DR. HERBERT L. BONKOVSKY (Orcid ID : 0000-0001-7387-3230)



Article type : Original

**Genetic Polymorphisms Implicated in Non-Alcoholic Liver Disease [NAFLD] or Selected other Disorders have no Influence on Drug-Induced Liver Injury [DILI]**

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

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

*Dr Severson's current address is Department of Medicine, St. Luke's Hospital, Duluth, MN

Email addresses of authors: Bonkovsky—hbonkovs@wakehealth.edu; Severson—tyler.severson@gmail.com; Nicoletti—paola.nicoletti@msssm.edu; Barnhart—huiman.barnhart@duke.edu; Serrano—serranoj@extra.niddk.nih.gov; Chalasani—nchalsa@iu.edu; Fontana—rfontana@med.umich.edu; Watkins—pbwatkins @email.unc.edu; Navarro—navarrov@einstein.edu; Stolz—astolz@hsc.usc.edu; Daly-- a.k.daly@newcastle.ac.uk; Aithal--guru.aithal@nottingham.ac.uk; Odin—joseph.odin@mountsinai.org

**Running Title:** Selected gene polymorphisms and DILI

**Key words:** idiosyncratic drug-induced liver injury; non-alcoholic fatty liver disease; outcomes of liver injury; genetic susceptibility.

**Footnote Page:**

**Address for Correspondence:** Herbert L. Bonkovsky, MD, Professor of Medicine; Director of Liver Services and the Liver and Metabolic Disorders Laboratory, Wake Forest University School of Medicine, E-112, NRC, 1 Medical Center Blvd, Winston-Salem, NC 27157
Phone: 336 713 7341; Fax: 336 713 7322; email: hbonkovs@wakehealth.edu

**List of Abbreviations Used:** AF, allele frequency; ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; CI, confidence interval; DILI[N], drug-induced liver injury [network]; FTO, fat mass obesity associated protein [also known as alpha-ketoglutarate-dependent dioxygenase; GNPAT, glyceroneophophate O-acyltransferase; HFE, homeostatic iron regulator gene, the gene that is mutated in the HLA-linked form of hereditary hemochromatosis; HLA, human leukocyte antigen; HMOX1, heme oxygenase 1; HSD17B13, hydroxysteroid 17-beta dehydrogenase 13; IFNL4, interferon lambda -4; LIPA, lipase A, lysosomal acid type 1 [also called cholesterol ester hydrolase]; MAF, minor allele frequency; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; PNPLA3, patatin like phospholipase domain containing 3; TBR, total bilirubin; SERPINA1, serpin peptidase inhibitor, clade A, member 1, the gene that is mutated in alpha-1 antitrypsin deficiency; TM6SF2, transmembrane 6 superfamily member 2;

**Financial Support:** Supported by Cooperative agreements among NIH/NIDDK and US DILIN Centers and by CTSA Awards. This study was funded as a cooperative agreement with the NIDDK under grant numbers: U01-DK065176, DK065184, DK065193, DK065201, DK065211, DK065238, DK082992, DK083020, DK083023, DK083027 and DK100928. Additional funding is provided by CTSA grants UL1 RR025761 (Indiana), UL1 RR025747 (UNC), UL1 RR024986 (UMich), DK065176 (Duke), DK100928 (Icahn Schl. of Medicine at Mount Sinai), and UL1 TR001420 (Wake Forest University). GP Aithal is the gastrointestinal and liver disorder theme lead for the NIHR Nottingham BRC. Reference no: BRC-1215-20003.

**Introduction:** 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 non-alcoholic fatty liver disease [NAFLD] have been associated with the p. I148M variant in the patatin-like LA3 phosphatase [*PNPLA3]* gene and p. E167K variant of transmembrane 6 super family 2 [*TM6SF2]* [1,2]. In 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 HLA region or other immune pathways, including those associated with NAFLD, may influence development, severity, or outcomes of DILI.

**Methods**: Thirteen variants previously associated with NAFLD and/ 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) and 56 cases due to HDS products. All cases had 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 4 additional variants (identified by *) 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, identified by **). For the latter variants, the allele frequencies for European (non-Finnish) control samples in the Gnomad database were used (http://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 trait. For genotyped variants and binary traits, the associations were compared to European control samples listed in Gnomad database and by Fisher exact test.

Any variant that passed the Bonferroni threshold of P <0.0004 (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 Drug-Induced Liver Injury Consortium [iDILIC] [5] and in African Americans (169 DILIN cases vs 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 (Table S1). Among the variants studied, rs1421085, found in the *FTO* gene, showed a marginal *protective* effect (OR= 0.8, 95%CI [0.77-0.95], P=0.005), [similar trend also in Hispanic and African-American cohort], but Caucasian replication cases showed higher frequency of the variants (Table S1).

**Conclusion:** None of the genetic polymorphisms tested was significantly associated with the risk of development, severity or outcome of DILI. These data suggest that single nucleotide polymorphisms [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, which 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.

**References:**

1. Speliotes EK, Yerges-Armstrong LM, Wu J, Hernaez R, Kim LJ, Palmer CD, Gudnason V, et al. Genome-wide association analysis identifies variants associated with nonalcoholic fatty liver disease that have distinct effects on metabolic traits. PLoS Genet 2011; 7: e1001324.

2. Severson TJ, Besur S, :Bonkovsky HL. Genetic factors that affect nonalcoholic fatty liver disease: a systematic clinical review. World J Gastroenterol 2016; 22 (29): 6742-56.

3. Lucena MI, Molokhia M, Shen Y, Urban TJ, Aithal GP, Andrade RJ, Day CP, et al. Susceptibility to amoxicillin-clavulanate-induced liver injury is influenced by multiple HLA class I and II alleles. Gastroenterology 2011; 141 (1): 338-47.

4. Bonkovsky HL, Shedlofsky SI, Jones DP, Russo M. Drug-induced liver injury. In Boyer TD, Manns MP, Sanyal A (Eds), Zakim and Boyer's Hepatology—a Textbook of Liver Disease, 6th Edition, Saunders-Elsevier, Philadelphia, pp 417-461, 2012.

5. Cirulli ET, Nicoletti P, Abramson K, Andrade RJ, Björnsson ES, Chalasani N, Fontana RJ, Hallberg P, et al. A missense variant in *PTPN22* is a risk factor for drug-induced liver injury. Gastroenterology 2019 Jan 18. Pii: S0016-5085(19)30094-0. Doi: 10.1053/j.gastro.2019.01.034 [Epub ahead of print].

**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, | 6.9% | 7.5% | 0.37 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | decreased serum cholesterol | | | |
| | 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 |

Abbreviations used: MAF, minor allele frequency; NAFLD, non-alcoholic liver disease