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Prevalence and Determinants of Liver Disease in Relatives of Italian Patients With Advanced MASLD

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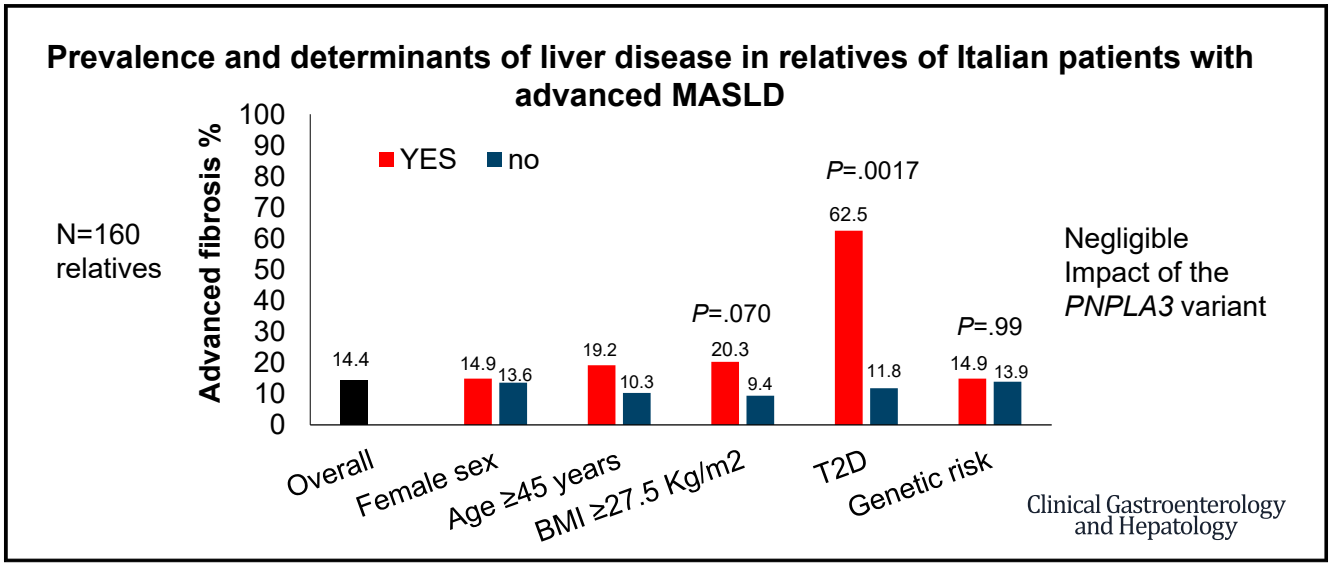

BACKGROUND & AIMS: **Metabolic dysfunction associated steatotic liver disease (MASLD) has a strong genetic component. The aim of this study was to examine noninvasively the prevalence of MASLD and of advanced fibrosis in relatives of patients with advanced MASLD and the risk factors for liver involvement, with a focus on the contribution of common genetic risk variants.**

METHODS: **We prospectively enrolled 98 consecutive probands with advanced fibrosis and/or hepatocellular carcinoma caused by MASLD and 160 nontwin first-degree relatives noninvasively screened for MASLD and advanced fibrosis at 4 Italian centers. We evaluated common genetic determinants and polygenic risk scores of liver disease.**

RESULTS: **Among relatives, prevalence of MASLD was 56.8% overall, whereas advanced fibrosis was observed in 14.4%. At multivariable analysis in relatives, MASLD was associated with body mass index (odds ratio [OR], 1.31 [1.18–1.46]) and tended to be associated with diabetes (OR,**

Abbreviations used in this paper: BMI, body mass index; HCC, hepatocellular carcinoma; LSM, liver stiffness measurement; MASLD, metabolic dysfunction associated steatotic liver disease; NAFLD, nonalcoholic fatty liver disease; PRS, polygenic risk score; SLD, steatotic liver disease; T2D, type 2 diabetes.

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5.21 [0.97–28.10]), alcohol intake (OR, 1.32 [0.98–1.78]), and with female sex (OR, 0.54 [0.23–1.15]), whereas advanced fibrosis was associated with diabetes (OR, 3.13 [1.16–8.45]) and nearly with body mass index (OR, 1.09 [1.00–1.19]). Despite that the *PNPLA3* risk variant was enriched in probands ($P = .003$) and overtransmitted to relatives with MASLD ($P = .045$), evaluation of genetic risk variants and polygenic risk scores was not useful to guide noninvasive screening of advanced fibrosis in relatives.

CONCLUSIONS: **We confirmed that about 1 in 7 relatives of patients with advanced MASLD has advanced fibrosis, supporting clinical recommendations to perform family screening in this setting. Genetic risk variants contributed to liver disease within families but did not meaningfully improve fibrosis risk stratification.**

Keywords: Advanced Fibrosis; Family Study; Genetics; NAFLD; PNPLA3.

Metabolic dysfunction associated steatotic liver disease (MASLD), whose hallmark is excess accumulation of fat in hepatocytes, is the leading cause of liver disease, with possible evolution to advanced fibrosis and hepatocellular carcinoma (HCC).[1] MASLD has a strong genetic component, highlighted by multiethnic epidemiologic studies, but also twin studies and family studies.[2-4] Steatosis shares heritability with fibrosis,[5] the main prognostic determinant in patients with MASLD.[6]

An increased and clinically relevant risk of advanced liver fibrosis has been reported in pilot studies in relatives of patients with MASLD-cirrhosis as compared with that in relatives of patients with simple MASLD or no MASLD.[7,8] This heightened susceptibility to severe liver disease was independent of classical and metabolic risk factors, suggesting a specific role of genetic risk variants predisposing to steatotic liver disease (SLD). The reported prevalence was 14.0%–15.6% in first-degree relatives of patients with MASLD with advanced fibrosis from the United States (n = 128) and Finland (n = 57). Based on these initial data, the American Association for the Study of the Liver clinical practice guidelines already recommends screening for fibrotic MASLD in relatives of patients with advanced forms of the disease.[9] However, there is still very limited validation to support this recommendation, which was based on results in small cohorts not stratified by genetic relatedness (eg, monozygotic twins vs nontwin siblings), and not fully characterized for the familial determinants of progressive MASLD.

The familial aggregation of progressive MASLD (advanced fibrosis and HCC) can be at least partly accounted for by shared genetic variants, both common and rare, and epigenetic modifications.[3] During the last years, genome-wide association studies have identified the main common inherited variants of MASLD, showing a consistent impact on hepatic fat accumulation, inflammation, fibrosis, and HCC.[10] The impact of the main common genetic risk variants for MASLD can be summarized by polygenic risk scores (PRS) that predict the development, severity, and evolution of liver disease in the general population and in clinical cohorts.[11,12] Therefore, based on the main common genetic risk variants in the *PNPLA3*, *TM6SF2*, *GCKR*, *MBOAT7*, and *HSD17B13* genes, it is possible to predict the risk of cirrhosis, HCC, and liver events.[11,12]

The aim of this study was therefore to examine noninvasively the prevalence of MASLD and of advanced fibrosis in relatives of Italian patients with advanced fibrosis and/or HCC caused by MASLD and the risk factors for liver involvement, with a focus on the contribution of common genetic risk variants. As a reference group, we used a locally matched cohort of individuals with metabolic dysfunction.

Patients and Methods

Study Cohort

The "Finalizzata-2023" cohort encompassed 98 consecutive Italian probands with advanced fibrosis and/or HCC caused by MASLD enrolled at 4 centers (years 2016–2022) and 160 first-degree relatives who consented to undergo a liver disease screening. The study flow chart is presented in Supplementary Figure 1 and detailed enrolment criteria in the Supplementary Material.

Abdominal ultrasonography was offered to relatives with altered liver enzymes[13] and/or increased controlled attenuation parameter levels (≥275 dB/m) and/or liver stiffness measurement (LSM; ≥8 kPa), and/or metabolic dysfunction (at least 1 metabolic alteration defining MASLD). SLD was diagnosed when controlled attenuation parameter ≥275 dB/m and/or ultrasonography evidence of steatosis.[14] The possible presence of advanced liver fibrosis was determined noninvasively by FibroScan, imaging, and biomarkers (Supplementary Material).[14] The clinical features of the probands and relatives are reported in Table 1. No twin siblings of the probands were observed nor enrolled in the study. As a reference for the prevalence of liver damage and of genetic risk variants for SLD we used the locally matched Liver-Bible-2022 cohort of 1144 middle aged individuals with metabolic dysfunction who were recruited and

evaluated at the Milan center during the same period (Supplementary Methods); this cohort has previously been described.[15]

The study conforms to the Declaration of Helsinki and was approved by the Ethical Committee of Fondazione IRCCS Ca' Granda Milano as a part of the Ricerca Finalizzata 2016, RF-2016-02364358, "Impact of whole exome sequencing on the clinical management of patients with advanced nonalcoholic fatty liver and cryptogenic liver disease" (CE 125_2018bis) multicenter prospective family study project and ratified by all participating centers. All participants signed a written informed consent.

Genotyping

Participants were genotyped for the rs738409 (*PNPLA3* p.I148M variant), rs58542926 (*TM6SF2* p.E167K), rs641738 C>T variant at *MBOAT7*, rs1260326 (*GCKR* p.P446L), and rs72613567 (*HSD17B13*:TA).[3] Genotyping was performed in duplicate by TaqMan 5'-nuclease assays (ThermoFisher, Waltham). The polygenic risk score of hepatic fat content (PRS-HFC) and polygenic risk score of MASLD-5 (PRS-5) were calculated as previously described.[11]

Statistical Analysis

For descriptive statistics, categorical variables are shown as number and proportion. Continuous variables are shown as mean and standard deviation or median and interquartile range, as appropriate. When appropriate, results were reported in a sex-specific fashion according to the SAGER guidelines.[16]

Observational associations were performed by fitting data to generalized linear models. Logistic models were fit to examine binary traits, such as presence of SLD and of advanced liver fibrosis. Analyses were adjusted for the main clinical and genetic confounders, as reported in the Results section. PRS-5 was used to summarize the genetic risk because of common variation in multivariable models, because more comprehensive than PRS-hepatic fat content score.

The nonalcoholic fatty liver disease (NAFLD)/MASLD familial score was tested to predict advanced fibrosis in relatives.[17]

To examine the specific contribution of common SLD genetic risk variants to the inheritance of SLD and advanced fibrosis, we used the Haploview software version 4.2 (http://www.broadinstitute.org) to perform association analysis and transmission disequilibrium test, testing the overtransmission of risk alleles to affected family members as compared with chance inheritance.[18,19]

Statistical analysis was carried out using the JMP Pro 16.0 Statistical Analysis Software (SAS Institute, Cary, NC), and R statistical analysis software version 4.3.2 (http://www.R-project.org/). P values $< .05$ (2-tailed) were considered significant.

What you Need to Know

Background

MASLD has a strong heritable component and the risk of liver disease is high in first-degree relatives of patients with advanced fibrosis, which supports screening in this setting.

Findings

Among relatives of patients with advanced MASLD, 56.8% had MASLD and 14.4% advanced liver fibrosis, more frequent in those with overweight and diabetes. Evaluation of genetic risk variants was not useful to guide fibrosis screening.

Implications for patient care

Relatives of patients with advanced MASLD should be screened for liver fibrosis, in particular if they have metabolic alterations.

Results

Cohort Composition

The clinical features of the probands with advanced MASLD and their relatives are shown in Table 1. Probands were diagnosed because of high liver stiffness (LSM $\geq$8 kPa) in 77 cases (78.6%), to the histologic or clinical presence of cirrhosis in 10 (10.2%), and 11 (11.2%) because of HCC (10; 90.9%, with advanced fibrosis). Relatives were most frequently offspring (121; 75.6%), followed by sibling (33; 20.6%) and parent (6; 3.8%). No twins were observed among siblings. Relatives were on average 20 years younger (43.8 $\pm$ 12 vs 63.9 $\pm$ 14 years; $P < .0001$), more frequently women ($P = .026$) and had lower body mass index (BMI) and prevalence of type 2 diabetes (T2D) ($P < .0001$ for both). Expectedly, liver damage severity, as determined by controlled attenuation parameter, LSM and FIB-4, was also lower in relatives than in probands ($P < .0001$ for all).

Concerning genetic risk variants, probands showed an enrichment in the *PNPLA3* p.I148M ($P = .0003$) and *GCKR* P446L ($P = .046$) variants as compared with their relatives, and higher PRS-HFC and PRS-5 scores ($P = .0003$ for both). No significant difference was observed in the frequency distribution of *TM6SF2*, *MBOAT7*, and *HSD17B13* variants.

In turn, relatives had a higher prevalence of the *PNPLA3* ($P < .0001$) and *TM6SF2* genetic risk variants ($P = .011$), lower prevalence of the *HSD17B13* protective variant ($P = .008$), and higher polygenic risk score PRS-5 ($P < .0001$) as compared with local control subjects with metabolic dysfunction (Supplementary Table 1).

Table 1. Clinical Features of the Finalizzata-2023 Cohort (n = 258), Including 98 Probands With MASLD and Advanced Fibrosis and/or HCC and 160 Relatives

	Probands	Relatives	
n	98	160	*P* value[a]
Age, *y*	63.9 ± 12.0	43.8 ± 14.0	< .0001
Sex, female	48 (49.0)	101 (63.1)	.026
BMI, kg/m^2	30.6 ± 4.5	27.5 ± 5.3	< .0001
Overweight and/or increased WC, ≥102/88 cm in M/F	92 (93.9)	111 (69.8)	< .0001
Low HDL, <45/55 mg/dL in M/F	58 (59.2)	61 (38.1)	.001
High triglycerides, ≥150 mg/dL	27 (27.6)	21 (13.1)	.005
Arterial hypertension, ≥130/85 mm Hg or therapy	59 (60.2)	21 (13.3)	< .0001
T2D	59 (60.2)	8 (5.0)	< .0001
At risk alcohol intake, 30/20 g/day in M/F	0	1 (0.6)	.43
CAP[b], *dB/m*	290.3 ± 55.9	251.9 ± 57.3	< .0001
LSM[b], *kPa*	22.3 ± 15.3	5.6 ± 2.8	< .0001
FIB-4 score[c]	4.07 ± 4.24	0.83 ± 0.57	< .0001
MASLD[d], CAP ≥275 dB/m	98 (100)	91 (56.9)	< .0001
Advanced fibrosis, LSM ≥8 kPa	97 (99.0)	23 (14.4)	< .0001
HCC	11 (11.2)	0	< .0001
Genetic factors			
PNPLA3 I148M, genotype	24/27/47 (24.5/27.5/48.0)	50/78/32 (31.2/48.7/20.0)	.0003
TM6SF2 E167K, genotype	79/18/0 (81.4/18.6/0)	139/19/2 (86.9/11.9/1.2)	.42
MBOAT7 rs641738, genotype	25/50/22 (25.8/51.6/22.7)	40/86/34 (25.0/53.8/21.2)	.94
GCKR P446L, genotype	17/49/31 (17.5/50.5/32.0)	43/78/39 (26.9/48.7/24.4)	.047
HSD17B13, rs72613567 genotype	64/32/1 (66.0/33.0/1.0)	115/41/4 (71.9/25.6/2.5)	.64
PRS-HFC, score	0.51 ± 0.26	0.40 ± 0.22	.0003
PRS-5, score	0.49 ± 0.27	0.37 ± 0.22	.0003
Relationship			
Parent	—	6 (3.8)	—
Sibling	—	33 (20.6)	—
Offspring	—	121 (75.6)	—

NOTE. Data are presented as mean ± standard deviation and number (%), as appropriate.
BMI, body mass index; CAP, controlled attenuation parameter; HCC, hepatocellular carcinoma; HDL, high-density lipoprotein; LSM, liver stiffness measurement; MASLD, metabolic dysfunction associated steatotic liver disease; PRS-5, polygenic risk score 5; PRS-HFC, polygenic risk score–hepatic fat content; T2D, type 2 diabetes; WC, waist circumference.
[a]At logistic regression models.
[b]Available in 227.
[c]Available in 216.
[d]All cases of steatotic liver disease could be classified as MASLD.

Prevalence and Risk Factors of Steatotic Liver Disease in Relatives

The prevalence of SLD in the overall cohort of relatives and clinically relevant subgroups is presented in Figure 1.

The prevalence of SLD in relatives was 56.8% overall and all relatives with SLD could be classified as being affected by MASLD, except for 1 man with reported alcohol intake of 30 g/day who could be classified as MetALD. MASLD was numerically more frequent in males (39/59; 66.1% vs 52/101; 51.5%; $P = .098$) and was more prevalent in those with severe overweight ($P < .0001$), and in those with T2D ($P = .011$). The prevalence of MASLD was not different according to age, genetic risk caused by carriage of common variants (PRS-5), and familial relationship (P = NS).

As compared with local control subjects, relatives had an increased risk of SLD (odds ratio, 3.06; 95% confidence interval, 1.96–4.79; $P < .0001$ after adjustment for age, sex, BMI, and ethnicity) (Supplementary Table 1).

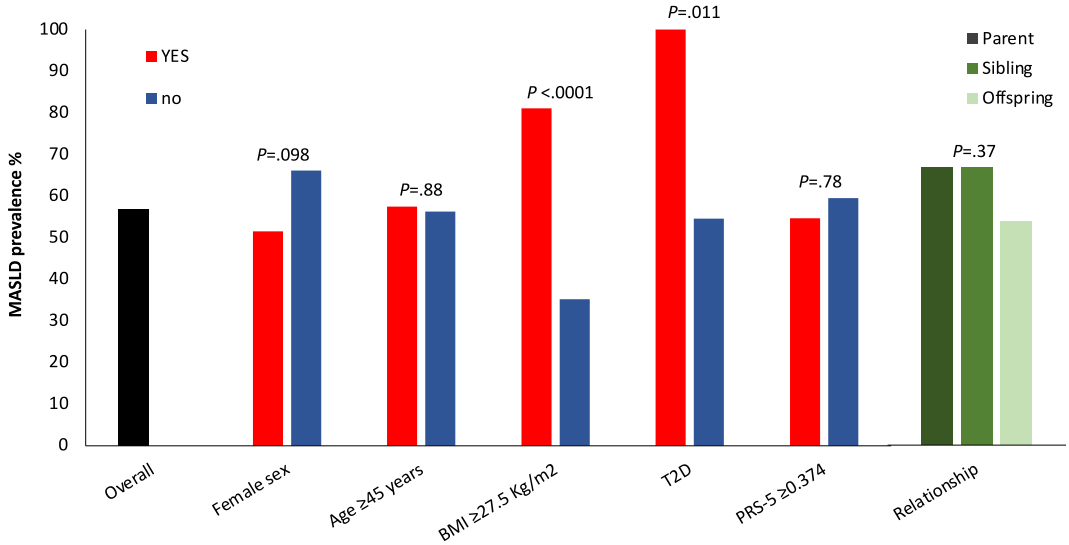

Figure 1. Prevalence of MASLD in relatives of patients with advanced MASLD (n = 160) in the overall cohort and main subgroups.

The independent determinants of SLD in relatives are shown in Table 2. MASLD was significantly associated with BMI ($P < .0001$) and tended to be associated with T2D ($P = .059$), alcohol intake ($P = .07$), and with male sex ($P = .010$), whereas no significant impact of age and PRS-5 was detected, even after stratification for *PNPLA3* genotype of the probands. The type of familial relationship with the proband was not associated with MASLD when included in the model. No single genetic risk variant was independently associated with MASLD.

Prevalence and Risk Factors of Advanced Liver Fibrosis in Relatives

The prevalence of possible advanced fibrosis in the overall cohort of relatives and clinically relevant subgroups is presented in Figure 2. In 151/160 cases (94.4%) liver fibrosis assessment was based on LSM, in 9 cases (5.6%) the presence of advanced fibrosis was corroborated by imaging studies, biomarkers, or clinically determined portal hypertension. The overall prevalence of possible advanced fibrosis was 14.4%. Among all relatives, 9 (5.6%) had FAST score >0.35, consistent with at-risk MASH, whereas 11 (6.9%) had LSM ≥10 or clinical evidence of cirrhosis, consistent with compensated advanced liver disease. Possible advanced fibrosis tended to be more frequent in relatives with overweight ($P = .070$) and according to the familial relationship (parents over siblings over offspring; $P = .059$) and was significantly and markedly more prevalent in those with T2D ($P = .0017$). The prevalence of advanced fibrosis was not different according to age, sex (8/59; 13.6% vs 15/101; 14.9%; $P = 1.0$), and PRS-5.

Table 2. Independent Determinants of SLD and of Possible Advanced Fibrosis in 160 Relatives of Patients With Advanced MASLD in the Finalizzata-2023 Cohort

	MASLD		Advanced fibrosis	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Sex, female	0.54 (0.23–1.15)	.10	1.00 (0.61–1.64)	.89
Age, *y*	1.01 (0.98–1.04)	.55	1.02 (0.98–1.06)	.24
BMI, kg/m^2	1.31 (1.18–1.46)	< .0001	1.09 (1.00–1.19)	.052
T2D	5.21 (0.97–28.10)	.059	3.13 (1.16–8.45)	.014
Alcohol intake, 10 g/day	1.32 (0.98–1.78)	.07	1.11 (0.81–1.52)	.53
PRS-5, score	1.23 (0.23–6.54)	.81	3.74 (0.41–33.82)	.20

NOTE. Results of multivariable logistic regression analysis, including as covariates those shown in the Table, are reported. At univariable analysis, low high-density lipoprotein levels, elevated circulating triglycerides, and presence of arterial hypertension were associated with MASLD and advanced fibrosis ($P < .05$ for all), but because of the strong collinearity with BMI and T2D, none remained independently associated at multivariable analysis ($P > .05$, not shown). Only 1 relative reported at-risk alcohol intake.

BMI, body mass index; CI, confidence interval; MASLD, metabolic dysfunction associated steatotic liver disease; OR, odds ratio; PRS-5, polygenic risk score-5; SLD, steatotic liver disease; T2D, type 2 diabetes.

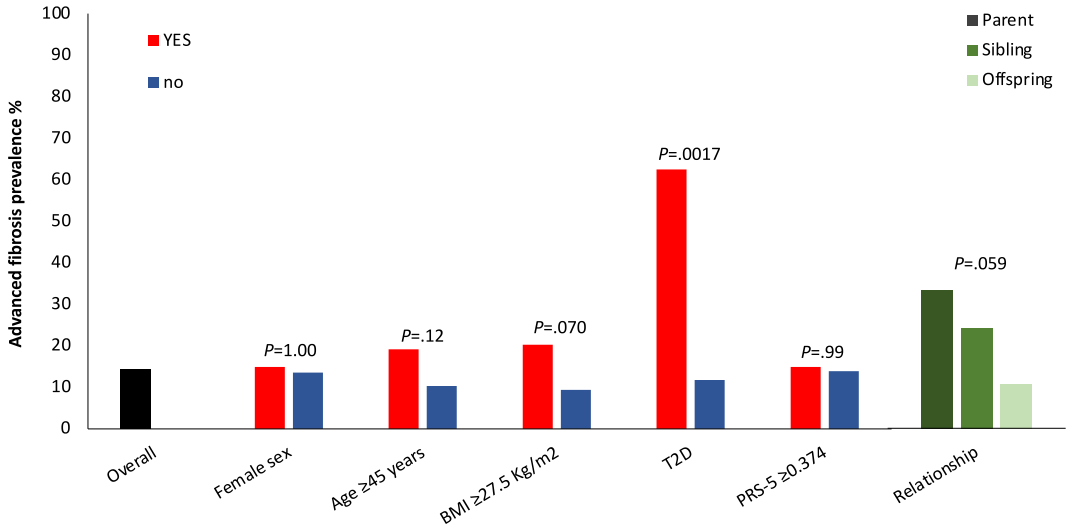

Figure 2. Prevalence of possible advanced liver fibrosis in relatives of patients with advanced MASLD (n = 160) in the overall cohort and main subgroups.

As compared with local control subjects, relatives had a significant increase in the risk of potential advanced liver fibrosis despite younger age and more favorable metabolic features (adjusted odds ratio, 17.06; 95% confidence interval, 7.64–38.06; $P < .0001$) (Supplementary Table 1).

The independent determinants of possible advanced fibrosis in relatives are shown in Table 2. Advanced fibrosis was associated with T2D ($P = .014$) and tended to be associated with BMI ($P = .052$), whereas no significant impact of age, sex, and PRS-5 was detected. The type of familial relationship with the proband was not associated with advanced fibrosis when included in the model. No single genetic risk variant was independently associated with advanced fibrosis.

In relatives, advanced fibrosis was significantly associated with the NAFLD (MASLD) familial risk score (estimate 0.48 ± 0.21; odds ratio, 1.62; 95% confidence interval, 1.06–2.47; $P = .026$), albeit the accuracy was poor overall (area under the receiver operating characteristic curve, 0.63), as reported in Table 3. The FIB-4 score was also associated with advanced fibrosis in relatives (Table 3; $P = .003$), but the accuracy was also poor (area under the receiver operating characteristic curve, 0.66).

Genetic Linkage With Liver Phenotypes

The genetic linkage of common genetic risk variants for SLD with liver phenotypes (MASLD and advanced fibrosis) in the Finalizzata-2023 is presented in Table 4. Despite the limited power (low number of informative families), we detected a significant overtransmission of the *PNPLA3* rs738409 G allele, encoding for the p.I148M variant, in offspring with MASLD ($P = .045$), whereas no significant association was observed with advanced fibrosis and for the other genetic risk variants.

Table 3. Diagnostic Performance of the NAFLD (MASLD) Familial Risk Score for Advanced Fibrosis and of FIB-4 in Relatives of Patients With Advanced MASLD (n = 160; Area Under the Receiver Operating Characteristic Curve, 0.063; $P = .026$)

	Familial risk score, threshold ≥3		Familial risk score, threshold ≥4		FIB-4	
Measure	Estimate	95% CI	Estimate	95% CI	Estimate	95% CI
Prevalence	0.145	0.098–0.208	0.145	0.098–0.208	0.143	0.092–0.215
Sensitivity	0.696	0.491–0.844	0.391	0.222–0.592	0.444	0.246–0.663
Specificity	0.537	0.453–0.618	0.765	0.687–0.828	0.917	0.849–0.956
PPV	0.203	0.129–0.304	0.220	0.120–0.367	0.471	0.262–0.690
NPV	0.913	0.830–0.957	0.881	0.811–0.928	0.908	0.839–0.949
Positive LR	1.502	1.085–2.079	1.663	0.919–3.009	5.333	2.37–12.003
Negative LR	0.567	0.300–1.072	0.796	0.566–1.119	0.606	0.399–0.920

CI, confidence interval; LR, likelihood ratio; MASLD, metabolic dysfunction associated steatotic liver disease; NAFLD, nonalcoholic fatty liver disease; NPV, negative predictive value; PPV, positive predictive value.

Table 4. Genetic Linkage of Common Risk Variants Examined With Liver Phenotypes in the Finalizzata-2023 Cohort (98 Probands and 160 Relatives)

						Steatotic liver disease			Advanced fibrosis		
Gene	Variant	Chr	Position	AA change	Allele	Case, control frequencies	T,U	*P* value[a]	Case, control frequencies	T,U	*P* value[a]
PNPLA3	rs738409 C>G	22	43928847	p.I148M	G	0.641, 0.389	4,0	.045	0.625, 0.650	2,0	.16
TM6SF2	rs58542926 C>T	19	19268740	p.E167K	T	0.222, 0.161	1,0	.32	0.200, 0.150	1,0	.32
MBOAT7	rs641738 C>T	19	54173068	NA	T	0.468, 0.389	7,2	.096	0.625, 0.475	3,1	.32
GCKR	rs1260326 C>T	2	27508073	p.P446L	T	0.550, 0.375	3,2	.65	0.560, 0.454	2,1	.57
HSD17B13	rs72613567 T>TA	4	7310240	NA	T	0.828, 0.722	1,1	1.00	0.806, 0.800	2,0	.16

T, transmitted; U, untransmitted.
[a]At transmission disequilibrium test.

The increased risk of SLD and of advanced fibrosis in relatives as compared with local control subjects were not attenuated by correction for PRS-5 (Supplementary Table 1), suggesting that other factors contribute to mediating this association.

Discussion

In this study, we examined the prevalence and the clinical and genetic determinants of liver disease in 160 relatives of 98 Italian patients with advanced MASLD from a multicenter prospective study. First, we found that the prevalence of SLD and possible advanced fibrosis in relatives was 56.8% and 14.4%, respectively. The number needed to screen to detect 1 relative with possible advanced fibrosis was therefore 7. We also showed that relatives had about 3-fold higher risk of SLD and 14.5-fold higher risk of advanced fibrosis independently of demographic and metabolic risk factors, including T2D, matching previous estimates derived from family studies.[7,8] These first data from a European Mediterranean population are in line with those recently obtained in similar cohorts from the United States and Finland,[7,8] and support the clinical utility and recommendation to propose family screening for liver disease in relatives of patients with MASLD and advanced liver fibrosis.[9] However, it should be noted that the prevalence of compensated advanced liver disease (LSM $\geq$10 kPa or clinical evidence of cirrhosis) was lower than in previous studies (6.9%). However, the present cohort did not include twins, suggesting results may be generalizable to clinical practice, and reported the risk of advanced fibrosis after stratification for the type of familial relationship.

Second, we confirmed that adiposity and T2D are main risk factors for liver disease in this population,[17] with the presence of T2D being independently associated with advanced fibrosis. Male sex tended to be associated with SLD, whereas age, sex, and the type of familial relationship with the proband were not independently associated with advanced fibrosis. However, the risk of potential advanced fibrosis was nonsignificantly higher in parents than in siblings than in the offspring. It should be noted the application of the NAFLD/MASLD familial fibrosis score and of FIB-4 (despite high FIB-4 being a criterion to define advanced fibrosis) to the relatives of patients with advanced MASLD showed poor accuracy in the detection of advanced fibrosis. These data, in addition to the relatively limited study power, suggest that family-based screening strategies should be further refined in larger studies.

Overall, together with previous results,[7,8] this evidence suggests that advanced liver fibrosis tends to cluster in families along with MASLD.[2,3] Inherited factors contribute to the shared familial predisposition between SLD and liver fibrosis through genetic mechanisms.[5,10] Indeed, during the last few years genome-wide studies have identified the main common genetic determinants of fibrosing SLD,[3] which can be summarized in PRS to predict liver disease.[11,12]

A strength and novelty of the present study was the evaluation of the contribution of the main common determinants of MASLD in the *PNPLA3*, *TM6SF2*, *MBOAT7*, *GCKR*, and *HSD17B13* genes to the risk of liver disease in the relatives. In probands with advanced MASLD, we detected a high prevalence of genotypes at risk. In particular, prevalence of homozygosity for the *PNPLA3* p.I148M variant was 48.0% and that of carriage of the

TM6SF2 p.E167K variant was 18.6%, as compared with 8.1% and 8.2% of local control subjects, respectively.[20,21] In the case of *PNPLA3*, this represents an impressive 8-fold enrichment as compared with healthy individuals, suggesting this variant had a large contribution in determining the severe phenotype of probands. In keeping with these data, the prevalence of these MASLD risk variants remained high, but was expectedly significantly diluted in first-degree relatives for the *PNPLA3*, and overall as captured by the PRS-5 scores, as compared with local control subjects. However, neither carriage of the *PNPLA3* variant, nor of other variants or PRS was helpful in predicting liver disease at the time of evaluation in the overall cohort of relatives. This observation does not rule out a contribution of SLD genetic risk variants to the phenotypes of probands. Indeed, despite the limited power of the cohort, at the level of individual families, we were able to detect a significant overtransmission (beyond chance) of the *PNPLA3* from parents to the offspring affected by SLD. These results suggest that knowledge of the *PNPLA3* genotype may improve risk stratification within individual families, in keeping with previous data obtained in family trios of children with MASLD.[22] Indeed, carriage of the *PNPLA3* variant was associated with increased risk of steatosis in relatives of patients with lean nonalcoholic SLD.[4] However, we could not detect a significant association with advanced fibrosis and these cross-sectional data do not currently lend support to the clinical utility of PRS determination to guide noninvasive screening of liver fibrosis in relatives, because of the poor performance of the available clinical scores. Furthermore, adjustment for common genetic risk variants did not appreciably attenuate the increased risk of liver disease in relatives. These data indicate that additional factors including shared environmental exposures, epigenetic factors, epigenome, and carriage of rare genetic risk variants with a large impact on protein function may contribute to the liver disease phenotype in these families.[3]

Additional validation in larger multiethnic cohorts, including also relatives of patients with less severe MASLD and healthy individuals, is still necessary to strengthen these conclusions. Further limitations of the present study include the heterogeneity of approaches for the evaluation of liver damage,[23] the young age of many participants and lack of follow-up hampering the evaluation of the lifelong risk of liver disease, the relatively low participation rate among relatives because of logistic problems, and the lack of power to discriminate the risk of advanced fibrosis in relatives of probands who developed HCC without cirrhosis. Future studies should also consider larger panels of common risk variants and the evaluation of rare variants determining increased risk of fibrosing MASLD.[20,24-26]

In conclusion, we confirmed that about 1 in 7 relatives of patients with advanced MASLD has advanced fibrosis in a Southern European cohort that did not include twin siblings, supporting clinical recommendations to perform family screening in this setting. In relatives, the presence of liver disease was associated with adiposity and T2D, but currently available risk scores were not helpful in guiding noninvasive evaluation of liver damage. Genetic factors and in particular the *PNPLA3* p.I148M variant contributed to the liver disease phenotype in patients and relatives, but their determination was not useful to further optimize fibrosis screening.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Clinical Gastroenterology and Hepatology* at www.cghjournal.org, and at https://doi.org/10.1016/j.cgh.2023.12.033.

References

1. Rinella ME, Lazarus JV, Ratziu V, et al. A multi-society Delphi consensus statement on new fatty liver disease nomenclature. J Hepatol 2023;79:1542–1556.
2. Long MT, Gurary EB, Massaro JM, et al. Parental non-alcoholic fatty liver disease increases risk of non-alcoholic fatty liver disease in offspring. Liver Int 2019;39:740–747.
3. Trepo E, Valenti L. Update on NAFLD genetics: from new variants to the clinic. J Hepatol 2020;72:1196–1209.
4. Niltwat S, Limwongse C, Charatcharoenwitthaya N, et al. Familial clustering of nonalcoholic fatty liver disease in first-degree relatives of adults with lean nonalcoholic fatty liver disease. Liver Int 2023;43:2713–2726.
5. Cui J, Chen CH, Lo MT, et al. Shared genetic effects between hepatic steatosis and fibrosis: a prospective twin study. Hepatology 2016;64:1547–1558.
6. Taylor RS, Taylor RJ, Bayliss S, et al. Association between fibrosis stage and outcomes of patients with nonalcoholic fatty liver disease: a systematic review and meta-analysis. Gastroenterology 2020;158:1611–1625.
7. Caussy C, Soni M, Cui J, et al. Nonalcoholic fatty liver disease with cirrhosis increases familial risk for advanced fibrosis. J Clin Invest 2017;127:2697–2704.
8. Tamaki N, Ahlholm N, Luukkonen PK, et al. Risk of advanced fibrosis in first-degree relatives of patients with nonalcoholic fatty liver disease. J Clin Invest 2022;132:e162513.
9. Rinella ME, Neuschwander-Tetri BA, Siddiqui MS, et al. AASLD Practice Guidance on the clinical assessment and management of nonalcoholic fatty liver disease. Hepatology 2023;77:1797–1835.
10. Romeo S, Sanyal A, Valenti L. Leveraging human genetics to identify potential new treatments for fatty liver disease. Cell Metab 2020;31:35–45.
11. Bianco C, Jamialahmadi O, Pelusi S, et al. Non-invasive stratification of hepatocellular carcinoma risk in non-alcoholic fatty liver using polygenic risk scores. J Hepatol 2021;74:775–782.
12. Bianco C, Tavaglione F, Romeo S, et al. Genetic risk scores and personalization of care in fatty liver disease. Curr Opin Pharmacol 2021;61:6–11.
13. Valenti L, Pelusi S, Bianco C, et al. Definition of healthy ranges for alanine aminotransferase levels: a 2021 update. Hepatol Commun 2021;5:1824–1832.

14. European Association for the Study of the Liver. EASL Clinical Practice Guidelines on non-invasive tests for evaluation of liver disease severity and prognosis: 2021 update. J Hepatol 2021; 75:659–689.
15. Mantovani A, Pelusi S, Margarita S, et al. Adverse effect of PNPLA3 p.I148M genetic variant on kidney function in middle-aged individuals with metabolic dysfunction. Aliment Pharmacol Ther 2023;57:1093–1102.
16. Heidari S, Babor TF, De Castro P, et al. Sex and gender equity in research: rationale for the SAGER guidelines and recommended use. Res Integr Peer Rev 2016;1:2.
17. Huang DQ, Ahlholm N, Luukkonen PK, et al. Development and validation of the nonalcoholic fatty liver disease familial risk score to detect advanced fibrosis: a prospective, multicenter study. Clin Gastroenterol Hepatol 2024;22:81–90.
18. Barrett JC, Fry B, Maller J, et al. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005;21:263–265.
19. Donati B, Motta BM, Pingitore P, et al. The rs2294918 E434K variant modulates patatin-like phospholipase domain-containing 3 expression and liver damage. Hepatology 2016;63:787–798.
20. Baselli GA, Jamialahmadi O, Pelusi S, et al. Rare ATG7 genetic variants predispose patients to severe fatty liver disease. J Hepatol 2022;77:596–606.
21. Valenti L, Tripodi A, La Mura V, et al. Clinical and genetic determinants of the fatty liver-coagulation balance interplay in individuals with metabolic dysfunction. JHEP Rep 2022;4:100598.
22. Valenti L, Al-Serri A, Daly AK, et al. Homozygosity for the patatin-like phospholipase-3/adiponutrin I148M polymorphism influences liver fibrosis in patients with nonalcoholic fatty liver disease. Hepatology 2010;51:1209–1217.
23. Boursier J, Guillaume M, Bouzbib C, et al. Non-invasive diagnosis and follow-up of non-alcoholic fatty liver disease. Clin Res Hepatol Gastroenterol 2022;46:101769.
24. Pelusi S, Baselli G, Pietrelli A, et al. Rare pathogenic variants predispose to hepatocellular carcinoma in nonalcoholic fatty liver disease. Sci Rep 2019;9:3682.
25. Verweij N, Haas ME, Nielsen JB, et al. Germline mutations in CIDEB and protection against liver disease. N Engl J Med 2022; 387:332–344.
26. Haas ME, Pirruccello JP, Friedman SN, et al. Machine learning enables new insights into genetic contributions to liver fat accumulation. Cell Genom 2021;1:100066.

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Supplementary Methods

The study cohort was recruited in the following centers: Milan (19 probands and 48 relatives), Turin (29 probands and 37 relatives), Palermo (46 probands and 61 relatives), and Udine (4 probands and 14 relatives).

These were adult patients prospectively and consecutively enrolled based on (1) MASLD, based on imaging evidence of steatosis, presence of at least 1 metabolic alteration, and alcohol intake $\leq$30 g/day[e1]; (2) absence of other chronic liver diseases including viral hepatitis B virus and hepatitis C virus hepatitis, autoimmune hepatitis, other immune-mediated liver disorders, celiac disease, hemochromatosis, and α_1-antitrypsin deficiency; (3) evidence either of the possible/likely presence of advanced liver fibrosis, as determined by liver histology or noninvasively by LSM $\geq$8 kPa by vibration controlled transient elastography with Fibroscan, to be consistent with previous literature in the field,[e2,e3] or FIB-4 index $\geq$2.67[e4] and/or clinical evidence of portal hypertension/hepatic decompensation and/or HCC diagnosed by standard clinical approaches (advanced MASLD)[e5]; and (4) willing to sign an informed consent and to involve relatives in the study. Participation in the family study was offered to all consecutive patients diagnosed with these criteria.

When patients and at least 1 first-degree relative consented, these relatives underwent MASLD screening with determination of anthropometric parameters, alcohol intake, biochemical liver tests (aspartate aminotransaminase, alanine aminotransaminase, γ-glutamyltransferase), metabolic parameters (BMI/abdominal circumference, arterial blood pressure, total and HDL cholesterol, triglycerides, fasting glucose and insulin levels),[e1] and noninvasive assessment of liver damage with determination of LSM and controlled attenuation parameter by FibroScan. Arterial hypertension, altered glucose metabolism, and T2D were determined by standard approaches, as previously described.[e6]

The FAST score was also calculated to define at-risk metabolic steatohepatitis.[e7]

The Liver-Bible-2022 consisted of 1142 middle-aged individuals, who were consecutively enrolled from July 2019 to July 2022, and for whom information on genomic data and liver damage was concurrently available.[e8-e10] These were apparently healthy blood donors, aged 40–65 years, who were selected for a comprehensive liver disease, metabolic, and cardiovascular screening, because of the presence of at least 3 metabolic risk abnormalities, among overweight/obesity, hypertension (blood pressure $\geq$130/85 mm Hg or antihypertensive treatment), dysglycemia (fasting glucose level $\geq$100 mg/dL or use of glucose-lowering agents), low plasma HDL-cholesterol ($<$45 mg/dL in men and $<$55 mg/dL women), or high plasma triglycerides ($\geq$150 mg/dL or lipid-lowering treatment).[e11] Individuals with chronic degenerative diseases, except for well-controlled arterial hypertension, treated hypothyroidism, and well-compensated T2D not requiring pharmacotherapy (except for metformin), were excluded from the cohort at first evaluation. The overall goal of this ongoing biobank study was primarily to examine the role of genetic factors and other noninvasive biomarkers of NAFLD in the risk prediction of cardiometabolic diseases, to provide the framework to design precision medicine approaches to prevent these cardiometabolic conditions. The clinical and genetic features of this cohort have previously been described.[e10]

Metabolic alterations, the prevalence of liver damage, and thresholds to define liver damage by vibration controlled transient elastography by FibroScan in this cohort were the same used for relatives, evaluated at the same center (Milan) during the same years (see the main methods).

Supplementary Results: Sensitivity Analyses

Adiposity (BMI) remained the only significant independent determinant of SLD in a sensitivity analysis when dichotomizing the continuous variables ($P <$ .0001; not shown), and in the offspring ($P <$ .0001; not shown).

T2D remained the only significant independent determinant of possible advanced fibrosis in a sensitivity analysis when dichotomizing the continuous variables ($P =$ .0046; not shown). Because of the low prevalence, no significant independent predictor of possible advanced fibrosis could be identified in the offspring.

Supplementary References

e1. Rinella ME, Lazarus JV, Ratziu V, et al. A multi-society Delphi consensus statement on new fatty liver disease nomenclature. J Hepatol 2023;79:1542–1556.

e2. Caussy C, Soni M, Cui J, et al. Nonalcoholic fatty liver disease with cirrhosis increases familial risk for advanced fibrosis. J Clin Invest 2017;127:2697–2704.

e3. Tamaki N, Ahlholm N, Luukkonen PK, et al. Risk of advanced fibrosis in first-degree relatives of patients with nonalcoholic fatty liver disease. J Clin Invest 2022;132:e162513.

e4. European Association for the Study of the Liver. EASL Clinical Practice Guidelines on non-invasive tests for evaluation of liver disease severity and prognosis: 2021 update. J Hepatol 2021;75:659–689.

e5. European Association for the Study of the Liver. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. J Hepatol 2012;56:908–943.

e6. Tomasi M, Cherubini A, Pelusi S, et al. Circulating interlukin-32 and altered blood pressure control in individuals with metabolic dysfunction. Int J Mol Sci 2023;24:7465.

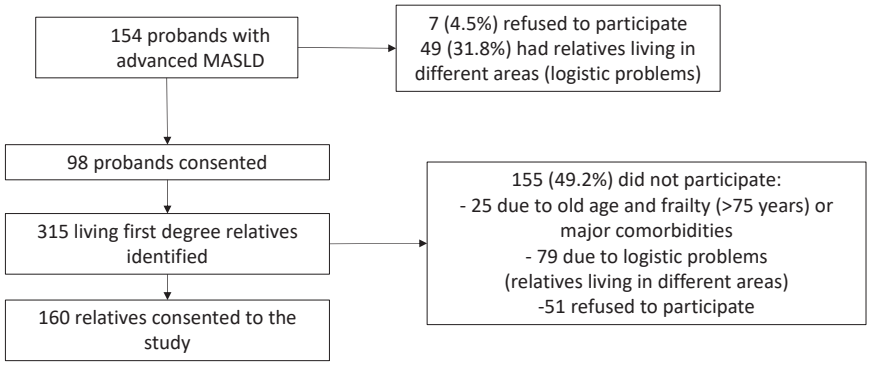
e7. Newsome PN, Sasso M, Deeks JJ, et al. FibroScan-AST (FAST) score for the non-invasive identification of patients with non-alcoholic steatohepatitis with significant activity and fibrosis: a prospective derivation and global validation study. Lancet Gastroenterol Hepatol 2020;5:362–373.

e8. Valenti L, Pelusi S, Bianco C, et al. Definition of healthy ranges for alanine aminotransferase levels: a 2021 update. Hepatol Commun 2021;5:1824–1832.

e9. Valenti L, Tripodi A, La Mura V, et al. Clinical and genetic determinants of the fatty liver-coagulation balance interplay in individuals with metabolic dysfunction. JHEP Rep 2022;4:100598.

e10. Mantovani A, Pelusi S, Margarita S, et al. Adverse effect of PNPLA3 p.I148M genetic variant on kidney function in middle-aged individuals with metabolic dysfunction. Aliment Pharmacol Ther 2023;57:1093–1102.

e11. European Association for the Study of the Liver, European Association for the Study of Diabetes, European Association for the Study of Obesity. EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. J Hepatol 2016;64:1388–1402.



Supplementary Figure 1. Study flow chart.

Supplementary Table 1. Comparison of the Prevalence of Common Genetic Risk Factors, and the Prevalence of Liver Damage (MASLD and Probable Advanced Fibrosis) Between First-Degree Relatives of Patients With Advanced MASLD (n = 160) and Locally Matched Middle-Aged Individuals With Metabolic Dysfunction (Liver-Bible-2022 Cohort, n = 1142)

	Relatives	Metabolic dysfunction	OR[a], 95% CI (estimate ± SE)	*P* value[a]	OR[b], 95% CI (estimate ± SE)	*P* value[b]	OR[b], 95% CI (estimate ± SE)	*P* value[c]
n	160	1142						
Steatotic liver disease	91 (56.9)	552 (48.3)	3.08, 1.97–4.79	< .0001	3.06, 1.96–4.79	< .0001	2.96, 1.87–4.67	< .0001
Advanced fibrosis	23 (14.4)	26 (2.3)	14.55, 6.73–31.46	< .0001	17.06, 7.64–38.06	< .0001	15.39, 2.96–30.91	< .0001
PNPLA3 p.I148M alleles	50/79/31 (31.2/49.4/19.4)	605/441/92 (53.1/38.8/8.1)	0.148 ± 0.032	< .0001				
TM6SF2 p.E167K alleles	139/19/2 (86.9/11.9/1.2)	1045/92/1 (91.8/8.1/0.1)	0.037 ± 0.014	.011				
MBOAT7 rs641738 alleles	40/86/34 (25.0/53.8/21.2)	239/570/329 (21.0/50.1/28.9)	-0.060 ± 0.034	.081				
GCKR p.P446L alleles	44/78/38 (27.5/48.7/23.8)	328/561/249 (28.8/49.3/21.9)	0.044 ± 0.035	.21				
HSD17B13 rs72613567 alleles	115/41/4 (71.9/25.6/2.5)	674/406/58 (59.2/35.7/5.1)	-0.077 ± 0.029	.008				
PRS-5	0.371 ± 0.224	0.261 ± 0.297	0.049 ± 0.010	< .0001				

Additional clinical features			
	Relatives	Metabolic dysfunction	*P* value[d]
n	160	1142	
Age, *y*	43.8 ± 14.0	53. ± 6.4	< .0001
Sex, female	101 (63.3)	192 (16.8)	< .0001
BMI, kg/m^2	27.4 ± 5.0	28.6 ± 3.1	< .0001
T2D	17 (1.5)	8 (5.0)	.007
Total cholesterol, *mg/dL*	185 ± 50	203 ± 33	< .0001
HDL, *mg/dL*	56 ± 14	43 ± 10	< .0001
Triglycerides, *mg/dL*	102 ± 55	163 ± 83	< .0001

NOTE. Data are shown as n (%) or mean ± standard deviation, as appropriate.
BMI, body mass index; CI, confidence interval; HDL, high-density lipoprotein; MASLD, metabolic dysfunction associated steatotic liver disease; OR, odds ratio; PRS-5, polygenic risk score 5; SE, standard error; T2D, type 2 diabetes.
[a]ORs and *P* values (corrected for age, sex, BMI, ethnicity) of having steatotic liver disease or advanced fibrosis and for being at genetic risk for being a first-degree relative of a patient with advanced MASLD are reported (at multivariable logistic regression analysis adjusted for the covariates shown in the upper part of the Table).
[b]Further corrected for PRS-5 genetic risk score.
[c]Further corrected for the presence of T2D.
[d]At unadjusted logistic regression analysis.