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***APOB* and *PNPLA3* Double Heterozygosity in a Father-Son Pair with Advanced
Nonalcoholic Fatty Liver Disease**

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Abbreviations: APOB: apolipoprotein (apo) B; FHBL: familial hypobetalipoproteinemia; HCC: hepatocellular carcinoma; LDL: low-density lipoprotein cholesterol; NAFLD: non-alcoholic fatty liver disease; NASH: non-alcoholic steatohepatitis; PNPLA3: patatin-like phospholipase domain-containing protein 3, HSD17B13: 17-beta hydroxysteroid dehydrogenase 13, TM6SF2: transmembrane 6 superfamily member 2, MBOAT7: Membrane-bound O-acyltransferase domain-containing 7, GCKR: Glucokinase (hexokinase 4) regulator

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Introduction

Patients with homozygous familial hypobetalipoproteinemia (FHBL) may develop non-alcoholic steatohepatitis (NASH) and fibrosis; heterozygous FHBL is typically not associated with clinically significant nonalcoholic fatty liver disease (NAFLD).¹

Case Presentations

A Caucasian father-son pair with well characterized NAFLD and very low low-density lipoprotein (LDL) cholesterol is followed in our hepatology clinic. The father is a 71-year-old with well characterized NAFLD cirrhosis who exhibited abdominal obesity and splenomegaly. His serum LDL was 28 mg/dL while not on any lipid lowering therapy. His other labs are shown in **Table 1**. Liver histology revealed active steatohepatitis with cirrhosis and hepatic venous pressure gradient was 12 mmHg. Recent surveillance for hepatocellular carcinoma (HCC) with triple phase CT showed two new small arterially enhancing lesions

concerning for early HCC. Targeted exome sequencing revealed a heterozygous mutation in apolipoprotein (apo) B (*APOB*) gene, c.1830 -1G>A, which is a pathogenic splicing variant in intron 13 reported to cause truncated apo B resulting in FHBL.² Genotyping for other genetic variants of interest showed heterozygosity for *PNPLA3*rs738409 (CG) and *HSD17B13* rs6834314 (AG), but ancestral genotypes for *TM6SF2* rs58542926 (CC), *MBOAT7* rs626283(GG), *GCKR* rs1260326 (CC)³,

The 46-year-old son was evaluated for elevated transaminases, hepatic steatosis by imaging, and very low LDL cholesterol. Physical exam was remarkable for abdominal obesity, hepatomegaly, and mild acanthosis at the elbow and nuchal areas. His fasting LDL-C was 15 mg/dL. Liver biopsy showed 80% macrovesicular steatosis, lobular inflammation and moderate ballooning, and stage 2 fibrosis (NAFLD activity score 5). He had genetic testing performed with identical results to his father's except for having one risk allele for *GCKR* rs1260326 (CT).

Discussion

This case presentation describes a father and a son with double heterozygosity for *APOB* and *PNPLA3* rs738409 in the setting of obesity. FHBL results in elevated levels of intrahepatic triglyceride content because of impaired VLDL metabolism and thus defective triglyceride export from the liver, but does not result in insulin resistance.' In patients who are heterozygous, the normal allele should allow for sufficient transport and secretion of primordial lipoprotein particles resulting in mobilization of fat from the liver. This lipoprotein assembly machinery and transport is, however, crippled in homozygotes. Fat retention in the liver has been implicated to play a causal role in chronic liver disease according to a recent a Mendelian randomization study.⁴ Furthermore, individuals with HCC related to NAFLD have an enrichment in inherited pathogenic variants, in particular *APOB*.⁵

The G allele in *PNPLA3* rs738409 is strongly associated with liver inflammation and elevated intrahepatic fat levels.⁷ In patients with NAFLD, *PNPLA3* GG homozygosity is associated with ~ 3-fold higher prevalence of NASH³, but *PNPLA3* CG heterozygosity alone may not be sufficient to cause advanced NAFLD. Our father-son pair were heterozygous for *HSD17B13* rs6834314 variants which are associated with lower risk of inflammation, ballooning, and cirrhosis.⁶ But, in case of our father-son pair, they did not protect them against advanced NASH, presumably due to their carriage pathogenic APOB and *PNPLA3* genotypes.

This father-son case series shows NAFLD phenotype to be the result of interacting effects of metabolic and disease modifying genetic variants. We believe the combination of *APOB* and *PNPLA3* heterozygosity resulted in clinically significant NAFLD in our obese father-son pair despite having a favorable *HSD17B13* allele. Very low serum LDL-C levels were the bedside hint for us to search for these genetic variants in our two patients.

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Table 1: Laboratory and Genotyping Results Comparing Father and Son

	Father	Son
BMI (kg/m ²)	35.6	34.5
Weight (kg)	114	116
Alkaline phosphatase (IU/L)	61	61
AST (IU/L)	40	41
ALT (IU/L)	29	109
Total bilirubin (mg/dL)	1.7	0.7
Albumin (g/dL)	3.9	4.6
Total cholesterol (mg/dL)	104	60
HDL cholesterol (mg/dL)	64	39
Triglycerides (mg/dL)	62	30
LDL cholesterol (mg/dL)	28	15
Platelets (k/mm ³)	83	192
INR	1.34	1.01
Fasting blood glucose (mg/dL)		70
Fasting serum insulin (mcu/ml)		8.09
Hemoglobin A1c (%)		5.1

Abbreviations: BMI: body mass index, AST: aspartate aminotransferase, ALT: alanine aminotransferase, HDL: high-density lipoprotein, LDL: low-density lipoprotein, INR: international normalized ratio