



HAL
open science

Combination of heterozygous APOB gene mutation with PNPLA3 and TM6SF2 variants promotes steatotic liver disease, cirrhosis and HCC development

Y. Chouik, M. Di Filippo, S. Radenne, J. Dumortier, P. Moulin, M. Levrero

► To cite this version:

Y. Chouik, M. Di Filippo, S. Radenne, J. Dumortier, P. Moulin, et al.. Combination of heterozygous APOB gene mutation with PNPLA3 and TM6SF2 variants promotes steatotic liver disease, cirrhosis and HCC development. *Liver International*, 2024, 44 (6), pp.1474-1477. 10.1111/liv.15837 . inserm-04661850

HAL Id: inserm-04661850

<https://inserm.hal.science/inserm-04661850>

Submitted on 25 Jul 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

RESEARCH LETTER

Combination of heterozygous *APOB* gene mutation with *PNPLA3* and *TM6SF2* variants promotes steatotic liver disease, cirrhosis and HCC development

Metabolic dysfunction-associated steatotic liver disease (MASLD) is the most common liver disease worldwide. In recent years, several single nucleotide polymorphisms (SNPs) in genes involved in hepatic lipid metabolism (*PNPLA3*, *TM6SF2*, *GCKR*, *MBOAT7* and *HSD17B13*) have been shown to significantly contribute to the emergence of the disease in association with environmental factors.¹⁻³ Monogenic disorders leading to a decreased VLDL secretion such as bi-allelic rare inactivating variants in the gene encoding for Apolipoprotein B (ApoB) leading to familial hypobetalipoproteinemia (FHBL-SD2) can also result in steatohepatitis.⁴ Additionally, heterozygous *APOB* mutations (Hz-FHBL-SD2) confer a sixfold higher risk of steatosis in subjects from the UK Biobank.⁵ An enrichment in pathogenic *APOB* variants has also been detected in MASLD-HCC patients.⁶ Here, we report how a heterozygous mutation of *APOB*, combined with two frequent MASLD-related SNPs (*PNPLA3* and *TM6SF2*), cosegregates with a steatotic liver disease (SLD) phenotype evolving towards cirrhosis and HCC in a pedigree of 11 members.

The proband was a 55-year-old man referred to our liver transplantation centre for HCC developed on cryptogenic cirrhosis. The patient had no previous medical records or history of alcohol abuse. All the following values were normal: ceruloplasmin, serum and 24-h urine copper; lysosomal acid lipase. At the time of diagnosis,

his body mass index (BMI) was 23.9 kg/m². He was tested negative for all viral hepatitis, iron overload and autoimmunity. Low-density lipoprotein cholesterol serum concentration was below the 5th percentile for age and sex. Liver histology revealed liver cirrhosis with mild micro- and macro-vesicular steatosis in 10% of hepatocytes with a moderate parenchymal lymphoplasmacytic inflammatory infiltrate.

The brother of the proband was transplanted 2 years earlier at the age of 57 for a multifocal HCC developed on cryptogenic cirrhosis. He had no features of metabolic syndrome except overweight (BMI 27.1 kg/m²) and no excessive alcohol consumption. Liver explant analysis showed four HCCs developed in a macronodular cirrhosis with hepatocellular ballooning but no steatosis.

The patient was suspected of heterozygous familial hypobetalipoproteinemia (Hz-FHBL-SD2). The family pedigree includes one sister, one half-brother and eight children from this sibling (Figure 1). The patients were recruited in Lyon (GENELIP/ASAP cohort study; clinical trial registration number: NCT03939039). Written informed consent from the proband and family members was obtained, according to French bioethical laws. The study was carried out according to the Code of Ethics of the World Medical Association (Declaration of Helsinki) and obtained the agreement of the Ethics Committee of

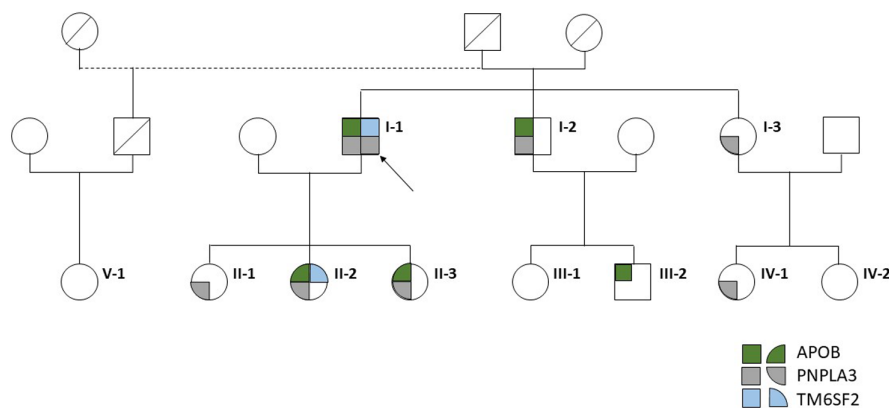


FIGURE 1 Family pedigree with clinical and biochemical data. Arrow: index case. Members carrying the *APOB* mutation are represented with a green-filled symbol. The grey-filled symbol represents the I148M *PNPLA3* carriers (at the heterozygous or homozygous state) and the blue-filled symbol indicates the presence of the minor allele of *TM6SF2* (E167K).

Abbreviations: BMI, body mass index; FHBL-SD2, familial hypobetalipoproteinemia; HBL, hypobetalipoproteinemia; HCC, hepatocellular carcinoma; HDL, high-density lipoprotein cholesterol; Hz-FHBL-SD2, heterozygous familial hypobetalipoproteinemia; LDL-C, low-density lipoprotein cholesterol; LSM, liver stiffness measurement; MASLD, metabolic dysfunction-associated steatotic liver disease; SLD, steatotic liver disease; SNP, single nucleotide polymorphism; TC, total cholesterol; TG, triglycerides.

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2024 The Authors. *Liver International* published by John Wiley & Sons Ltd.

TABLE 1 Clinical and biochemical data of all family members studied.

Subject	Age (y)	Sex	BMI (kg/m ²)	HCC	APOB gene mutation	PNPLA3 rs738409C>G	TM6SF2 rs58542926 G>A	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	ApoB (g/L)	Vitamin E (μmol/L)	AST (U/L)	ALT (U/L)	LSM (kPa)	CAP (db/m)
I-1 (WP)	55	M	23.9	Present	Present	GG	GA	2.24	.95	.12	1.69	.70	/	18	20	/	/
I-2 (WJ)	45 (before LT)	M	27.1	Present	Present	CG	GG	1.9	.84	.34	1.18	/	/	28	27	/	/
	57 (after LT)							4.83	.59	1.57	2.96	/	25.8	78 ^a	70 ^a	/	/
I-3 (DM)	69	F		Absent	Absent	CG	GG	/	/	/	/	/	/	/	/	4.4	261 ^a
II-1 (WE)	29	F	30.5	Absent	Absent	CG	GG	3.30	.92	1.29	1.59	.65	21.9	31	54 ^a	4.3	347 ^a
II-2 (WC)	26	F	19.7	Absent	Present	CG	GA	3.40	.52	2.27	.89	.39	14.0 ^a	29	29	4.2	246 ^a
II-3 (WL)	22	F	24.8	Absent	Present	CG	GG	2.50	.51	1.69	.58	.32	14.2 ^a	18	20	4.3	190
III-1 (WV)	29	F		Absent	Absent	CC	GG	4.60	.55	1.56	2.79	.94	/	20	25	/	/
III-2 (WJ)	25	M	24.5	Absent	Present	CC	GG	2.10	.21	1.50	.50	<.26	12.3 ^a	27	64 ^a	8.7 ^a	329 ^a
IV-1 (DC)	43	F		Absent	Absent	CG	GG	5.95	.90	1.78	3.76	/	/	21	18	2.6	238
IV-2 (DF)	41	F		Absent	Absent	CC	GG	5.41	1.36	1.84	2.95	.87	/	17	21	/	/
V-1 (BK)	41	F	35.1	Absent	Absent	CC	GG	5.30	.70	1.59	3.39	.97	/	12	13	3.6	321 ^a

Note: G and A alleles are, respectively, the minor alleles of PNPLA3 and TM6SF2 genes. Laboratory reference ranges were 4.0–6.0, 0–1.7, 1.3–2.0, 2.0–4.1 mmol/L for, respectively, TC, TG, HDL-C and LDL-C. The laboratory reference range for ApoB was .55–1.25 g/L and 20.0–35.0 μmol/L for plasma vitamin E concentration. Bold type: <5th percentile for age and sex of the laboratory (<https://www.lipidtools.com/calculator-pages/reference/>) for lipid profile and <https://drmikemacdonald.com/apob-percentile-calculator-normal-range/>) for ApoB concentration.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CAP, controlled attenuation parameter; F, female; HCC, hepatocellular carcinoma; HDL, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; LSM, liver stiffness measurement; M, male; TC, total cholesterol; TG, triglycerides.

^aAbnormal values.

the 'Commission Nationale de l'Informatique et des Libertés' (CNIL) (N° 920434).

Venous blood samples were collected after an overnight fast. Serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL) cholesterol triglyceride, and ApoB concentrations were measured using commercial kits. Low-density lipoprotein cholesterol was calculated with the Friedewald equation. Imaging (ultrasound and transient elastography using Fibroscan®) was performed systematically for all offspring. Histological analysis (liver biopsy and/or explant analysis) was available in the proband and his older brother only. Fibrosis grade was assessed by Masson trichrome and Sirius red staining. Genomic DNA was isolated from 5 mL of EDTA-anticoagulated blood of all family members. Exons and intron-exon junctions of APOB, PCSK9 and ANGPTL3 were sequenced using the DysliSEQ custom NGS panel. Rare variants were confirmed by Sanger sequencing. Previously reported SNPs of PNPLA3 (rs738409, NM_025225.3:c.444C>G p.[Ile148Met]), TM6SF2 (rs58542926, NM_001001524.3:c.499G>A p.[Glu167Lys]), GCKR (rs1260326, P446L), MBOAT7 (rs641738, C>T) and HSD17B13 (rs72613567) were sequenced. PRS-HFC and PRS-5 were calculated using the previously published formulas.^{7,8}

The diagnosis of Hz-FHBL-SD2 was confirmed in the proband, revealing a heterozygous pathogenic nonsense mutation of APOB (c.7600C>T, p.[Arg2534*]) with a 55.5% truncated protein. This mutation, previously associated with FHBL-SD2 and liver steatosis,⁹ explained the low LDL-cholesterol concentrations. No mutation was identified in the PCSK9 and ANGPTL3 genes. PNPLA3 sequencing revealed the presence of four SNPs on exon 3, including the p.Ile148Met in a homozygous state. TM6SF2 sequencing revealed the minor allele for p.Glu167Lys at the heterozygous state. Robust polygenic risk scores indicated a high risk of HCC in case of MASLD (PRS-HFC and PRS-5 both at .934).⁷ A complete lipid profile and genotypic analysis were performed on all family members (one sister, one brother and eight children from this sibling). Hz-FHBL-SD2 was diagnosed in the brother, two of his daughters and his nephew. All the children and the brother of the proband carried the PNPLA3 polymorphism, but only one of his daughters (II-2) displayed the TM6SF2 variant (Figure 1). The APOB mutation segregated with low TC and LDL-cholesterol levels, <5th percentile for age and sex (Table 1).

The proband underwent liver transplantation, and the graft was functional with no HCC or steatosis recurrence on imaging follow-up 5 years after liver transplantation, as for his brother. Low-density lipoprotein cholesterol and ApoB levels normalized after liver transplantation in both (Table 1).

Regarding the other family members, the three carrying the APOB mutation were all aged <30 years. Two had steatosis based on controlled attenuation parameter (CAP), including one with elevated liver stiffness measurement (LSM) and abnormal liver function tests. The 69-year-old sister, carrying only the I148M PNPLA3 variant, showed isolated steatosis with normal LSM (Table 1). In the absence of detailed recommendations, in the patients carrying the APOB mutation a monitoring of plasma vitamin E concentration and a follow-up with LSM and US was proposed with a frequency adjusted according to the APOB and the PNPLA3 and TM6SF2 genotype.

FHBL represents a clinically complex and heterogeneous group of disorders characterized by a decrease of LDL-cholesterol and ApoB concentrations below the 5th age- and sex-specific percentile.⁹ Among monogenic inherited FHBLs, the most frequent is FHBL-SD2, caused by pathogenic variants in the APOB gene. Apolipoprotein B encodes the principal protein responsible for the assembly and secretion of very low-density lipoproteins (VLDL) and chylomicrons. A loss-of-function mutation of APOB results in liver steatosis and may lead to impaired intestinal absorption of fatty acids and antioxidant lipophilic vitamins when the size of truncated APOB is below 48%.⁴ Homozygous or compound heterozygous mutations of APOB (bi-allelic FHBL-SD2) lead to a severe phenotype, present from birth, associating steatorrhea, failure to thrive with hepatomegaly and steatosis.⁹ However, depending on the size of the truncated protein, FHBL-SD2 is sometimes diagnosed later and liver abnormalities can be isolated, even in bi-allelic FHBL-SD2.⁴ Conversely, Hz-FHBL-SD2 patients are often asymptomatic, identified in the setting of routine lipid profiling. However, an increased incidence liver steatosis and the occurrence of cryptogenic cirrhosis have been described in Hz-FHBL-SD2 adults with short truncated ApoB.^{8,10}

The risk of MASLD attributable to genetics is estimated at around 50% and is involved at all stages of the natural history of the disease, particularly fibrosis progression and HCC development (reviewed in 11). The PNPLA3 I148M variant is the principal contributor to genetic susceptibility in MASLD onset and progression.¹¹ Other genes such as TM6SF2, MBOAT7, GCKR or HSD17B13^{7,11} have also been reported. While the molecular mechanisms underlying the association of APOB heterozygous mutation with cirrhosis and HCC are unclear, PNPLA3 and TM6SF2 have been demonstrated to promote fibrogenesis and carcinogenesis in MASLD patients.¹¹ Since our patients had none of the classical components of the metabolic syndrome, the combination of heterozygous APOB mutation with PNPLA3 and TM6SF2 pathological variants has likely promoted steatosis, cirrhosis and HCC. If the genetic screening of common SNPs involved in MASLD is still not recommended, a complete lipid profile including ApoB dosage seems mandatory in cryptogenic SLD. Indeed, since the frequency of pathogenic APOB mutations has been estimated between 1:700 and 1:3000, FHBL-SD2 may be underdiagnosed and participate in SLD development and progression notably in patients without metabolic syndrome components as recently suggested by others.^{12,13} Lipid profiles need to be evaluated with caution in cirrhotic patients knowing that low LDLc and HDLc serum levels may be related to liver failure.

In conclusion, we show how a heterozygous nonsense mutation of the APOB gene may result in the development of steatosis, cirrhosis and HCC when associated with common genetic variants (PNPLA3 and TM6SF2). We also describe one of the few reported cases of liver transplantation for FHBL-SD2 and confirm the feasibility and efficacy of this treatment to cure both the underlying liver disease and the dyslipidaemia. Finally, our study highlights the benefits of genetic analysis in cryptogenic cirrhosis and atypical cases of MASLD. The identification of SLD related to APOB deleterious variants must lead to family screening and careful monitoring of these patients at high risk of fibrosis progression.

KEYWORDS

Apolipoprotein B, familial hypobetalipoproteinemia, FHBL, FHBL-SD2, MASLD, NAFLD, NASH, SNP

AUTHOR CONTRIBUTIONS

Yasmina Chouik, Mathilde Di Filippo, Philippe Moulin and Massimo Levrero wrote the manuscript. Mathilde Di Filippo and Philippe Moulin performed the genetic analyses. Sylvie Radenne and Jérôme Dumortier were involved in the patient's medical care, and all the authors approved the final version of the manuscript.

FUNDING INFORMATION


None.

CONFLICT OF INTEREST STATEMENT

The authors do not have any disclosures to report.


DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Yasmina Chouik^{1,2,3,4} 

Mathilde Di Filippo^{5,6}

Sylvie Radenne⁴

Jérôme Dumortier^{1,2,3,7} 

Philippe Moulin^{6,8}

Massimo Levrero^{1,2,3,4,9,10}

¹INSERM U1052, CNRS UMR_5286, Cancer Research Center of Lyon, Lyon, France

²University of Lyon, Université Claude-Bernard 1, UMR_S1052, Lyon, France

³Institute of Hepatology Lyon, Lyon, France

⁴Department of Hepatology, Hôpital Croix-Rousse, Hospices Civils de Lyon, Lyon, France

⁵Department of Biochemistry and Molecular Biology, Hospices Civils de Lyon, Lyon, France

⁶CarMeN Laboratory, UMR INSERM U1060/INRAE U1397, Claude Bernard Lyon1 University, Bron, France

⁷Department of Hepatology, Hospices Civils de Lyon, Hôpital Universitaire Edouard Herriot, Lyon, France

⁸Department of Endocrinology, Metabolic Disease, Diabetes and Nutrition, Hospices Civils de Lyon, Lyon, France

⁹Department of Medicine SCIAC, University of Rome La Sapienza, Rome, Italy

¹⁰The Italian Institute of Technology (IIT) Center for Life Nanosciences (CLNS), University of Rome La Sapienza, Rome, Italy

Correspondence

Yasmina Chouik, Hôpital Croix-Rousse, Hospices Civils de Lyon, Lyon, France.

Email: yasmina.chouik@inserm.fr

ORCID

Yasmina Chouik  <https://orcid.org/0000-0001-7548-3703>

Jérôme Dumortier  <https://orcid.org/0000-0002-7824-5396>

REFERENCES

- Romeo S, Sanyal A, Valenti L. Leveraging human genetics to identify potential new treatments for fatty liver disease. *Cell Metab.* 2020;31(1):35-45. doi:[10.1016/j.cmet.2019.12.002](https://doi.org/10.1016/j.cmet.2019.12.002)
- Holmer M, Ekstedt M, Nasr P, et al. Effect of common genetic variants on the risk of cirrhosis in non-alcoholic fatty liver disease during 20 years of follow-up. *Liver Int.* 2022;42(12):2769-2780. doi:[10.1111/liv.15438](https://doi.org/10.1111/liv.15438)
- Seko Y, Yamaguchi K, Shima T, et al. The greater impact of PNPLA3 polymorphism on liver-related events in Japanese non-alcoholic fatty liver disease patients: a multicentre cohort study. *Liver Int.* 2023;43(10):2210-2219. doi:[10.1111/liv.15678](https://doi.org/10.1111/liv.15678)
- Di Filippo M, Moulin P, Roy P, et al. Homozygous MTTP and APOB mutations may lead to hepatic steatosis and fibrosis despite metabolic differences in congenital hypocholesterolemia. *J Hepatol.* 2014;61(4):891-902. doi:[10.1016/j.jhep.2014.05.023](https://doi.org/10.1016/j.jhep.2014.05.023)
- Haas ME, Pirruccello JP, Friedman SN, et al. Machine learning enables new insights into genetic contributions to liver fat accumulation. *Cell Genom.* 2021;1(3):100066. doi:[10.1016/j.xgen.2021.100066](https://doi.org/10.1016/j.xgen.2021.100066)
- Pelusi S, Baselli G, Pietrelli A, et al. Rare pathogenic variants predispose to hepatocellular carcinoma in nonalcoholic fatty liver disease. *Sci Rep.* 2019;9(1):3682. doi:[10.1038/s41598-019-39998-2](https://doi.org/10.1038/s41598-019-39998-2)
- 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(4):775-782. doi:[10.1016/j.jhep.2020.11.024](https://doi.org/10.1016/j.jhep.2020.11.024)
- Cefalù AB, Pirruccello JP, Noto D, et al. A novel APOB mutation identified by exome sequencing cosegregates with steatosis, liver cancer, and hypocholesterolemia. *Arterioscler Thromb Vasc Biol.* 2013;33(8):2021-2025. doi:[10.1161/ATVBAHA.112.301101](https://doi.org/10.1161/ATVBAHA.112.301101)
- Bredfeldt C, Peretti N, Hussain MM. Medical advisory panel. New classification and management of abetalipoproteinemia and related disorders. *Gastroenterology.* 2021;160(6):1912-1916. doi:[10.1053/j.gastro.2020.11.040](https://doi.org/10.1053/j.gastro.2020.11.040)
- Bonnefont-Rousselot D, Condat B, Sassolas A, et al. Cryptogenic cirrhosis in a patient with familial hypocholesterolemia due to a new truncated form of apolipoprotein B. *Eur J Gastroenterol Hepatol.* 2009;21(1):104-108. doi:[10.1097/MEG.0b013e3282ff9f8](https://doi.org/10.1097/MEG.0b013e3282ff9f8)
- Eslam M, Valenti L, Romeo S. Genetics and epigenetics of NAFLD and NASH: clinical impact. *J Hepatol.* 2018;68(2):268-279. doi:[10.1016/j.jhep.2017.09.003](https://doi.org/10.1016/j.jhep.2017.09.003)
- Zheng M, Huang DQ, Konkwo C, et al. Genomic analysis of lean individuals with NAFLD identifies monogenic disorders in a prospective cohort study. *JHEP Rep.* 2023;5(4):100692. doi:[10.1016/j.jhepr.2023.100692](https://doi.org/10.1016/j.jhepr.2023.100692)
- Buryska S, Ahn JC, Allen AM, Simha V, Simonetto DA. Familial hypobetalipoproteinemia: an underrecognized cause of lean NASH. *Hepatology.* 2021;74(5):2897-2898. doi:[10.1002/hep.31988](https://doi.org/10.1002/hep.31988)