Genetic Polymorphisms Implicated in Nonalcoholic Liver Disease or Selected Other Disorders Have No Influence on Drug-Induced Liver Injury

Herbert L. Bonkovsky ,[1] Tyler Severson,[1] Paola Nicoletti,[2,3] Huiman Barnhart,[4] Jose Serrano,[5] Naga Chalasani,[6] Robert J. Fontana,[7] Paul B. Watkins,[8] Victor Navarro,[9] Andrew Stolz,[10] Ann K. Daly,[11] Guruparasad P. Aithal,[12] and Joseph Odin[2], for the US DILIN Investigators

With the application of genetic testing to contemporary medical diagnostics and practice, it has become apparent that the phenotypes of many disorders are modulated by host genetic factors. The aim of the current study was to determine whether selected single nucleotide polymorphisms (SNPs) unrelated to the human leukocyte antigen region or other immune pathways, including those associated with nonalcoholic fatty liver disease (NAFLD), may influence development, severity, or outcomes of drug-induced liver injury (DILI). Thirteen variants previously associated with NAFLD and/or selected other liver diseases were tested in 832 Caucasian DILI cases and 10,397 Caucasian population controls. DILI cases were attributed to multiple agents (177 individual drugs), with 56 cases due to herbal/dietary supplement products. Allele frequencies were imputed from recent genome-wide association studies and compared to those for European control samples from the Gnomad database. Significance was tested by linear regression or logistic regression, depending on the nature of the trait. Any variant that passed the Bonferroni threshold of $P < 0.0004$ ($\frac{0.05}{13}$) was considered a significant association. None of the variants proved to be significantly associated with DILI as phenotype nor with any of the selected severity traits. Among the variants studied, rs1421085, found in the fat mass and obesity associated (*FTO*) gene, showed a marginal protective effect (odds ratio, 0.8; 95% confidence interval, 0.77-0.95; $P = 0.005$). None of the genetic polymorphisms tested were significantly associated with the risk of development, severity, or outcome of DILI. *Conclusion:* SNPs implicated in common liver diseases, such as NAFLD, do not play a substantial role in DILI pathogenesis across agents. It remains possible that these variants could be involved with DILI due to single agents, but this will require the evaluation of larger numbers of *bona fide* cases. (*Hepatology Communications* 2019;3:1032-1035).

With the application of genetic testing to contemporary medical diagnostics and practice, it has become apparent that the phenotypes of many disorders are modulated by host genetic factors. For example, the susceptibility to and progression of nonalcoholic fatty liver disease (NAFLD) have been associated with the p. I148M variant in the patatin-like phospholipase domain-containing protein 3 (*PNPLA3*) gene and the p. E167K variant of transmembrane 6 super family 2 (*TM6SF2*).[1,2] In

Abbreviations: DILI, drug-induced liver injury; DILIN, Drug-Induced Liver Injury Network; FTO, *fat mass and obesity associated; NAFLD, nonalcoholic fatty liver disease; SNP, single nucleotide polymorphism.*

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Present address for Tyler Severson is Department of Medicine, St. Luke's Hospital, Duluth, MN.

Guruparasad P. Aithal is the gastrointestinal and liver disorder theme lead for the National Institute for Health Research, Nottingham Biomedical Research Centre (reference no. BRC-1215-20003).

contrast, susceptibility to idiosyncratic drug-induced liver injury (DILI), a rare form of liver disease, has been associated primarily with genes that influence innate or adaptive immune responses.[3,4]

The aim of the current study was to determine whether selected single nucleotide polymorphisms (SNPs) unrelated to the human leukocyte antigen region or other immune pathways, including those associated with NAFLD, may influence development, severity, or outcomes of DILI.

Materials and Methods

Thirteen variants previously associated with NAFLD and/or selected other liver diseases were tested in 832 Caucasian DILI cases and 10,397 Caucasian population controls (Table 1).[5] DILI cases were attributed to multiple agents (177 individual drugs), with 56 cases due to herbal/dietary supplement products. All cases had DILI Network (DILIN) causality scores equal to or higher than probable (judged 51%-100% likely due to a drug). None of the subjects from DILIN had been enrolled as acute cases (within 14 days of onset). Eight variants were imputed from the most recent genome-wide association study,[5] and four additional variants were directly genotyped only in DILI cases, except for the hydroxysteroid dehydrogenase 17B13 (HSD17B13) splice variant, which was typed only in a subset of the DILI cases (n = 384). For the latter variants, the allele frequencies for European (non-Finnish) control samples in the Gnomad database were used (https://gnomad.broadinstitute.org/).

The DILI cases were also categorized by severity and chronic DILI. Chronic DILI was defined as evidence of ongoing liver injury 6 months after DILI onset, as described.[3,5] The significance was tested by linear regression or logistic regression, depending on the nature of the trait. For genotyped variants and binary traits, associations were compared to European control samples listed in the Gnomad database and by Fisher's exact test.

Any variant that passed the Bonferroni threshold of $P < 0.0004$ ($\frac{0.05}{13}$) was considered a significant association. Follow-up analyses were done for the most strongly associated variants by testing in an independent Caucasian cohort of 974 DILI cases from the International DILI Consortium[5] and in African

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ARTICLE INFORMATION:

From the [1]Department of Medicine, Gastroenterology and Hepatology Section, Wake Forest University School of Medicine, Winston-Salem, NC; [2]Icahn School of Medicine at Mount Sinai Medical Center, New York, NY; [3]Sema4, Stamford, CT; [4]Duke Clinical Research Institute, Durham, NC; [5]National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD; [6]Division of Gastroenterology, Indiana University School of Medicine, Indianapolis, IN; [7]Division of Gastroenterology, University of Michigan School of Medicine, Ann Arbor, MI; [8]Departments of Pharmacology and Medicine, University of North Carolina School of Medicine, Chapel Hill, NC; [9]Department of Medicine, Einstein Medical Center, Philadelphia, PA; [10]Division of Gastroenterology and Hepatology, University of Southern California School of Medicine, Los Angeles, CA; [11]Newcastle University, Newcastle-upon-Tyne, United Kingdom; [12]National Institute for Health Research, Nottingham Biomedical Research Centre, Nottingham University Hospitals National Health Service Trust and the University of Nottingham, Nottingham, United Kingdom.

ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO:

Herbert L. Bonkovsky, M.D.
Director of Liver Services and the Liver
and Metabolic Disorders Laboratory
Wake Forest University School of Medicine, E-112, NRC
1 Medical Center Boulevard
Winston-Salem, NC 27157
E-mail: hbonkovs@wakehealth.edu
Tel.: +1-336-713-7341

TABLE 1. SNPS INVESTIGATED IN DILIN CAUCASIAN DILI CASES AND POPULATION CONTROLS

Gene Name	Genetic Variant	Coding DNA Change	Amino Acid Change	Putative Effect of Variant in NAFLD	DILI Case MAF	Control MAF	*P*
PNPLA3	rs738409	444C>G	I148M	Increased hepatocyte triglyceride content	21.1%	23.4%	0.5
	rs6006460*	1531G>T	S453I	Lower than average hepatic triglyceride accumulation	0.06%	0.02%	0.2
TM6SF2	rs58542926	499A>G	E167K	Elevated AST/ALT, increased hepatic triglyceride levels, decreased serum cholesterol	6.9%	7.5%	0.37
	rs10401969	613+80A>G	Intron	Lower hepatic TM6SF2 mRNA levels correlate with larger hepatocellular lipid droplets	6.9%	7.7%	0.2
LIPA	rs116928232*	894G>A	E8SJM	Cholesterol ester storage disease often resulting in fibrosis→cirrhosis	0.1%	0.1%	1
IFNL4	rs12979860*	151-152G>A	Intron	Increased degree of hepatic inflammation and fibrosis	32.3%	32.0%	0.9
HFE	rs1800562	845G>A	C282Y	Increased hepatic iron uptake, associated with greater NAFLD risk/severity	6.0%	5.5%	0.74
	rs1799945	187C>G	H63D	Increased hepatic iron uptake, associated with greater NAFLD risk/severity	15.3%	15.9%	0.8
HMOX1	rs2071746*	−413A>T	Affects promoter	Higher HMOX1 activity correlated with less frequent and less severe NAFLD	42.7%	43.5%	0.9
FTO	rs1421085	46-43098T>C	Affects repressor	Adipocytic phenotype shift from beige (energy dissipating) to white (energy storing)	38.2%	41.7%	0.005
GNPAT	rs11558492	1556A>G	D519G	Worsened iron overload in patients with *HFE* genetic variations	21.1%	23.3%	0.7
SERPINA1	rs28929474	1096G>A	E342K	Associated with deficiency of alpha-1 antitrypsin and with increased risk of liver diseases	2%	2%	0.73
HSD17B13	rs72613567†	4:88231392;T>TA	Splice variant	Associated with a reduced risk of chronic liver disease and of progression from steatosis to steatohepatitis	28%	27%	0.52

*Variants directly genotyped only in DILI cases; †Variant genotyped only in a subset of the DILI cases (n = 384).
Abbreviations: ALT, alanine, aminotransferase; AST, aspartate aminotransferase; GNPAT, glyceroneophophate O-acyltransferase; HFE, homeostatic iron regulator; HMOX1, heme oxygenase 1; HSD17B13, hydroxysteroid 17-beta dehydrogenase 13; IFNL4, interferon lambda 4; LIPA, lipase A, lysosomal acid type 1; MAF, minor allele frequency; mRNA, messenger RNA; PNPLA3, patatin-like phospholipase domain-containing protein 3; SERPINA1, serpin peptidase inhibitor, clade A, member 1; TM6SF2, transmembrane 6 super family 2.

Americans (169 DILIN cases versus 1,314 controls) and Hispanic cohorts (109 DILIN cases and 718 controls).[5]

Results

None of the variants proved to be significantly associated with DILI as phenotype (Table 1) nor with any of the selected severity traits (Supporting Table S1). Among the variants studied, rs1421085, found in the fat mass and obesity associated (*FTO*) gene, showed a marginal protective effect (odds ratio, 0.8; 95% confidence interval, 0.77-0.95; $P = 0.005$, with similar trends also in Hispanic and African-American cohorts), but Caucasian replication cases showed a higher frequency of the variants (Supporting Table S1).

Discussion

None of the genetic polymorphisms tested were significantly associated with the risk of development, severity, or outcome of DILI. These data suggest that

SNPs implicated in common liver diseases, such as NAFLD, do not play a substantial role in DILI pathogenesis across agents. However, it remains possible that these variants could be involved with DILI risk or outcome due to a single drug, but this will require the evaluation of larger numbers of *bona fide* cases due to specific drugs. In addition, rare variants may play a role in DILI pathogenesis, but additional studies using whole exome or genome testing are required.

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Author names in bold designate shared co-first authorship.

Supporting Information

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