Whole-Exome Sequencing Study of Extreme Phenotypes of NAFLD

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Nonalcoholic fatty liver disease (NAFLD) is a heterogeneous disease with highly variable outcomes. Patients with simple steatosis typically experience a benign course, whereas those with more advanced liver injury, nonalcoholic steatohepatitis (NASH), and advanced stage fibrosis suffer increased risk for complications such as cirrhosis, hepatic decompensation, and liver cancer. Genetic variants in patatin-like phospholipase domain-containing 3 (PNPLA3) and transmembrane 6 superfamily member 2 (TM6SF2) and clinical factors including diabetes, obesity, and older age increase a patient’s risk for NASH, advanced fibrosis, and worse outcomes. Despite substantial investigation and identification of some common variants associated with NAFLD and advanced fibrosis, the genetics and functional mechanisms remain poorly understood. This study aimed to identify genetic variants by whole-exome sequencing of NAFLD phenotypes to provide novel insights into mechanisms behind NAFLD pathogenesis and variability. We sequenced 82 patients with liver biopsy–confirmed NAFLD and 4455 population controls. NAFLD patients were divided into extreme phenotypes based on liver fibrosis stage and clinical risk factors to investigate rare variants that might predispose to or protect from advanced NAFLD fibrosis. We compared NAFLD extremes to each other and individually to population controls, exploring genetic variation at both the single-variant and gene-based level. We replicated known associations with PNPLA3 and TM6SF2 and advanced fibrosis, despite sample-size limitations. We also observed enrichment of variation in distinct genes for progressor or protective NAFLD phenotypes, although these genes did not reach statistical significance. Conclusion: We report the first whole-exome sequencing study of genetic variation in liver biopsy–confirmed NAFLD susceptibility and severity, using a small cohort of extreme NAFLD phenotypes and a large cohort of population controls. (Hepatology Communications 2018;2:1021-1029)
Obesity, male gender, and older age) are associated with hepatic fibrosis risk. However, not all NAFLD patients with risk factors develop advanced liver disease, suggesting a genetic contribution. Genome-wide association studies (GWAS) have reproducibly identified patatin-like phospholipase domain-containing 3 (PNPLA3) I148M as associated with NAFLD susceptibility and severity. Although several additional genes have been implicated, including hydroxysteroid 17-beta dehydrogenase 13 (HSD17B13) most recently, currently the only other independently reproducible association is with transmembrane 6 superfamily member 2 (TM6SF2) and few signals have been tracked to causal variants.

In contrast to GWAS, next-generation sequencing interrogates rare variation and can often directly pinpoint causal variants including for complex diseases. Understanding the full spectrum of NAFLD genetic variation predisposing to or protecting from advanced fibrosis may facilitate biomarker discovery or assist with novel treatment development. We used whole-exome sequencing (WES) to examine the entire coding portion of the genome to identify potential causal variants for NAFLD fibrosis progression and protection. To accurately define different risk categories within the NAFLD spectrum, we used gold standard liver biopsy for NAFLD fibrosis staging and common clinical measurements related to NASH and advanced fibrosis. We sampled NAFLD phenotypic distribution extremes to enrich for selection of rare causal variants with potentially larger effect sizes.

We defined two extreme NAFLD fibrosis phenotypes: “protective” and “progressor.” We hypothesized that protective patients (i.e., those without advanced fibrosis despite being high risk [older, obese, and diabetic]) might harbor genetic variants that protect them from fibrosis progression, whereas progressor patients (i.e., those with advanced fibrosis despite lacking this clinical risk profile) might carry genetic variants enhancing their fibrosis vulnerability. Here we report a comprehensive WES study that investigates genetic variation underlying NAFLD fibrosis risk and progression.

Materials and Methods

PATIENT SELECTION

We selected two cohorts of NAFLD patients from the Duke University Health System NAFLD Biorepository using an extreme phenotype design. The NAFLD Biorepository, details of which have been published previously, contains specimens and clinical data from NAFLD patients who underwent diagnostic liver biopsy to grade and stage NAFLD severity as standard of care. The Biorepository has the Duke Institutional Review Board’s approval, and patients consented to genomic analyses.

We defined two extreme phenotypes of NAFLD: protective and progressor, based on the development of advanced liver fibrosis (fibrosis stage, F3–F4). The protective phenotype included NAFLD patients expected to have significant liver injury and fibrosis based on clinical risk factors (age <50 years, body mass index [BMI] <30 kg/m², and type 2 diabetes mellitus).
but with no or little fibrosis on liver biopsy (fibrosis stage, F0–F1). At the other extreme, the pro-
geressor phenotype included NAFLD patients expected
to have little fibrosis based on a lack of clinical risk fac-
tors (age <55 years, BMI <35 kg/m², no T2DM), but
biopsy showed advanced liver fibrosis or cirrhosis.

Final analyses included Caucasian individuals, the
majority of both NAFLD cohorts. We compared the
two extreme NAFLD phenotypes as well as each
extreme phenotype to previously sequenced Caucasian
population controls from unrelated Duke University
studies with available consent for use.

SEQUENCING AND QUALITY
CONTROL

WES was performed on 103 NAFLD patients with
available stored genomic DNA. Standard protocols
and Illumina HiSeq platforms were used to sequence
NAFLD patients and population controls (Supporting
Information). Following quality control, 82 Caucasian
NAFLD samples (54 protective, 28 progressor) and 4455
Caucasian population controls were available for analysis.

DATA ANALYSIS AND
ASSOCIATION TESTING

We performed downstream statistical analyses using
in-house pipeline ATAV version 5.8 software (https://
github.com/igm-team/atav/). Three primary com-
parisons were conducted: (1) NAFLD progressor
versus NAFLD protective; (2) NAFLD protective ver-
sus population controls; and (3) NAFLD progressor
versus population controls. For each comparison, we
performed single-variant (Fisher’s exact test) and gene-
based collapsing analyses (progressor versus protective
λ = 0.50-0.99, protective versus controls λ = 1.16-2.23,
progressor versus controls λ = 1.16-2.50; representa-
tive quantile–quantile plots in Supporting Figs. S1-S3).
Statistical significance was based on Bonferroni-
corrected thresholds for the number of variants or
genes tested, respectively (Supporting Table S1).

Results

Eighty-two Caucasian NAFLD patients were
included for analysis. Given the extreme phenotype
design, all protective phenotype patients were age 50
or older with T2DM and obesity (median BMI = 41
kg/m²), whereas progressors were age 55 or younger
without T2DM and lower median BMI (32 kg/m²). Most
of the progressors were male and had a NAFLD
activity score greater than or equal to 4 (Supporting
Table S2).

NAFLD PROGRESSOR VERSUS
NAFLD PROTECTIVE

No variants or genes reached genome-wide statis-
tical significance after quality control and multiple
testing correction in the progressor versus protective
extreme phenotypes comparison. However, there was
nonsignificant enrichment of the known PNPLA3
I148M (rs738409, P = 8.42E-05) and TM6SF2
E167K (rs58542926, P = 4.10E-03) polymor-
phisms under single-variant allelic models among the
NAFLD progressors (5–9,11) An adjacent synonymous
variant in PNPLA3, P149 (rs738408, P = 8.42E-05),
in perfect linkage disequilibrium (r² = 1) with I148M,
also neared significance. PNPLA3 I148M is in a com-
mon haplotype block and, although variants in nearby
genes PARVB W37R and SAMM50 G453 neared the
genome-wide significance threshold, adjustment for
PNPLA3 I148M completely eliminated any associa-
tion signal in this region (data not shown), consistent
with previous literature. (16)

Among the top nonsignificant variants in our
analysis, several were enriched in genes that differed
between the NAFLD cohorts. These associations
highlight genes and pathways that may either promote
or protect against fibrosis progression; however, with
current available evidence, their involvement remains
uncertain. Nonsignificant variants enriched among
progressors in biologically plausible, although not pre-
viously implicated, NAFLD genes included several
immune-related findings (Table 1 and Supporting
Tables S3 and S4). Common variants with nonsignif-
icant enrichment under allelic models included S31R
(rs1801270, P = 1.30E-03) in cyclin-dependent kinase
inhibitor 1A (CDKN1A) and L439V (rs11465927,
P = 6.70E-03) in interleukin 1 (IL1) receptor asso-
ciated kinase 2 (IRAK2). CDKN1A encodes p21, a
senescence marker involved in innate immunity,
whose hepatocyte expression has been associated with
NAFLD fibrosis stage. (17) A previous candidate gene
study linked several CDKN1A variants with NAFLD
fibrosis development, including S31R, although S31R
was not associated with fibrosis and only borderline
associated with steatohepatitis. (17) IRAK2 is part of
the innate immune response, acting in IL1R-mediated
and IL1-mediated signaling. Under recessive models,
| Gene       | Variant | rs#      | NAFLD Progressors With Variant, N (MAF) | NAFLD Protective With Variant, N (MAF) | PolyPhen Prediction | ExAC Global MAF | Genetic Model | P Value | Enrichment |
|------------|---------|----------|----------------------------------------|---------------------------------------|---------------------|----------------|---------------|---------|------------|
| PNPLA3     | I148M   | rs738409 | 24 (0.61)                              | 24 (0.28)                             | Probably damaging   | 0.26           | SV allelic   | 8.42E-05 | Progressor |
| PNPLA3     | P149    | rs738408 | 24 (0.61)                              | 24 (0.28)                             | NA                  | 0.26           | SV allelic   | 8.42E-05 | Progressor |
| SAMM50     | G453    | rs7587   | 1 (0.04)                               | 26 (0.27)                             | NA                  | 0.22           | SV allelic   | 4.23E-04 | Protective |
| PTX4       | G36C    | rs1040499| 11 (0.20)                              | 39 (0.47)                             | Unknown             | 0.41           | SV allelic   | 6.26E-04 | Protective |
| PTX4       | R276K   | rs2745098| 12 (0.21)                              | 37 (0.46)                             | Benign              | 0.41           | SV allelic   | 2.10E-03 | Protective |
| CDKN1A     | S31R    | rs1801270| 9 (0.18)                               | 3 (0.03)                              | Benign              | 0.15           | SV allelic   | 1.30E-03 | Progressor |
| GBP1       | A409G   | rs1048443| 9 (0.16)                               | 36 (0.41)                             | Probably/possibly damaging | 0.30           | SV allelic   | 1.40E-03 | Protective |
| TM6SF2     | E167K   | rs58542926| 5 (0.09)                              | 0 (0)                                 | Probably/possibly damaging | 0.02           | SV allelic   | 4.10E-03 | Protective |
| IRAK2      | L439V   | rs11465927| 5 (0.11)                              | 1 (0.01)                              | Benign              | 0.03           | SV allelic   | 6.70E-03 | Progressor |
| PARVB      | W37R    | rs1007863| 39 (0.44)                              | 39 (0.44)                             | Benign              | 0.44           | SV recessive | 2.28E-04 | Progressor |
| VRK2       | I167V   | rs1051061| 21 (0.57)                              | 36 (0.37)                             | Probably damaging   | 0.36           | SV recessive | 7.63E-04 | Progressor |
| SEC31B     | L1071V  | NA       | 1                                      | 0                                     | Benign              | 0.006          | GB dominant  | 0.006   | Progressor |
| V504M      | G86R    | NA       | 1                                      | 0                                     | Benign              | 0.03           | GB dominant  | 0.006   | Progressor |

Abbreviations: ExAC, exome aggregation consortium; GB, gene-based; MAF, minor allele frequency; NA, not available; SV, single-variant.
I167V (rs1051061, \( P = 7.63 \times 10^{-4} \)) was enriched in vaccinia-related kinase 2 (\( VRK2 \)), an IL1-mediated signaling effector.

Among the protective phenotype, in which variants might reduce fibrosis progression risk, several immune genes were among the top results, although not in the IL1 pathway. We saw nonsignificant enrichment in all genetic models of G36C (rs1040499, \( P = 6.26 \times 10^{-4} \)) and R276K (rs2745098, \( P = 2.10 \times 10^{-3} \)) in long pentraxin 4 (\( PTX4 \)), which are in high linkage disequilibrium (\( r^2 = 0.93 \)) as well as A409G (rs1048443, \( P = 1.40 \times 10^{-3} \)) in interferon (IFN)-inducible guanylate binding protein 1 (\( GBP1 \)). PTX4 is a potential functional antibody ancestor that acts in innate immunity, whereas GBP1 is a guanosine triphosphatase that regulates IL2 secretion and acts in cytokine, IFN-\( \gamma \), and T-cell receptor-mediated signaling pathways.

**NAFLD PROTECTIVE VERSUS POPULATION CONTROLS**

The protective versus population control comparison investigated susceptibility to this NAFLD phenotype, as well as possible protective variants against advanced fibrosis. We did not discover any significant, high-quality variants. Several nonsignificant rare variants in genes involved in immune-, liver-, lipid-, or fibrosis-related pathways were identified, although their NAFLD role remains unconfirmed (Table 2 and Supporting Tables S5 and S6).

Three extremely rare variants in immune-related genes were nonsignificantly enriched among the NAFLD protective phenotype relative to population controls under single-variant all genetic models: I13V (\( P = 1.42 \times 10^{-4} \)) in SLAM (signaling lymphocytic activation molecule) family member 7 (\( SLAMF7 \)), C161 (\( P = 1.42 \times 10^{-4} \)) in IL6, and T151 (\( P = 4.24 \times 10^{-4} \)) in orosomucoid 1 (\( ORM1 \)). Under a dominant gene-based model, D162V (rs2069860, \( P = 0.004 \)) in IL6 was also enriched. IL6 influences inflammation-associated disease states, including metabolic syndrome diseases such as diabetes. IL6 also upregulates IL32, which induces tumor necrosis factor-\( \alpha \) (TNF-\( \alpha \)) macrophage production and acts in oxidative damage response. Interestingly, IL32 can attenuate alcohol-induced liver injury, as well as lipid accumulation in mice on a high-fat diet.\(^{18,19} \) Also within the IL6 pathway, ORM1 is an acute phase plasma reactant protein involved in immunosuppression, including negative regulation of IL6 and TNF-\( \alpha \), with a potential role in alcoholic liver cirrhosis.\(^{20} \) SLAMF7 activates natural killer cells, inhibits pro-inflammatory

### Table 2. NAFLD Protective (n = 54) Versus Population Controls (n = 4455) Comparison: Top Associated Variants in Biologically Relevant Genes

| Gene          | Variant | rs#           | NAFLD Protective With Variant, N (MAF) | Controls With Variant, N (MAF) | PolyPhen Prediction | ExAC Global MAF | Genetic Model | \( P \) Value |
|---------------|---------|---------------|----------------------------------------|--------------------------------|---------------------|----------------|--------------|--------------|
| E168K         | NA      | rs2069860     | 5 (0.05)                               | 75                             | Benign              | 0.006          | GB dominant  | 0.004        |
| THEM5         | NA      | rs2069860     | 1 (0.02)                               | 0                               | Benign              | 0.0006         | GB dominant  | 0.006        |
| CYP26B1       | V456L   | NA            | 1 (0.02)                               | 0                               | Benign              | 0.0006         | GB recessive | 0.012        |

Abbreviations: ExAC, exome aggregation consortium; GB, gene-based; MAF, minor allele frequency; NA, not available; SV, single-variant.
cytokines including TNF-α, and has been associated with several autoimmune diseases including diabetes.\(^\text{(21)}\)

**NAFLD PROGRESSOR VERSUS POPULATION CONTROLS**

In the progressor versus population control comparison, PNPLA3 variants I148M and P149 reached statistical significance (both \(P = 2.10\times10^{-9}\) allelic) despite small progressor sample size. Although there were no other robust, significant associations, we observed non-significant enrichment in several NAFLD-associated genes: TM6SF2 E167K \((P = 8.88\times10^{-4}\) allelic), PARVB, and SAMM50. The enrichment of PNPLA3 I148M and TM6SF2 E167K in both the progressor versus protective and progressor versus control comparisons, but not in the protective versus control comparison, provides further evidence that these variants are important for NAFLD fibrosis progression independent of T2DM and obesity.

Among the top nonsignificant gene-based associations, several genes were associated with metabolic syndrome and lipids (Table 3 and Supporting Tables S7 and S8). Under a dominant gene-based model, we observed nonsignificant enrichment of rare variation in the major histocompatibility complex I immune response molecule alpha-2-glycoprotein 1 zinc-binding \((AZGP1; P = 0.007, H214Q \text{ and } A46V)\), which regulates fatty acid synthesis and cell adhesion. AZGP1 is implicated in metabolic syndrome and insulin sensitivity, is elevated in kidney injury, and is a lipid catabolism biomarker. Additionally, under recessive single-variant and gene-based models, a single nonsynonymous variant, I717V \((rs2759, P = 7.76\times10^{-4}\) single-variant; \(P = 7.75\times10^{-4}\) gene-based) was enriched in myeloperoxidase \((MPO)\), a major component of neutrophil granules that acts in low-density lipoprotein remodeling.\(^{22}\)

**TABLE 3. NAFLD PROGRESSOR (N = 28) VERSUS POPULATION CONTROLS (N = 4455) COMPARISON: TOP ASSOCIATED VARIANTS IN BIOLOGICALLY RELEVANT GENES**

| Gene         | Variant | rs#            | NAFLD Progressors | ExAC Global | Genetic Model | PValue     |
|--------------|---------|----------------|-------------------|-------------|---------------|------------|
|              |         |                | With Variant, N (MAF) |            |               |            |
|              |         |                | Controls With Variant, N (MAF) |            |               |            |
|              |         |                | PolyPhen Prediction |            |               |            |
|              |         |                | ExAC Global MAF |            |               |            |
|              |         |                | Genetic Model |            |               |            |
|              |         |                | PValue           |            |               |            |
| PNPLA3       | P149    | rs738408       | 24 (0.61)         | 1826 (0.23) | NA            | 0.26 SV allelic (and recessive) | **2.09E-09** |
| PNPLA3       | I148M   | rs738409       | 24 (0.61)         | 1826 (0.23) | Probably damaging | 0.26 SV allelic (and recessive) | **2.10E-09** |
| PARVB        | W37R    | rs1007863      | 23 (0.70)         | 2691 (0.40) | Benign        | 0.44 SV allelic (and recessive) | 8.20E-06     |
| SAMM50       | D110G   | rs3761472      | 17 (0.38)         | 1329 (0.16) | Benign        | 0.21 SV allelic | 1.45E-04 |
| TM6SF2       | E167K   | rs58542926     | 9 (0.20)          | 549 (0.06)  | Probably/possibly damaging | 0.07 SV allelic | 8.88E-04     |
| MPO          | I717V   | rs2759         | 4 (0.11)          | 237 (0.03)  | Benign        | 0.02 SV recessive | 7.76E-04 |
| HIST1H2BC    | FS (chr6: 26124019 insT) | NA | 1 | 1 | NA | 0 GB dominant | 0.002 |
|              | A22S    | NA             | 1                 | 1           | Unknown       | 2.00E-05 GB dominant | 0.007 |
| AZGP1        | H214Q   | NA             | 1                 | 1           | Benign        | 2.00E-05 GB dominant | 0.007 |
|              | A46V    | rs142669146    | 1                 | 0           | Benign        | 1.00E-04 GB recessive | 7.75E-04 |
| MRGPRX1      | Q307R   | rs138752944    | 1                 | 0           | Benign        | 3.00E-04 GB recessive | 7.75E-04 |
|              | Q307*   | rs140371088    | 1                 | 0           | NA            | 3.00E-04 GB recessive | 7.75E-04 |
|              | Y272C   | NA             | 1                 | 0           | Probably damaging | 1.00E-04 GB recessive | 7.75E-04 |
| CYP26B1      | A420G and R191H | rs7568553 and rs76025186 | 1 | 0 | Benign and probably damaging | 0.006 and 0.001 GB compound-heterozygous | 0.006 |
|              | EFCAB13 | K244* and T577R/T481R | NA and rs142664574 | 1 | 0 | NA and possibly damaging/benign | 2.00E-05 and 0.005 GB compound-heterozygous | 0.007 |

Note: Italicized and bolded variants reached statistical significance.
Abbreviations: ExAC, exome aggregation consortium; GB, gene-based; MAF, minor allele frequency; NA, not available; SV, single-variant
**Discussion**

A major goal in caring for patients with NAFLD is determining who is at risk for fibrosis and thus poor outcomes. We aimed to determine the contribution of genetic variants to this risk by using WES in patients at NAFLD extremes. We confirmed the significance of PNPLA3 I148M in NAFLD fibrosis progression as identified in previous studies.\(^{(8,9)}\) Importantly, we were able to do this in a small NAFLD cohort through accurate phenotyping of biopsy-proven NAFLD patients. Our results support a role for PNPLA3 directly in NAFLD fibrosis severity, as previously implicated,\(^{(7)}\) and the recent suggestion that PNPLA3 potentiates the pro-fibrogenic features of hepatic stellate cells.\(^{(24)}\)

Similarly, the enrichment of TM6SF2 E167K observed among progressors is consistent with previous associations between E167K and hepatic fibrosis progression in NAFLD,\(^{(11)}\) although it did not reach significance in this study.

Although only PNPLA3 reached statistical significance, and only in the larger progressor versus control comparison, we observed several suggestive associations in biologically relevant genes broadly in line with a role for a pro-inflammatory state in NAFLD development. These findings are important for future hypothesis-driven research but require replication in independent NAFLD cohorts, as immune genes compose a substantial fraction of the human genome, enabling considerable narrative potential. Among the top associations in the NAFLD progressor versus protective comparison, several affected the immune genes, with distinct genes and biological processes observed for each phenotype. Among progressors, two variants were enriched in IL1 signaling pathway genes (IRAK2 and VRK2), which could conceivably reflect disruption of this innate immune and tissue regeneration pathway in advanced fibrosis progression.\(^{(25)}\)

In contrast, several variants enriched among high-risk, low-fibrotic NAFLD patients were observed in IL6-related genes (IL6, IL32, ORM1, and SLAMF7) in the NAFLD susceptibility investigation of protective versus population controls, suggesting a potential role for a pro-inflammatory immune response. This finding is supported by recently published NASH Clinical Research Network data implicating pro-inflammatory pathways, including common variants in IL1B and IL6, with NAFLD fibrosis risk and ballooning.\(^{(25)}\) However, as protective individuals were obese with T2DM as part of the study design, the IL6-related genes may also reflect T2DM risk.

Our findings are important for several reasons. First, we used gold standard liver biopsy-confirmed NAFLD to ensure accurate histologic phenotyping of patients. Second, we report the first WES investigation of genetic variation in NAFLD fibrosis. Third, the extreme phenotype design assisted in overcoming difficulties of accurate phenotyping in a highly heterogeneous disease such as NAFLD. This has been a major challenge for GWAS, as variable clinical factors contribute to diverse molecular pathogenesis even with similar histologic phenotypes. We selected extreme phenotypes to control clinical factors associated with risk or protection from advanced fibrosis including obesity, age, and T2DM. Due to the feasibility of having sufficient specimen availability meeting criteria, the histologic extremes could not be completely separated. This may have reduced our ability to detect fibrosis-associated variants specific to NASH and represents a study limitation. Additionally, as most patients had stages 0, 1, and 3 fibrosis, it was not possible to identify extremes with just isolated steatosis or cirrhosis. Although we included population controls from studies unrelated to viral hepatitis or liver disease to increase power and generalizability, detailed phenotyping information about NAFLD and risk factors, such as obesity and diabetes, was unavailable. As NAFLD is relatively common in the general population,\(^{(1)}\) another limitation is the potential for misclassification among controls, leaving our results conservative. Indeed, we did not observe any association with HSD17B13, likely due to a combination of sample-size constraints and differences in investigated phenotypes, as our study focused on histological fibrosis, whereas HSD17B13 initially associated with serum alanine and aspartate aminotransferases.\(^{(10)}\) Finally, as individuals with the protective phenotype were older, obese and diabetic, the protective phenotype comparison with population

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\(^{22}\) MPO has been linked to various metabolic syndrome diseases and is involved in stimuli responses including to food and lipopolysaccharides.\(^{(22)}\) Further, a MPO promoter polymorphism (−463G > A) was previously implicated in fibrosis severity in women with hepatitis C,\(^{(23)}\) consistent with its enrichment among advanced fibrosis observed here. MPO was also enriched among progressors in the gene-based recessive model for the progressor versus protective analysis, potentially implying a role in advanced fibrosis, if confirmed.
controls may have reflected an underlying genetic risk for NAFLD or diabetes susceptibility, whereas the progressor versus control comparison uniquely interrogated risks for advanced fibrosis among NAFLD patients without diabetes.

Although many of our results were not significant, we hope that this investigation will pave the way for further next-generation-sequencing studies. We have highlighted several biologically plausible genes among our top associations, although we currently lack the evidence necessary to determine their NAFLD fibrosis involvement. However, if confirmed in future studies, they may warrant further investigation in the search for pathogenic and therapeutic targets in NASH and liver fibrosis. This study suggests that “extreme” NAFLD phenotypes may represent distinct disease subtypes, perhaps accounting for the nonlinear nature of fibrosis progression. Improved delineation of these subtypes and genetic risks will require large, well-phenotyped follow-up studies. Ideally, the goal is to develop personalized variant profiles for NAFLD based on the risk of progression to fibrosis and fatal liver outcomes, enabling not only personalized treatment options for NAFLD but early identification and prevention of this extremely common disease.

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Supporting Information

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