HEPATOLOGY

HEPATOLOGY, VOL. 64, NO. 5, 2016

Nonalcoholic Fatty Liver Disease and Metabolic Syndrome: Shared Genetic Basis of Pathogenesis

SEE ARTICLE ON PAGE 1547

> Nature shows us only the tail of the lion. But I do not doubt that the lion belongs to it even though he cannot at once reveal himself because of his enormous size.
>
> *Albert Einstein*

A growing body of evidence indicates that nonalcoholic fatty liver disease (NAFLD) develops from a complex process that includes genetic susceptibility and environmental exposure. Regardless of whether it is the cause or the consequence of the metabolic syndrome (MetS), NAFLD often co-occurs with one or more MetS-associated phenotypes. There is also robust evidence in support of NAFLD and MetS sharing common pathogenic mechanisms.[1] Nevertheless, with the exception of the transmembrane 6 superfamily member 2 gene[2]—which illustrates an unexpected opposite association between NAFLD and cardiovascular disease, although it can be suspected—no compelling report demonstrating that NAFLD and MetS share a common genetic background presently exists.

In this issue, Cui et al. show not only that steatosis and fibrosis potentially share the same predisposing genes but also that these conditions have a significant shared gene effect with metabolic risk factors,[3] the latter being a truly remarkable finding. These interesting results prompt several reflections.

Twin Genetics

To explore the putative shared genetic effect among steatosis, fibrosis, and MetS, Cui et al. performed a cross-sectional study in a cohort of community-dwelling twins (45 monozygotic and 20 dizygotic twin pairs) and assessed the difference in the correlation of monozygotic and dizygotic twin pairs.[3] While the sample size is modest compared with some other current twin studies, this is a robust approach to study the shared heritability of NAFLD and MetS. The reproducibility of the reported findings in twins reared apart should, however, be explored.

Liver Imaging "Genetics"

Participants underwent magnetic resonance imaging for hepatic steatosis (proton density fat fraction) and hepatic fibrosis (magnetic resonance elastography).[3] The comparison of images of twins concordant for NAFLD and fibrosis suggests a promising tool for studying the genetic effects on longitudinal changes. Nevertheless, liver biopsy would have provided valuable information on relevant histological features, including hepatocyte ballooning and inflammation. In addition, because steatosis and fibrosis were recorded as dichotomous traits, there is a potential caveat in the assumption that the components of the NAFLD phenotype are normally distributed in the population.

Abbreviations: MetS, metabolic syndrome; NAFLD, nonalcoholic fatty liver disease; PNPLA3, phospholipase domain containing 3.

Received June 21, 2016; accepted July 6, 2016.

Supported by Agencia Nacional de Promoción Científica y Tecnológica (PICT 2014-0432 and PICT 2014-1816). S.S. and C.J.P. belong to Consejo Nacional de Investigaciones Científicas (CONICET).

Copyright © 2016 by the American Association for the Study of Liver Diseases.

View this article online at wileyonlinelibrary.com.
DOI 10.1002/hep.28746

Potential conflict of interest: Nothing to report.

ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO:

Silvia Sookoian, M.D., Ph.D., and
Carlos J. Pirola, Ph.D.
Instituto de Investigaciones Médicas, UBA-CONICET
Combatientes de Malvinas 3150, CABA-1427
Ciudad Autónoma de Buenos Aires, Argentina
E-mail: sookoian.silvia@lanari.fmed.uba.ar or pirola.carlos@lanari.fmed.uba.ar
Tel: +54-11-4514 8701 ext. 167

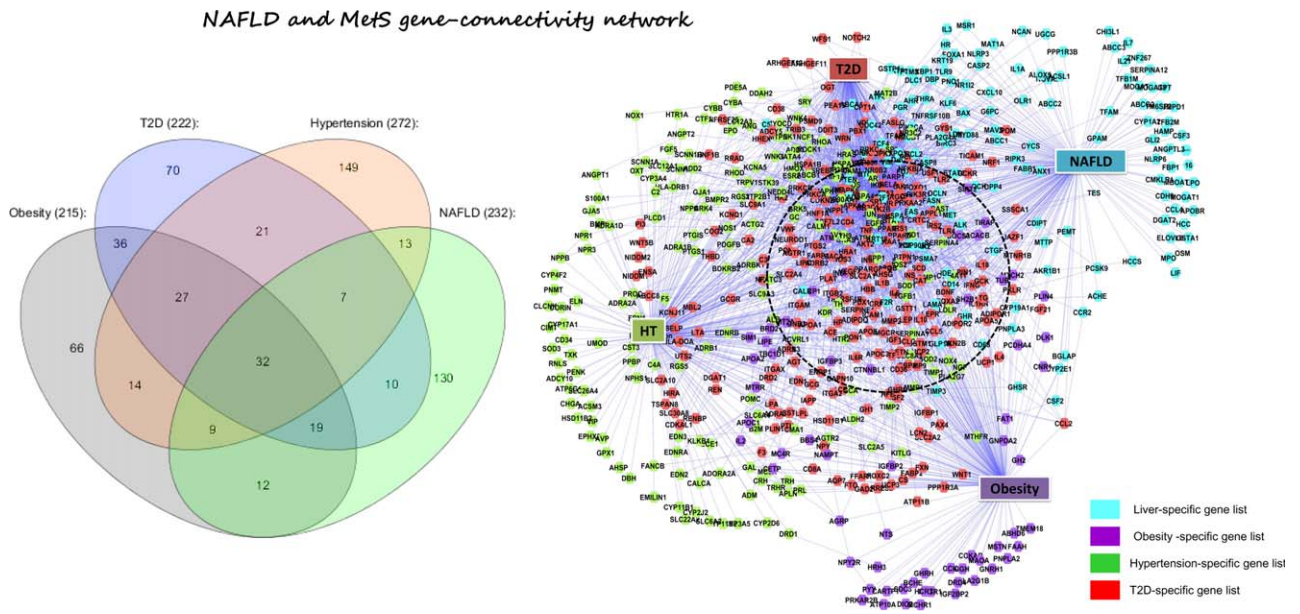

FIG. 1. Genetic commonality between NAFLD and MetS: a gene-based connectivity network. The figure shows an illustration of gene/protein co-occurrence and its relatedness to NAFLD and MetS-associated diseases. The gene-based analysis suggests a large interconnectivity network (dashed circle) that links nodes of NAFLD, obesity, type 2 diabetes, and arterial hypertension. A note of caution should be added as systematic integration of genetic data does not provide details on the direction (protection or risk-conferring) and magnitude of the effect(s) with putative opposites effects in the MetS components. As demonstrated,[5,6] the patatin-like phospholipase domain containing 3 (PNPLA3) gene is not represented in the shared core. The input consisted of multiple gene lists from existing reports, including 272 genes for hypertension, 214 genes for obesity, 222 genes for type 2 diabetes, and 232 genes for NAFLD. The text-mining tool T-HOD (http://bws.iis.sinica.edu.tw/THOD/) was used for collecting the available evidence on the genetic risk of type 2 diabetes, hypertension, and obesity; the gene list was restricted to loci with at least three replication studies. The bioinformatics resource ToppCluster (https://toppcluster.cchmc.org/) was used to predict shared genes between clusters. The network is shown as a Cytoscape graph. A Venn diagram shown at the left side of the figure represents the fraction of genes belonging to each subcategory according to major components of the MetS; 130 genes are exclusively associated with NAFLD, but 102 are shared with another MetS component, 32 of which are shared with type 2 diabetes, obesity, and hypertension. Abbreviations: HT, hypertension; T2D, type 2 diabetes.

Shared Gene Effects Among Hepatic Steatosis, Fibrosis, and MetS

Cui et al.[3] observed significant shared gene effects between steatosis and body mass index, blood pressure, triglycerides, glucose levels, homeostasis model assessment score, and hemoglobin A1c. These results are supported by the fact that 44% of genes associated with NAFLD are shared by other MetS components (almost 14% with type 2 diabetes, obesity, and hypertension combined; Fig. 1). Similarly, significant genetic covariance between fibrosis and body mass index, triglycerides, glucose, homeostasis model assessment score, and hemoglobin A1c was found. While the novelty of this work stems from the possibility of a shared genetic background between NAFLD and MetS, the intraclass correlation assessment between phenotypes, which could potentially cause an overestimation of the effects, was not included in the main analysis of results.

The heritability estimates provided by Cui et al.[3] are aligned with the reported heritability of all traits of the MetS, which range from 30% to 75% in large collections of twin registries worldwide.[4] Data-mining strategies and systems biology approaches strongly suggest the presence of genetic commonality between NAFLD and MetS (Fig. 1). In future work, the main challenge not only will stem from mapping the specific locus or loci involved in the genetic crosstalk between NAFLD and MetS but also will arise due to the need to provide mechanistic evidence on the underlying biological processes as well as specific measurement of the effect(s).

Shared Gene Effects Between Hepatic Steatosis and Fibrosis

Cui et al. found a high level (r_G 0.756) of shared genetic effect between steatosis and fibrosis, which probably mirrors a close and plausible biological partnership between injury and wound-healing response.[3] The patatin-like phospholipase domain containing 3 (PNPLA3) rs738409 is a consistent example of this observation; the variant is involved in the susceptibility to steatosis[5] and severity of NAFLD (inflammation and fibrosis).[6] However, not all reported variants (e.g., transmembrane 6 superfamily member 2 rs58542926) have as consistent effects as the rs738409.

The deterministic assumption that steatosis may itself portend a bad outcome seems hopeless if we consider that ~30% of the general population is affected by the disease.[1] Moreover, the reported results concerning the shared gene effects between steatosis and fibrosis were based on a sample of 26 patients affected by steatosis and only 10 diagnosed with fibrosis. It would have been desirable to adjust the effects for age, sex, and body mass index at the minimum as these are known modifiers of fibrosis. Still, the contribution of Cui et al. provides an interesting proof-of-principle concept to pursue in future long-term follow-up studies of the natural history of NAFLD.

Low Evidence of Shared Environment Effect Among Steatosis, Fibrosis, and MetS?

Surprisingly, Cui et al. did not observe any significant or relevant shared environmental effect between NAFLD phenotypes and MetS-associated risk factors, with the exception of serum ferritin.[3] Conceptually, the shared environmental effect between NAFLD and MetS should be significant; indeed, the role of diet or physical activity in NAFLD is unquestionable.[7] Environmental factors are also strongly implicated in the development of MetS; hence, one might speculate *a priori* that a strong shared environmental component would explain a large proportion of the correlation among phenotypes. Therefore, external validity and replication in large cohorts are required in order to allow for generalization of the results reported by Cui et al. Otherwise, a negligible environmental component would definitively challenge the classical definition of NAFLD and MetS as "complex diseases" in which the environment plays a definitive role.

Heritability Versus Genetic Susceptibility = Missing Heritability

There is a considerable disparity in the magnitude of heritability estimates of NAFLD and the proportion of variance explained by single-nucleotide polymorphisms uncovered from previous genome-wide association studies.[5,8] Hence, the study conducted by Cui et al. motivates us to consider all that we have learned from genetic studies and look for promising avenues for delineating future research agendas.

The heritability estimates of steatosis and fibrosis are as high as they seem to be (~50%),[3] yet the largest ever reported genetic effect for the phenotypic variance of NAFLD is ~5%.[6] Given that this percentage is explained by a single-nucleotide polymorphism (rs738409), it prompts us to consider what we are missing. The simplest and rational answer is "the missing heritability." It may be explained by not yet identified rare variants. If that were the case, a huge challenge remains ahead because future "extra large" genome-wide association studies are needed to eventually pick up the potentially undiscovered variants that occur in <5% of the population. Still, the cumulative number of rare variants needed in an individual would explain few disease cases.

Gene-gene interactions and structural variations might also account for the missing heritability of NAFLD; unfortunately we presently know nothing about that.

The missing heritability of NAFLD finally includes a myriad of other factors not limited to DNA sequence variation, such as epigenetic modifications of the nuclear[1] and mitochondrial[9] DNA. Changes in the epigenome also explain the crosstalk between NAFLD and MetS, specifically insulin resistance.[10]

As mentioned earlier, NAFLD and MetS depend not just on genetics but also on the environment and the interaction between the two. Hence, epigenetics would explain part of this interaction as well. Unfortunately, knowledge on the magnitude of the gene × environment component in the biology of NAFLD is

still elusive. We have hardly even seen the intricate nature of interaction among the epigenome, transcriptome, metabolome, proteome, microbiome, and NAFLD and the crosstalk with MetS. Most importantly, we must appreciate, but not be discouraged by, the fact that, so far, we have only seen the tail of the lion.

Silvia Sookoian, M.D., Ph.D.[1]
Carlos J. Pirola, Ph.D., F.A.H.A.[2]
[1]**Department of Clinical and Molecular Hepatology**
[2]**Department of Molecular Genetics and Biology of Complex Diseases, Institute of Medical Research A Lanari-IDIM**
University of Buenos Aires-National Scientific and Technical Research Council (CONICET)
Ciudad Autónoma de Buenos Aires, Argentina

REFERENCES

1) Brunt EM, Wong VW, Nobili V, Day CP, Sookoian S, Maher JJ, et al. Nonalcoholic fatty liver disease. Nat Rev Dis Primers 2015;1:15080.
2) Pirola CJ, Sookoian S. The dual and opposite role of the TM6SF2-rs58542926 variant in protecting against cardiovascular disease and conferring risk for nonalcoholic fatty liver: a meta-analysis. Hepatology 2015;62:1742-1756.
3) Cui J, Chen CH, Lo MT, Schork N, Bettencourt R, Gonzalez MP, et al. Shared genetic effects between hepatic steatosis and fibrosis: a prospective twin study. Hepatology 2016;64:1547-1558.
4) van Dongen J, Slagboom PE, Draisma HH, Martin NG, Boomsma DI. The continuing value of twin studies in the omics era. Nat Rev Genet 2012;13:640-653.
5) **Romeo S, Kozlitina J**, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. Nat Genet 2008;40:1461-1465.
6) Sookoian S, Pirola CJ. Meta-analysis of the influence of I148M variant of patatin-like phospholipase domain containing 3 gene (PNPLA3) on the susceptibility and histological severity of nonalcoholic fatty liver disease. Hepatology 2011;53:1883-1894.
7) Hannah WN Jr, Harrison SA. Lifestyle and dietary interventions in the management of nonalcoholic fatty liver disease. Dig Dis Sci 2016;61:1365-1374.
8) **Kozlitina J, Smagris E**, Stender S, Nordestgaard BG, Zhou HH, Tybjaerg-Hansen A, et al. Exome-wide association study identifies a TM6SF2 variant that confers susceptibility to nonalcoholic fatty liver disease. Nat Genet 2014;46:352-356.
9) Pirola CJ, Gianotti TF, Burgueno AL, Rey-Funes M, Loidl CF, Mallardi P, et al. Epigenetic modification of liver mitochondrial DNA is associated with histological severity of nonalcoholic fatty liver disease. Gut 2013;62:1356-1363.
10) Sookoian S, Rosselli MS, Gemma C, Burgueno AL, Fernandez GT, Castano GO, et al. Epigenetic regulation of insulin resistance in nonalcoholic fatty liver disease: impact of liver methylation of the peroxisome proliferator-activated receptor gamma coactivator 1alpha promoter. Hepatology 2010;52:1992-2000.

Author names in bold designate shared co-first authorship.