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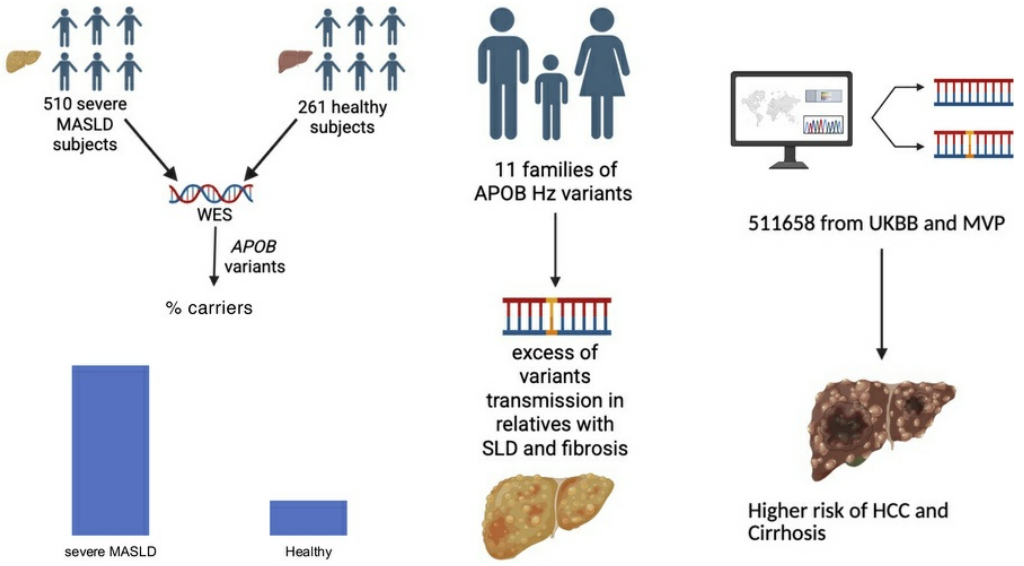
Graphical abstract



Carriage of rare *APOB* variants predisposes to severe steatotic liver disease and hepatocellular carcinoma

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Abstract

Background: MASLD has a substantial inherited component. Rare variants in Apolipoprotein B gene (*APOB*) have been implicated in susceptibility to liver steatosis, but their role in disease progression and outcomes is unclear.

Methods: We investigated *APOB* rare variants in a case-control cohort of people with advanced MASLD vs. healthy controls (n=510/261), a family-based study (n=43 and literature meta-analysis), the Million Veteran Program cohort (MVP, n=94,885) and the UK Biobank (UKBB, n=417,657).

Results: In the clinical cohort, *APOB* variants were enriched in people with advanced MASLD (OR 13.8, 95% c.i. 2.7-70.7, p=0.002) and associated with lower circulating lipids, but higher MASLD activity and fibrosis ($p<0.05$). In the family study, *APOB* variants segregated with hepatic steatosis and fibrosis ($p<0.05$). Cross-ancestry meta-analysis of the study cohorts yielded pooled ORs for cirrhosis and hepatocellular carcinoma of 1.82, 95% c.i. 1.33-2.49 and 3.53, 95% c.i. 2.09-5.98, respectively. Variants affecting specifically ApoB100 had a three-fold greater impact on hepatic lipid metabolism compared to those impairing also ApoB48 and were specifically protective against coronary artery disease ($p<0.05$). Variants affected cirrhosis risk similarly, but ApoB48/100 had a larger impact on hepatocellular carcinoma ($p<0.05$).

Conclusions: Rare *APOB* variants predispose to advanced MASLD and HCC, with distinct contributions from disrupted VLDL and chylomicrons secretion. These findings highlight the interplay between hepatic and intestinal lipid handling, suggesting that *APOB* genotyping may enhance MASLD risk stratification and case identification.

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Introduction

Driven by the global epidemics of obesity and type 2 diabetes (T2D), metabolic dysfunction-associated steatotic liver disease (MASLD) is now the primary cause of liver disease, affecting one in three individuals worldwide (1). MASLD can progress to steatohepatitis (MASH), which promotes hepatic fibrogenesis and is emerging as a major cause of cirrhosis and hepatocellular carcinoma (HCC) (2). At the cellular level, MASLD is defined by excessive accumulation of triglycerides within intracellular lipid droplets (LDs), reflecting a fundamental disturbance of lipid metabolism (3). Despite its high prevalence and clinical impact, noninvasive tools for accurate risk stratification remain a major unmet clinical need (4).

Heritability accounts for approximately 50% of MASLD interindividual variability, and genome-wide association studies (GWAS) have identified the major common genetic contributors to disease susceptibility (5). The genetic architecture of common variants indicates a largely linear relationship between genetic effects on hepatic fat accumulation and the risk of downstream outcomes, including cirrhosis and hepatocellular carcinoma (6-8). We previously demonstrated that the main genetic determinants of steatohepatitis dissociate hepatic and cardiometabolic complications of MASLD (9). Subsequent work highlighted that some genetic variants drive liver-specific outcomes, whereas others influence cardiometabolic complications (10). Nonetheless, a large portion of the variability of severe MASLD remains unexplained (11).

Apolipoprotein B (*APOB*) encodes for the major apolipoprotein responsible for triglyceride secretion in the liver and small intestine. Tissue-specific post-transcriptional mRNA editing (C->U deamination) introduces a premature stop codon resulting in the synthesis of two distinct isoforms: the full-length ApoB100 (~500 kDa) in hepatocytes, which is required for very low-density lipoprotein (VLDL) secretion, and the truncated ApoB48 (~240 kDa) in enterocytes, which is required for chylomicron formation (12). Heterozygous *APOB* loss of function (LoF) variants are associated with reduced VLDL secretion and protection against atherosclerosis (13). Further, homozygous familial hypobetalipoproteinemia, caused by *APOB* LoF mutations, leads to very low levels of ApoB-containing lipoproteins, lipid malabsorption and elevated risk of cirrhosis (14). Accordingly, heterozygous *APOB* LoF rare variants anecdotally co-segregated with cirrhosis and HCC in extended family pedigrees (15, 16). The association between *APOB* LoF variants and liver disease has been corroborated by studies in the population-based UK Biobank cohort (UKBB) (17, 18). However, the contribution of these variants to severe liver disease and HCC in clinical studies has not been systematically examined, especially for their potential to explain individual risk and to co-segregate with liver disease in families (15, 16, 18-20). Additionally, the impact of

mutation location, whether disrupting both ApoB48 and ApoB100 (upstream of amino acid 2154) or only ApoB100 (downstream), has not been investigated.

Here, we aimed to determine how rare *APOB* variants affecting ApoB48 and/or ApoB100 contribute to severe MASLD, defined as the presence of advanced fibrosis or HCC. To this end, we employed a comprehensive approach using clinical and histological, familial analysis, and large-scale population-based biobank data.

Results

APOB variants are enriched in people with severe MASLD

We started by examining a case-control study of people with severe MASLD (cases=510 and controls=261; Supplementary Table 3). Rare variants in *APOB* (definition A) were enriched more than 6-fold in cases compared to controls (4.5% vs. 0.7%, Figure 1A). After adjusting for age and sex, *APOB* variant carriers had an almost 14-fold higher risk for severe MASLD (Figure 1A-B and Supplementary Table 4 reporting burden test results; adjusted OR 13.8, 95% c.i. 2.7-70.7, p=0.002). In a sensitivity analysis, using the more stringent definition B, this risk increase was even larger (adjusted OR for definition B: 21.5, 95% c.i. 1.9-236, p=0.012; Figure 1 and Supplementary Table 4). Similar results were obtained for pure LoF variants (Supplementary Table 5).

Among people in the clinic, *APOB* variant carriers exhibited markedly reduced LDL cholesterol and triglyceride levels alongside modestly elevated HDL cholesterol in plasma ($p<0.05$; Supplementary Table 3), although they were less frequently on statins (p=0.059), supporting a LoF effect of the variants identified. In people who were not on statins, lower LDL cholesterol levels (71.7 vs. 104.1 mg/dl, p=0.002 for definition A; 48.6±30.0 vs. 103.9±41.6, $p<0.0001$ for definition B in carriers vs. non-carriers), allowed to predict the presence of *APOB* variants (AUROC 0.73, best threshold 55.4 mg/dl, 50% sensitivity and 42% specificity for definition A; AUROC 0.89, best threshold 70.6 mg/dl, 90% sensitivity and 72% specificity for definition B). *APOB* variant carriers had lower platelets (p=0.006, Supplementary Figure 1A) and higher FIB-4 score (p=0.04, Supplementary Figure 1B), as well as higher prevalence of advanced liver fibrosis (11/11, 100%, vs. 309/499, 61.9%, p=0.03). Among people with available liver biopsy (n=156), *APOB* variant carriers had higher grades of ballooning (p=0.014, Supplementary Table 3).

Inheritance of APOB variants is associated with the MASLD spectrum within families

Next, we characterized 11 families (Milan family study, Supplementary Figure 2 and Supplementary Table 2), whose probands had severe MASLD. Overall, we examined 32 first-degree relatives, 12 (37.5%) with steatosis, and 7 (21.8%) with liver fibrosis. Within these pedigrees, *APOB* variants co-segregated with liver steatosis (p=0.0045), severe steatosis (p=0.004), and fibrosis (p=0.034, Table 1). These associations were strengthened by including additional family pedigrees from previous studies in the analyses (Table 1).

Carriage of APOB variants is associated with cirrhosis and HCC in large population cohorts

Next, we sought to replicate our findings in two large independent cohorts, namely the Million Veteran Program biobank (MVP) and UK Biobank (UKBB). Considering the large size and the study design, in these cohorts we focused the analyses on LoF and LoF/deleterious missense variants. Among MVP participants (Supplementary Table 6), carriage of LoF *APOB* variants was associated with increased risk of cirrhosis in European, and of HCC in both African and European ancestries ($p<0.05$ for all). In the whole cohort, *APOB* variants conferred an increased risk of cirrhosis (OR 3.28, p 0.009) and HCC (OR 11.68, p 6.35 E-05). In the UKBB, rare variants were associated with a higher risk of cirrhosis and HCC (Supplementary Table 7). The impact of rare *APOB* variants on liver and cardiometabolic-related phenotypes in UKBB is presented in Figure 2 and Supplementary Table 8. Expectedly, *APOB* carriers had lower LDL-cholesterol, lower circulating triglycerides, higher HDL-cholesterol and were protected against coronary artery disease (CAD). Conversely, they had higher hepatic triglyceride content, liver enzymes, and prevalence of chronic liver disease ($p<0.05$ for all). Interestingly, *APOB* variant carriers had higher C-Reactive Protein (CRP), glucose, HbA1c, and prevalence of diabetes (all $p<0.05$). Finally, we performed a cross-ancestry meta-analysis on liver outcomes using harmonized definition of LoF and LoF/missense in the three studies, demonstrating that *APOB* carriers have a higher risk of cirrhosis (P_{Cauchy}= 1.79E-6; Figure 3A) and of HCC (P_{Cauchy}=7.59E-12, Figure 3B). The pooled OR for cirrhosis was 1.82 (95% c.i. 1.33-2.49) for LoF/missense, and 2.74 (95% c.i. 1.83-4.09) for LoF variants. The pooled OR for HCC was 3.53 (95% c.i. 2.09-5.98) for LoF/missense and 7.79 (95% c.i. 4.37-13.92) for LoF variants.

Differential impact of LoF variants on ApoB isoforms

Next, we examined the effect of *APOB* variants on liver outcomes based on the aminoacidic sequence location where the variants occur and therefore by their impact on the two ApoB isoforms. Specifically, if the mutation occurred downstream of the amino acid 2154 the LoF affects only ApoB100. Conversely, if the mutation occurred upstream of this position, both isoforms are affected, namely ApoB48/100 (Figure 1B and Figure 3). Results are reported in detail in Supplementary Table 6, 7 and 8 and in Figure 3. While both the LoF and LoF/missense mutations in ApoB48/100 and ApoB100 isoforms showed a similar level of significance in the association with cirrhosis (P_{Cauchy}=4.70E-4 for ApoB48/100 and P_{Cauchy}=2.59E-4 for ApoB100), mutations in ApoB48/100 were more strongly associated with HCC (P_{Cauchy}=6.07E-11) than mutations in ApoB100 alone (P_{Cauchy}=1.60E-3). Concerning ApoB48/100 variants, the pooled OR for cirrhosis was 1.98 (95% c.i. 1.24-2.14) for LoF/missense, and 2.90 (95% c.i. 1.64-5.14) for LoF variants. The pooled OR for HCC was 4.04 (95% c.i. 2.28-7.14) for LoF/missense and 10.26 (95% c.i. 5.16-20.38) for LoF variants.

For ApoB100 variants, the pooled OR for cirrhosis was 1.98 (95% c.i. 1.09-3.60) for LoF/missense, and 3.96 (95% c.i. 1.96-8.02) for LoF variants. The pooled OR for HCC was 2.30 (95% c.i. 0.59-8.91) for LoF/missense and 10.19 (95% c.i. 2.62-39.58) for LoF variants.

To gain insight as to whether the differential impact of different types of mutations on liver outcomes correlated with the modulation of lipid metabolism, we next examined the impact of *APOB* LoF isoforms on circulating lipoproteins and CAD risk. Carriers of ApoB100 disrupting variants had lower LDL-cholesterol, triglycerides and ALT, higher liver triglyceride content and HDL-cholesterol mirrored by protection against CAD (Figure 2). Interestingly, these differences were reduced in carriers of ApoB100/48 variants. Consistently, when we examined NMR metabolomics, carriers of ApoB variants had lower circulating lipids accompanied by differences in other metabolic species (Figure 4A). Carriers of ApoB100 disrupting variants had an approximately three-fold larger decrease in VLDL size, triglyceride levels within the VLDL subclasses, and biomarkers of lipoprotein metabolism, all suggesting a more severe impairment of VLDL secretion (Figure 4B-C). These data support a larger impact of variants affecting specifically ApoB100 on circulating lipoprotein levels. While carriage of *APOB* variants was negatively associated with statin use (OR 0.61 and 0.67, for LoF and LoF/damaging variants, respectively), this inverse relationship was larger for variants disrupting ApoB100 than for those affecting ApoB48/100 (OR 0.18 and 0.52 vs. OR 0.79 and 0.78 for LoF and LoF/damaging variants, respectively, Supplementary Table 9).

The impact of *APOB* variants on circulating proteomics is reported in the supplementary results (Supplementary Figure 3 and Supplementary Table 11). Altogether, these data suggest that the larger impact of mutations affecting both ApoB48/100 on HCC risk is mediated through mechanisms independent of lipid retention in hepatocytes that may involve impaired secretion of chylomicrons from enterocytes.

Discussion

The main finding of this study is that rare *APOB* LoF variants, causing heterozygous hypobetalipoproteinemia, confer a strong predisposition to severe MASLD, namely cirrhosis and HCC. This predisposition was consistently observed across clinical cohorts, familial co-segregation analyses, and population-based studies. Accordingly, the severity of clinical outcomes increased with the predicted functional impact of the variants and the elevated HCC risk was present in both people with European or African ancestry and across the entire SLD spectrum.

Here, we started by showing that *APOB* variants are markedly (13.8-fold) enriched in people with advanced MASLD compared to the general population. Although hypobetalipoproteinemia is rare, it accounted for a substantial fraction (4.5%) of people with advanced MASLD in our large tertiary care cohort, suggesting a role for targeted genetic testing in people with advanced fibrosis or HCC and low LDL-cholesterol levels. Clinically, *APOB* variant carriers had lower LDL-cholesterol, lower triglycerides and higher HDL-cholesterol, with lower circulating LDL-cholesterol identifying with high accuracy carriers of more severe *APOB* variants. Furthermore, they showed more severe hepatocyte ballooning, higher indices of fibrosis and portal hypertension.

While common genetic variants account for a substantial fraction of MASLD heritability, they do not explain familial progression to advanced fibrosis (11). In contrast, *APOB* variants segregated with severe liver steatosis and fibrosis, demonstrating that heterozygous familial hypobetalipoproteinemia contributes to familial MASLD clustering and suggesting a role for genetic cascade screening in proband families.

By combining multiple cohorts at a cross-ancestry level, we show that rare LoF *APOB* variants increase the risk of HCC and confirmed previous data on the association with cirrhosis and chronic liver disease. However, our analysis reveals that *APOB* variants confer a two-fold larger increase in the risk of HCC compared to cirrhosis in the MVP and UKBB cohorts. In line with previous observations (21, 22)these findings reinforce the notion that a) *APOB* variants cause chronic liver disease and b) hepatic lipid accumulation has a direct carcinogenic effect on liver. Moreover, they provide a granular evaluation of the impact of specific class of mutations depending on if only the hepatic isoform (ApoB100) or the intestinal and hepatic isoform (ApoB48/100) were affected.

Mechanistically, *APOB* variants drive MASLD by impairing lipid export and thus resulting in hepatic lipid accumulation, and inflammation. Carriers had elevated hepatic lipid, CRP and HbA1c levels, and higher risk of diabetes, despite similar BMI. These data are in line with the notion that hepatic lipid retention promotes inflammation, insulin resistance, and Type 2 diabetes, as previously shown by Mendelian randomization (8). The

underlying mechanism seems to be driven by lipotoxicity, and may mediated by reduction in insulin clearance, induction hepatic insulin resistance, oxidative stress and the release of inflammatory mediators (23, 24).

A key novel insight from this study is the marked phenotypic divergence between variants affecting both ApoB48 and ApoB100 and those selectively impacting ApoB100. Mutations disrupting both isoforms, which also may impair chylomicron assembly in enterocytes (25), were associated with more severe liver disease and elevated HCC risk, despite modest changes in circulating lipoproteins and diminished protection against CAD. Conversely, ApoB100 mutations led to greater reduction in lipoproteins, higher hepatic triglycerides content and circulating glucose levels. Notably, although these ApoB mutations conferred stronger protection against coronary artery disease (CAD), they showed only a weak association with hepatocellular carcinoma (HCC), despite comparable degrees of liver inflammation.

Taken all together, these findings suggest that: (a) in carriers of *APOB* LoF variants, hepatic lipid accumulation is necessary but not sufficient to drive HCC, (b) in carriers of ApoB48/100 variants additional extrahepatic tumor-promoter mechanisms, namely impaired chylomicron secretion leading to intestinal barrier dysfunction and alterations of the extracellular matrix, dysbiosis, and oxidative stress via malabsorption of lipids and fat-soluble antioxidants (e.g., vitamin E), may further contribute to hepatocarcinogenesis (26); even though, previous studies did not find lower lipid absorption following standardized meals in heterozygotes carrying ApoB48/100 variants (27); c) an impairment in mitochondrial activity in carriers of ApoB48/ApoB100 variants may underpin the higher HCC risk. Indeed, the increased ratio of beta-hydroxybutyrate over acetoacetate observed in carriers of these variants may reflect reduced NADH production, a mechanism linked to hepatic carcinogenesis and oxidative stress susceptibility (28), and to common genetic determinants of MASLD (29). However, more severe mutations affecting ApoB48 function may have been underrepresented or excluded, as they are likely to cause pronounced pediatric manifestations due to impaired lipid absorption during critical developmental periods; and d) in carriers of ApoB100 specific variants, who have a more favorable lipoprotein profile, higher HDL levels may confer protection against malignant transformation of liver disease (30). Further mechanistic validation and experimental data are required to test these hypotheses.

These findings have several clinical implications. First, they reinforce that hepatic lipoproteins retention drives MASLD (6, 31), and type 2 diabetes (8), while also protecting against cardiovascular disease (9). Second, they support genetic screening for *APOB* variants in people with advanced MASLD and low LDL-cholesterol, alongside family cascade screening (11, 32). Finally, combining rare variants with polygenic risk scores could further refine MASLD stratification, enabling precision medicine approaches (10, 33). In this respect, the present

study demonstrates higher penetrance of LoF ApoB48/100 mutations on hepatic carcinogenesis compared with LoF ApoB100. On the other hand, ApoB100 mutations protect against CAD likely by a more selectively impairment in lipoprotein metabolism.

This study has some limitations related to the retrospective design, although it is fair to say that germline mutations are randomized at birth and present during the entire lifespan. Moreover, liver histology was not systematically available, and the study populations were limited to European and African ancestry.

In conclusion, rare *APOB* LoF variants increase the risk of advanced MASLD, particularly HCC, with distinct effects based on their impact on ApoB isoforms. These insights highlight detection of *APOB* mutations as a promising tool for case finding, risk stratification and early intervention in advanced MASLD.

Methods

Sex as a biological variable

Both sexes were included in this study. In the Milan cohort, we enrolled a total of 498 Males and 273 females.

Study cohorts

Severe MASLD case-control cohort

The severe MASLD case-control cohort is made up of the EPIDEMIC-NAFLD (now MASLD), a cross-sectional Italian multicenter case-control study cohort aimed at the identification of genetic variants predisposing to the development of HCC in unrelated individuals with MASLD including ethnically matched controls, and the prospective "SERENA" study of consecutive patients with MASLD with advanced liver fibrosis without HCC at baseline. Part of this cohort has previously been described (34).

The enrolment, phenotyping and genotyping of this cohort are described in the supplementary methods; the clinical features of cases and controls are presented in Supplementary Table 1.

Family study

Evaluation of first-degree family members of probands with severe MASLD bearing rare coding *APOB* variants was carried on at the Milan center within the RF-2016-02364358 project (11), as described in the supplementary. Clinical features are reported in Supplementary Table 2.

For meta-analyzing the impact of rare *APOB* variants on MASLD-related phenotypes, the literature was systematically searched for papers published in English language containing "NAFLD", MAFLD", "steatosis", "fatty liver disease", "cirrhosis", "hepatocellular carcinoma" AND ["ApoB" OR "apob" OR "Apolipoprotein B"]. References of identified papers were also assessed for possible referral to other publications. Six papers were identified reporting independent families where the carriage of rare pathogenic *APOB* variants was linked to liver traits (15, 33, 35-38).

Million Veteran Program Cohort

Liver disease characterization was performed as described previously (39).

UK Biobank

Liver disease and genetic characterization were performed as described previously (10, 16) and reported in the supplementary material. Metabolomic, lipidomics and proteomics analyses are described in the supplementary material.

Definition of APOB variants

For the main analysis in the clinical cohort, we considered the following criteria to select likely LoF and damaging variants in *APOB* based on the literature (definition A more liberal and B more restrictive, see supplementary material).

In the UKBB and the MVP, we conducted a main analysis focusing on LoF mutation in *APOB,* either rare (MAF <0.01) or ultrarare (singletons). We conducted an additional analysis selecting likely damaging missense variant when at least four of the following in silico prediction criteria were consistent with high pathogenicity: REVEL ≥0.5, CADD ≥20, SIFT, PolyPhen, LRT, MutationTaster, M-CAP, AlphaMissense.

To discriminate whether the impact of *APOB* mutations differed according to the lipoproteins affected, we considered three sets of *APOB* variants: LoF in the whole *APOB* and stratified based on the genomic location according to predicted impact on both ApoB48/100 (upstream aminoacid 2154), or specifically on ApoB100 (downstream of amino acid 2154).

Statistical analysis

For descriptive statistics, categorical variables are shown as numbers and proportions. Continuous variables are shown as median and interquartile range (IQR), as appropriate. Observational associations were performed by fitting data to generalized linear models. Logistic models were fit to examine binary traits, such as presence of MASLD and of advanced liver fibrosis. Analyses were adjusted for the main clinical and genetic confounders. Non-normally distributed variables were log-transformed before entering the analyses. Missing values in <5% of cases were imputed based on sex-specific median.

To examine the specific contribution of common SLD genetic risk variants to the inheritance of SLD and advanced fibrosis, we used transmission disequilibrium test (TdT), testing the over-transmission of risk alleles to affected family members as compared to chance inheritance (40).

In UKBB and MVP, the association between liver outcomes or lipidomic and metabolic data and burden of rare (MAF <0.01) LoF *APOB* variants was tested using a whole-genome regression approach implemented in REGENIE, as detailed in the supplementary material.

Statistical analysis was carried out using the JMP Pro 18.0.2 Statistical Analysis Software (SAS Institute, Cary, NC), and R statistical analysis software version 4.3.2 (http://www.R-project.org/). P values <0.05 (two tailed) were considered significant.

Study approval

Informed consent was obtained for all the patient enrolled in the Milan Cohort and the family study. The Million Veteran Program biobank study (MVP) is a national multi-center biorepository linked to longitudinal electronic health record data from United States Veterans who provided informed consent to participate in the MVP Cohort study (MVP000), which was approved by Central Institutional Review Board of the Veterans Health Administration (VHA) Office of Research and Development. Data used in this study were obtained under MVP003/028 "Genetics of Cardiometabolic Diseases in the VA Population." The UK Biobank study has been approved by the North-West Multicenter Research Ethics Committee (reference number 11/NW/0382). Data used in this study were obtained under the Application Number 37142.

Data availability

Clinical and *APOB* genetic data of the Milan cohort are provided in the supporting data values; UK Biobank data are available upon request; the summary statistics for genetic analyses in the study cohorts are available at: https://github.com/Ojami/APOB-WES-analysis.

Code availability statement: All codes and scripts used for analyses are available at: https://github.com/Ojami/APOB-WES-analysis.

Authors Contributions

LV, SR, SP, MM conceptualized the work; LV, SR, MM, CVS, OJ, RMM, drafted the manuscript; PP edited the manuscript; SP, GP, LR, MM, CR, SP, LV, MS, VLM, RD, FPR, RDA, MF, SP, LM, EB enrolled the clinical cohort and collected clinical data; VM, LM, HE run genetic and bioinformatic analyses in the clinical cohorts; MM, SP, OJ, LM, MV performed the analysis in the clinical cohort; MM, SP, LR performed the family study; OJ and CVS performed analysis on UKBB; MV, CT, JL, PT performed analysis on MVP; LV, SR, CVS, KMC, DP, supervised the study conduct; LV, SR, SA funded the study. All authors read and approved the final manuscript version.

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FIGURES

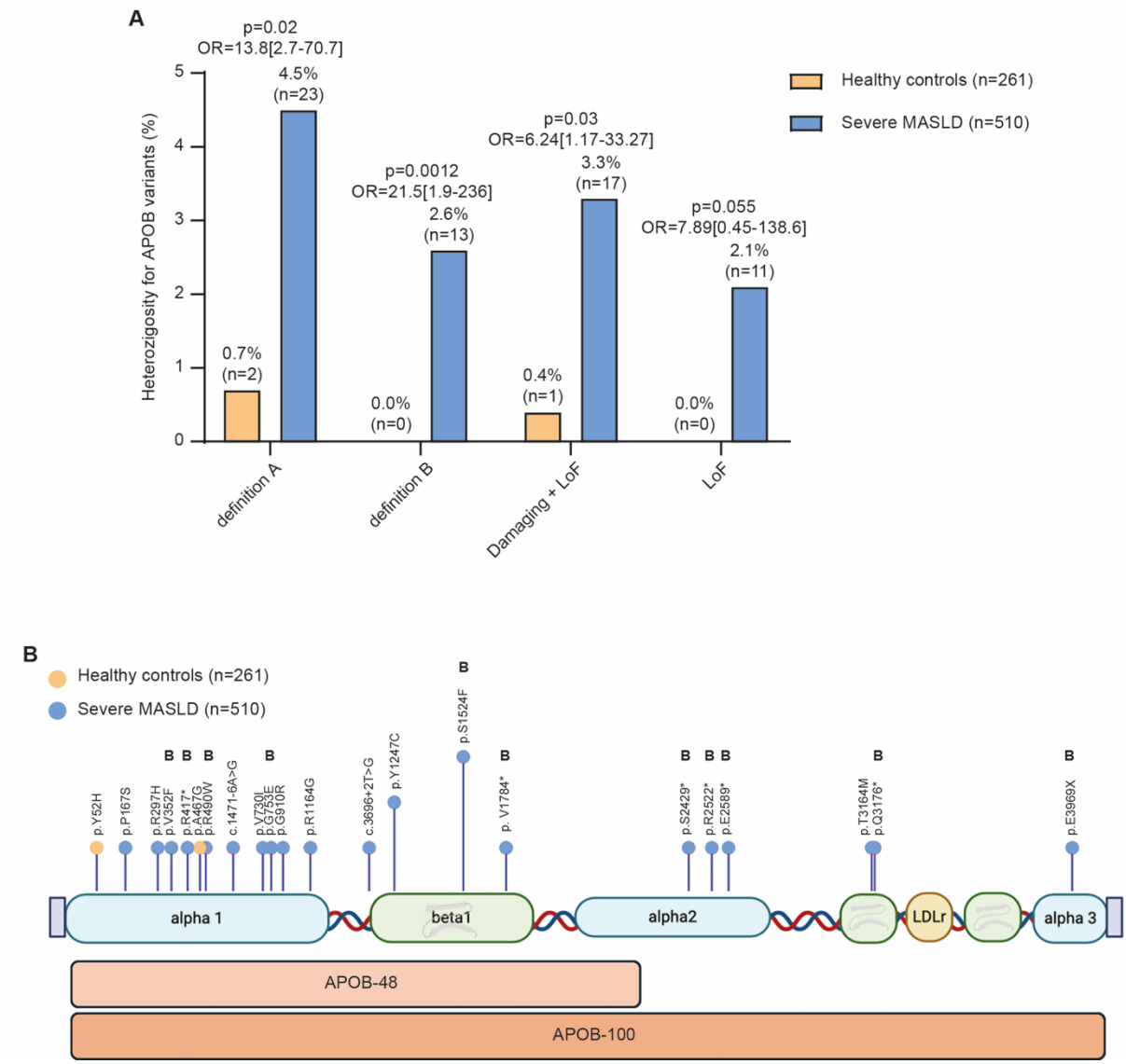

Figure 1. Risk associated with carriage of rare *APOB* variants in a severe MASLD case-control cohort according to definition A, definition B, damaging plus LoF variants, and LoF *APOB* variants. OR, 95% c.i., and p-values were calculated by logistic regression analysis adjusted for age and sex. **A**): prevalence of variants in cases vs. controls; **B**) Lollipop graph showing the aminoacidic residues affected by the mutations.

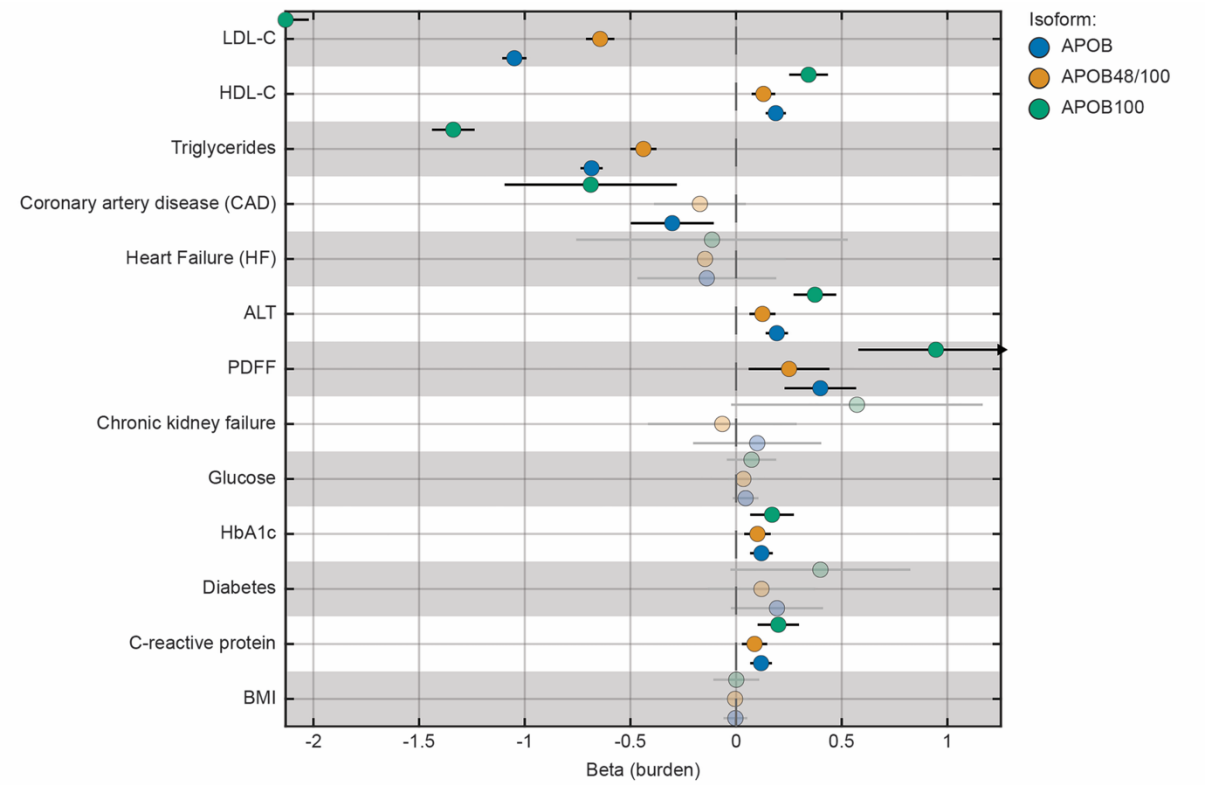

Figure 2. Risk of cardiometabolic and liver outcomes associated with rare pathogenic *APOB* LoF variants in individuals from UK Biobank (n=417,657)**.** X-axis shows either *beta* (continuous traits) or log Firth's odds ratio (binary traits) from a burden test while adjusting for age, sex, age×sex, age^2×sex, age^2, smoking, alcohol consumption, BMI, with additional adjustments for diabetes and hypertension for coronary artery disease. Blue, orange and green dots denote loss-of-function variants affecting the full *APOB* gene, both ApoB48/100, and exclusively ApoB100 protein isoforms, respectively. Transparent dots indicate non-significant associations.

LDL: low density lipoprotein cholesterol, HDL: high density lipoprotein cholesterol, ALT: alanine aminotransferases, HbA1c: glycated hemoglobin, BMI: body mass index.

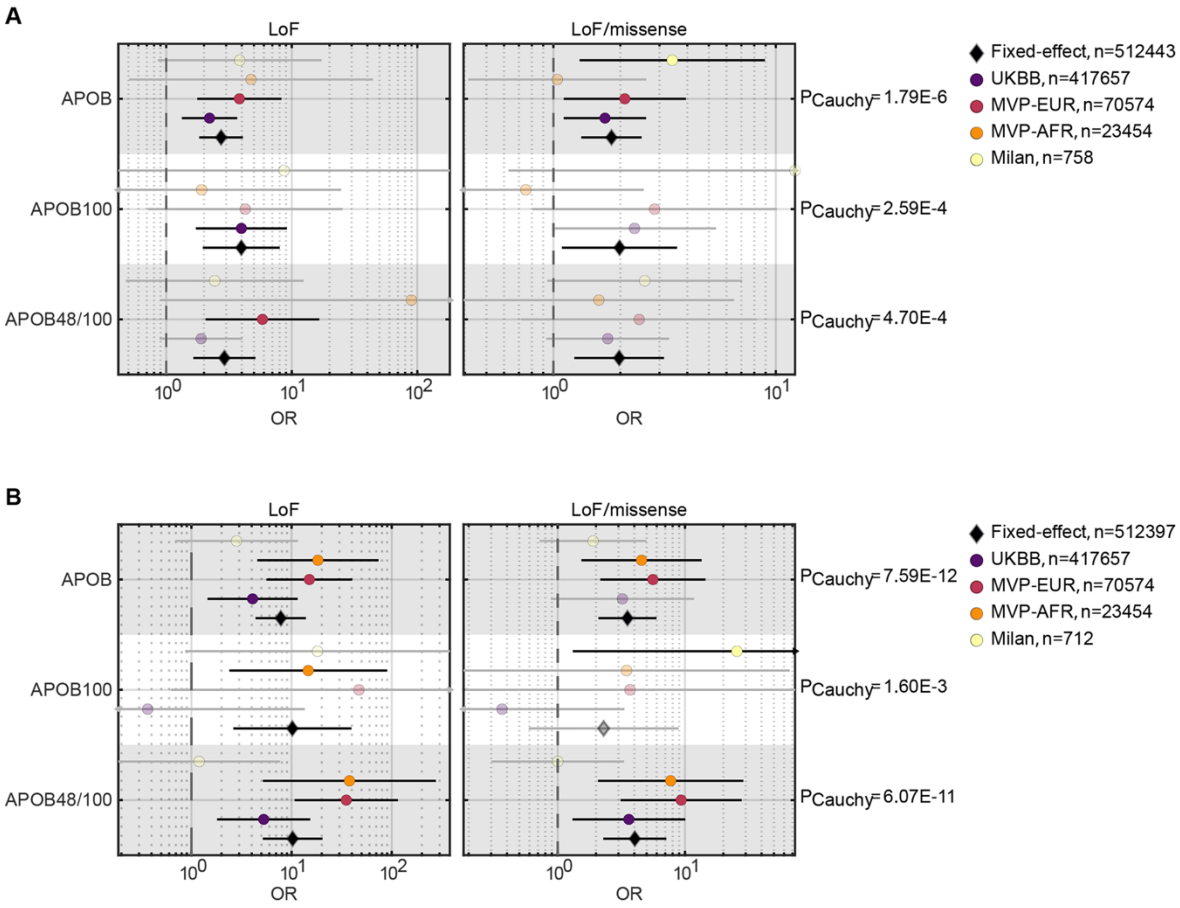

Figure 3. Cross-ancestry meta-analysis of rare *APOB* variants on liver outcomes in three study cohorts. A: cirrhosis, B: hepatocellular carcinoma (HCC). Firth's corrected odds ratio (OR) for each study is displayed for all variants affecting the *APOB* gene (ApoB), specifically impacting ApoB48/100, and exclusively ApoB100 protein isoforms. Diamond marker shows the fixed-effect meta-analysis of each mask-isoform pair. P-values per each mask were combined using Cauchy distribution as shown on the right. Transparent dots indicate non-significant associations.

Milan: Milan Biobank case-control cohort; UKBB: UK Biobank cohort; MVP-EUR: Million Veteran Program Cohort Biobank, European Americans; MVP-AFR: Million Veteran Program Cohort Biobank, African Americans.

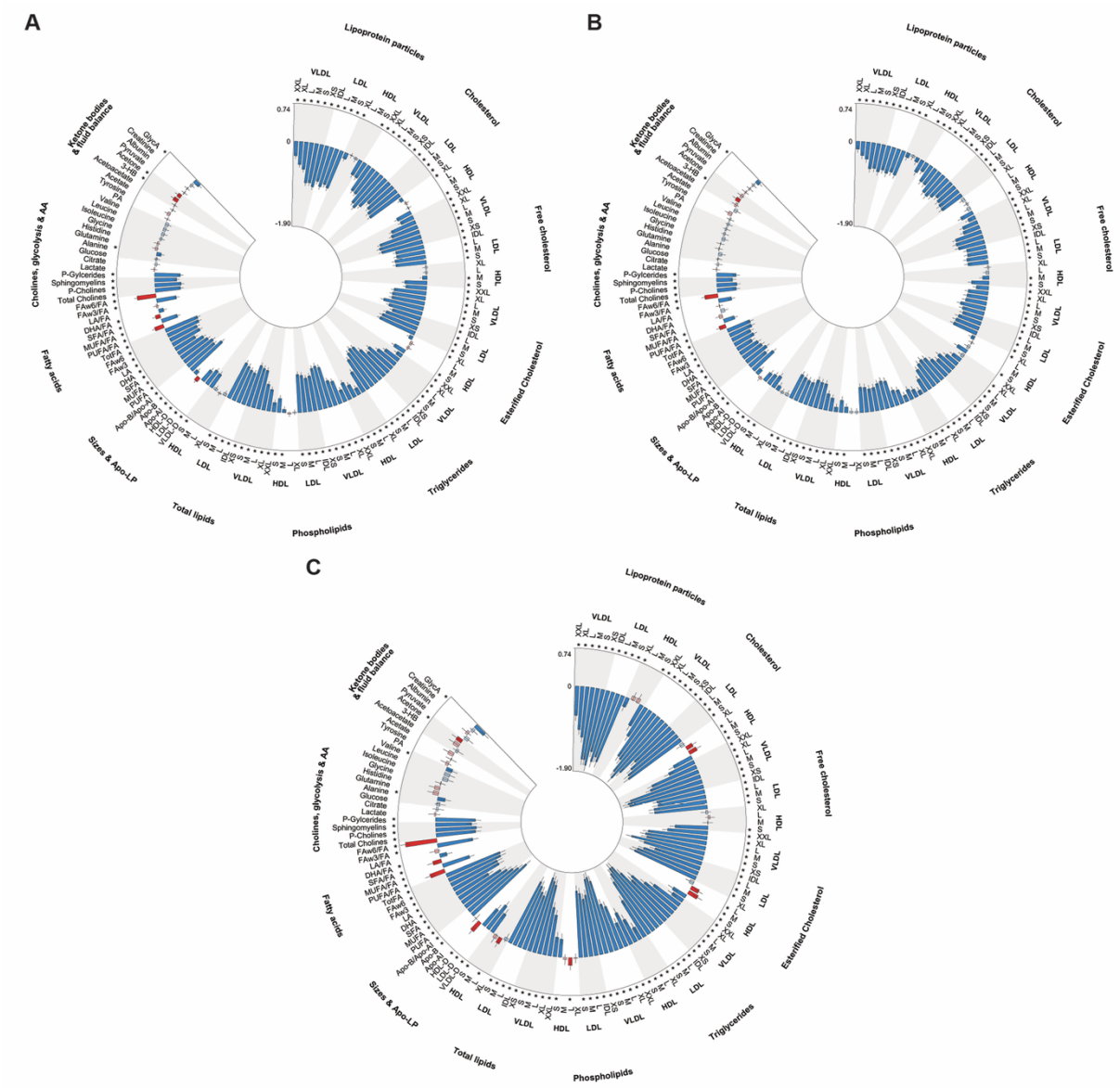

Figure 4. Circle plot for circulating lipoproteins, lipidomic and metabolomic analysis of *APOB* LoF variants carriers in the UKBB. Associations of metabolic biomarkers in carriers with European ancestry from the UKBB. Panel A): overall variants, B): affecting both ApoB48/100, C): affecting specifically ApoB100. P values were calculated using a whole-genome regression model as implemented in REGENIE; beta coefficients (with 95% CIs) are presented per 1-SD change in metabolic biomarker, adjusted for age, sex, age2, age×sex, age2×sex, BMI, first 10 PCs of ancestry and genotyping array. *Adjusted p<0.05 (False discovery rate–controlled). Positive associations are displayed in red, whereas negative associations are blue. Abbreviations: DHA, docosahexaenoic acid; FAw3, omega-3 fatty acid; FAw6, omega-6 fatty acid; HDL-D, high-density lipoprotein particle diameter; LA, linoleic acid; LDL-D, low-density lipoprotein particle diameter; LP, lipoprotein; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; VLDL-D, very low-density lipoprotein particle diameter.

TABLE 1. Over-transmission of rare *APOB* variants in relatives of probands with SLD-related phenotypes in family studies.

	Milan cohort (n=43)		Meta-analysis[a] (n=100)	
	T:U	***p* value**	**T:U**	***p* value**
SLD (CAP ≥275 dB/m or US evidence)	7:2	0.004	12:3	2.0E-4
Severe SLD (CAP ≥290 dB/m or steatohepatitis)	5:0	0.003	5:1	0.011
Liver fibrosis (LSM ≥6.5 KPa or histological stage ≥F1 or clinical cirrhosis)	4:1	0.034	5:1	0.020
Clinically significant fibrosis (LSM ≥8 KPa or histological stage ≥F2 or clinical cirrhosis)	3:1	0.100	4:1	0.060

p at parental transmission disequilibrium test. a) meta-analysis was conducted considering the 11 families enrolled in the Milan Cohort and 7 families with heterozygous hypobetalipoproteinemia and evaluation of liver damage in at least one relative (1, 21-25)

CAP: controlled attenuation parameter; LSM: liver stiffness measurement; SLD: steatotic liver disease; T: transmitted; U: un-transmitted; US: ultrasonographic.