underestimation for probabilities above .5 for wGRS and .6 for uGRS. As it can be seen in the bottom of the graph, most probabilities are under these values, showing a good calibration.

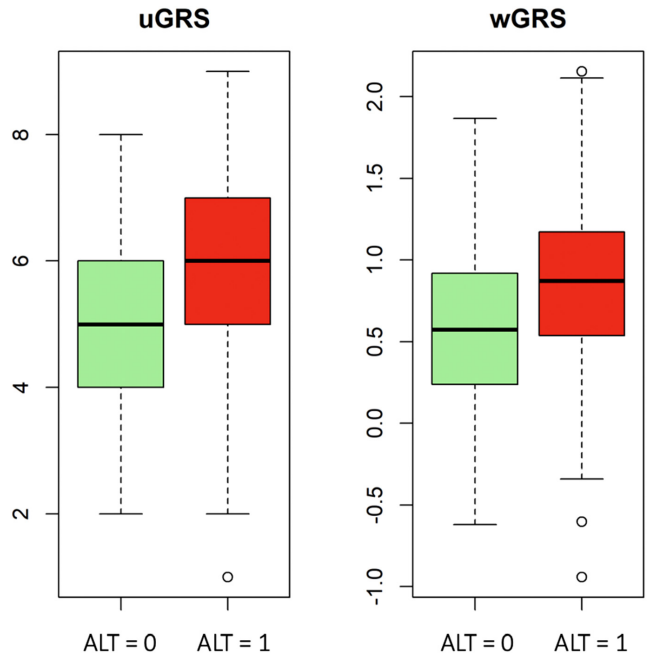

FIGURE 3 Boxplot of the distribution of unweighted genetic risk score (uGRS) and weighted genetic risk score (wGRS). Values for the groups indicate: 0 = no risk of hepatic damage versus 1 = risk of hepatic damage. The uGRS boxplot indicates number of risk alleles whereas wGRS boxplot indicates numeric values for weighted format of risk alleles. ALT, alanine aminotransferase.

4 | DISCUSSION

The present study associated ALT-specific GRSs (weighted and unweighted) with ALT levels in European adolescents. Our GRSs have considered protective and risk SNPs coexisting at the same time, conferring a more comprehensive approach in the prediction of genetic susceptibility to abnormal ALT levels. Both GRSs contained seven SNPs, of which five of them were significantly associated with elevated ALT enzyme concentrations. However, the ALT-GRSs showed a moderate ability to predict hepatic risk, so the use and applicability of these GRSs should be considered with caution.

To our knowledge, no studies on normo-weight European adolescents have previously focused on

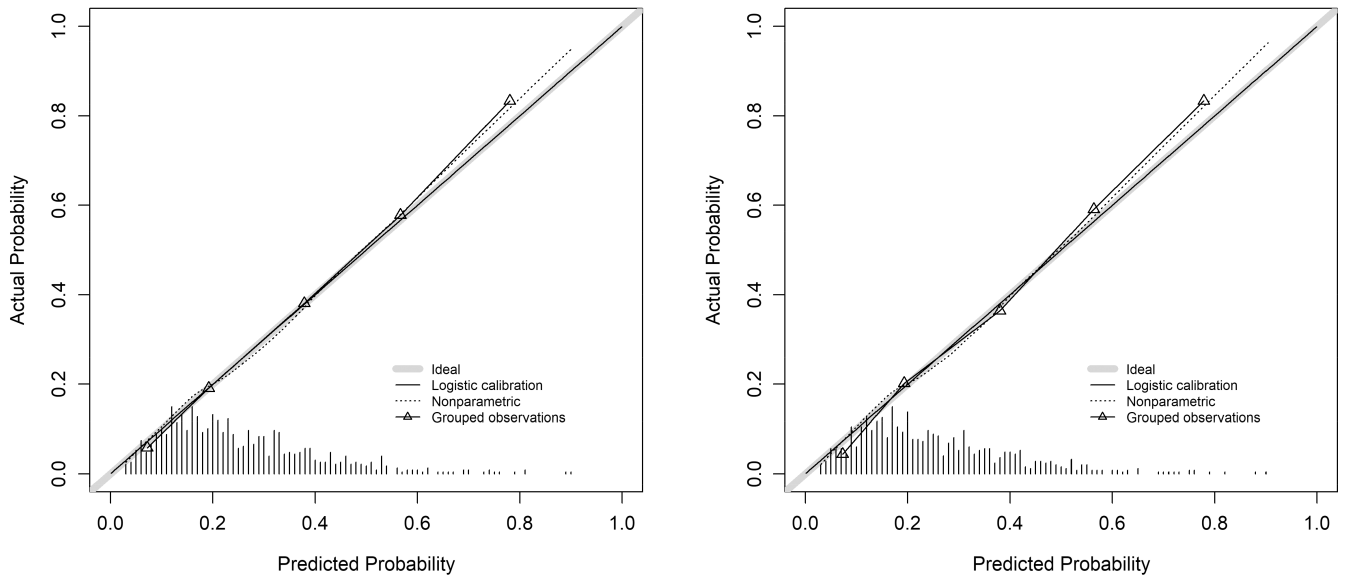

FIGURE 4 Calibration curves mean between predicted probabilities by unweighted genetic risk score (uGRS, left panel) and weighted genetic risk score (wGRS, right panel) and the real occurrence of the event.

ALT-specific GRSs to help in the early diagnosis of hepatic risk. Overall, a combination of SNPs associated with hepatic disorders identified in GWAS from European populations showed good ability to predict risk hepatic damage.[10,29] However, not all GRSs are able to show a great discriminatory capacity of hepatic risk, as it was shown in our GRSs and also with a hepatic specific GRS of 4 SNPs in a cohort of Spanish adolescents (67% sensitivity and 65% specificity).[30] All mentioned studies were performed in population with established obesity. Exceptionally, a study was conducted in normal-weight multi-ethnic children and adolescents, where the hepatic related five SNP GRS showed an association with higher hepatic fat fraction in lean Hispanic adolescents ($\beta = .20$; p-value $= .007$).[13]

Some of the SNPs included in our GRS have also been associated with a higher hepatic risk in previous studies. The *I148M* (rs738409 *C/G*) variant of the *PNPLA*3 gene has been consistently associated with higher hepatic damage in in adults,[31] adolescents,[29,32] and children[9,33] of European origin. The *PNPLA3* rs738409 usually shows the highest frequency among all available SNPs to predict hepatic risk in the literature. Thus, the *PNPLA3* rs738409 was also part of the ALT-GRS developed within the studied cohort. Moreover, the susceptibility of *PNPLA3* rs738409 to increased hepatic fat content in youth can be observed across different ethnic populations.[5,34]

Interestingly, the *PNPLA3 rs1883350* was newly identified to have a protective role within the GRS developed. Other previous studies also showed certain genes with single variants acting in opposite directions (i.e. *PCSK9*: hypercholesterolaemia[35]) and also producing changes in certain K ATP channels activating the insulin secretion function, which could result in either hyperinsulinism or neonatal diabetes (*KCNJ11*).[36] Since the present GRS was not externally validated, the results obtained, particularly in polygenic diseases, should be interpreted carefully.

In addition, other SNPs comprising our GRS were also observed to be somehow associated with cardiometabolic phenotypes. For example, the *PLAGL1* rs17073227 had been previously associated with transient neonatal Type 1 diabetes mellitus (T1DM).[37] In contrast to our findings, a recent GWAS of ALT serum concentrations performed in a large sample of adult subjects from the UK biobank did not show any specific associations with the *PLAGL1* and risk of hepatic damage.[38] These contradictory results could be explained by age differences in the studied participants. In any case, our results should be replicated in further cohorts of different age and ethnicity to test the reliability and validity of the association reported in the present manuscript.

Another study considering *LEPR* rs11208659, also included in the present GRS as risk variant, showed inverse associations with cardiometabolic parameters in Spanish children with obesity, reporting an influence on insulin resistance in both male and female youth.[39] Finally, a meta-analysis on different obesity genes showed that *LEPR* rs9436299 was associated with BMI in adult African Americans ($p = 2.71 \times 10^{-3}$).[40] To the best of our knowledge, the rest of SNPs included in our GRSs (*ENPP1* rs12209268 and *PNPLA3* rs1883350 as protector factors and *PLAGL1* rs2064495 as risk factor) could be considered as new predictive variants, as they have not previously been associated with hepatic related risk factors nor with other phenotypes. However, these variants not previously observed in the literature should be interpreted cautiously, as no association

with liver damage was found in the literature. Thus, replicating the approach of the present analysis in other cohorts of similar characteristics would help to confirm whether the effect observed is age or ethnic-specific dependent, or else, there is no relationship with liver damage at all.

Other SNPs proposed to be explored in our initial analysis were associated with hepatic disorders (*MBOAT7* rs641738,[41] *MBOAT7* rs626283[42] and *TM6SF2* rs58542926[10]). However, despite the evidence found in genes such as *MBOAT7* and *TM6SF2* and their association with hepatic risk among European adolescents, our study showed no combined effect with any SNP of the mentioned hepatic associated genes. On the contrary, the *ENPP1* gene was represented in our GRS, although the *ENPP1* rs1044498, which indicated a predisposition to hepatic damage in the literature, did not show a relevant association in the present analysis.[29]

In terms of GRS development, the gold standard technique is applying the use of external weights from meta-analysis, when available. If external weights are not available, the sum of risk alleles is commonly accepted when assessing genetic risk through an uGRS.[43] The present study used internal weights from the genetic effects obtained during the statistical analysis. In this case, the wGRS showed a similar predictive ability than the uGRS. However, the AUC of the ROC analysis was not strong to obtain sufficient clinical value for screening combined genetic factors alone (AUC <70). Due to the little predictive capacity of the ALT-related SNPs by themselves, the BMI, considered as related clinical hepatic risk factor, was included in the model, improving the ability to predict elevated ALT levels (uGRS: .7039 vs. wGRS: .7035) among the present cohort of European adolescents. Therefore, the predictive ability of the ALT-GRSs developed could be considered as moderate and BMI dependent. Similarly, other ROC curve results combining clinical risk factors and genetic susceptibility were obtained for hepatic related disorders (steatohepatitis) in another study in European children and adolescents with obesity, improving the AUC: .80 (95% CI .73–.87) ($p < .001$).[44]

The current study presents some limitations. Despite the confirmation of a successful internal validation of the model to form the GRSs, we acknowledge that the optimal situation would have been to perform an external validation in a different group of European adolescents. Furthermore, although PC analysis was performed to control the genetic variability among individuals, the present GRSs should not be replicated in other non-European populations, as the allele frequency and their effect size might differ across ethnicities. Moreover, although the present study has considered the Tanner stage as controlling factor for pubertal changes, it is assumable that periods of rapid growth could influence short-term changes in ALT levels.[41] Moreover, the discovery of new genetic variants associated with a disease in GWAS studies is still ongoing; thus, the results obtained might vary depending on the ethnicity and number of subjects included in the study.[45] The gold standard diagnostic technique is the liver biopsy.[46] However, it was not possible to perform a diagnostic test of invasive nature,[47] especially at this age range. Alternatively, less invasive methods, such ultrasonography or magnetic resonance imaging (MRI) have shown high accuracy for diagnosing liver fat content.[48,49] However, none of the mentioned imaging techniques were considered within the HELENA study. Other noninvasive serum biomarkers such fatty liver index or ALT enzyme concentrations are also considered to be useful complementary diagnostic tools for hepatic steatosis,[50] fibrosis[51] and also to observe associations between hepatic steatosis and common genetic variants.[52] In addition, studies observed associated the genetic predisposition to hepatic risk are predominantly conducted in subjects with established obesity whereas the present study considered the risk of elevated ALT levels in a cohort where subjects with overweight or obesity represent the 22.3% of the total sample size.

At the same time, there are also some strengths in the present study. Two GRSs were developed in a normally distributed cohort of normal-weight adolescents of 10 European cities, when only 25.3% of individuals had increased ALT levels. This fact allows the genetic tools to assess the potential risks of developing excess of hepatic fat levels when the advanced stages of the hepatic disease are not established yet. Most studies analysed in similar age populations were performed in subjects with overweight or obesity, where it is likely that some form of hepatic damage is already an additional cardiometabolic risk factor in the adolescents´ health status.

5 | CONCLUSIONS

In conclusion, the uGRS and wGRS developed to evaluate the genetic predisposition to elevated ALT levels could be considered as complementary diagnostic tools to identify hepatic damage risk from early stages in life. However, the ability of these GRSs to predict hepatic risk was moderate and needed the BMI to be considered for prediction improvement. These second line of diagnostic techniques, together with imaging technology as the main definitory hepatic assessment, could help progressing in the personalization of treatment strategies, particularly in youth.

AUTHOR CONTRIBUTIONS

Luis A. Moreno: design research study and supervision. Miguel Seral-Cortes and Idoia Labayen: contributed important reagents. Miguel Seral-Cortes, Luis Mariano

Esteban, Sergio Sabroso Lasa, Manon Muntaner and Aline Meirhaeghe: analized data, data curation and software. Miguel Seral-Cortes wrote paper. Marcela Gonzalez-Gross, Carlos Quesada-Gonzalez, Peter Stehle, Frederic Gottrand, Ascension Marcos, Ligia Esperanza-Diaz, Yannis Manios, Odysseas Androutsos, Kurt Widhalm, Denes Molnar, Inge Huybrechts, Diego Salazar-Tortosa and Jonatan R. Ruiz: review and editing. All authors read the draft and agreed on the final version.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results. The content of this article reflects the authors' views alone, and the European Community is not liable for any use that may be made of the information contained herein.

INFORMED CONSENT

Informed consent was signed by parents of all participants.

DATA AVAILABILITY STATEMENT

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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