HHS Public Access

Author manuscript

Obesity (Silver Spring). Author manuscript; available in PMC 2019 December 20.

Published in final edited form as:

Obesity (Silver Spring). 2019 August ; 27(8): 1331–1337. doi:10.1002/oby.22527.

Genome-Wide Association Study Identifies Loci for Liver Enzyme Concentrations in Mexican-Americans: The GUARDIAN Consortium.

Kendra A. Young[1], **Nicholette D. Palmer**[2], **Tasha E. Fingerlin**[1,3], **Carl D. Langefeld**[4], **Jill M. Norris**[1], **Nan Wang**[5], **Anny H. Xiang**[6], **Xiuqing Guo**[7], **Adrienne H. Williams**[4], **Yii-Der I. Chen**[7,8], **Kent D. Taylor**[7,8], **Jerome I. Rotter**[7,8], **Leslie J. Raffel**[9], **Mark O. Goodarzi**[9,10], **Richard M. Watanabe**[5], **Lynne E. Wagenknecht**[11]

[1.]Colorado School of Public Health, University of Colorado Denver, Aurora, CO USA

[2.]Department of Biochemistry at Wake Forest School of Medicine, Winston-Salem, NC USA

[3.]Center for Genes, Environment, and Health, National Jewish Health, Denver, CO, USA

[4.]Department of Biostatistical Sciences, Wake Forest School of Medicine, Winston-Salem, NC USA

[5.]Department of Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles CA USA

[6.]Research and Evaluation Branch, Kaiser Permanente of Southern California, Pasadena, CA USA

[7.]Institute for Translational Genomics and Population Sciences, Los Angeles Biomedical Research Institute at Harbor-University of California, Los Angeles Medical Center, Torrance, CA USA

[8.]Department of Pediatrics, Los Angeles Biomedical Research Institute at Harbor-University of California, Los Angeles, CA USA

[9.]Medical Genetics Research Institute, Cedars-Sinai Medical Center, Los Angeles, CA

[10.]Division of Endocrinology, Diabetes and Metabolism, Department of Medicine, Cedars-Sinai Medical Center, Los Angeles, CA USA

[11.]Division of Public Health Sciences, Wake Forest School of Medicine, Winston-Salem, NC USA

Abstract

Objective: Mexican-American ancestry populations are at increased risk of non-alcoholic fatty liver disease (NALFD). Our objective was to determine whether loci in known and novel genes were associated with variation of aspartate aminotransferase (AST, n=3644), alanine

Corresponding Author: Lynne E. Wagenknecht, Division of Public Health Sciences, Wake Forest School of Medicine, Winston Salem, NC 27157, Phone: 336-716-7652, lwgnkcht@wakeheath.edu.

Disclosure:
The authors declared no conflict of interest.

aminotransferase (ALT, n=3595) and gamma-glutamyl transferase (GGT, n=1577) by conducting the first genome-wide association study (GWAS) of liver enzymes, which commonly measure liver function, in Mexican-American ancestry.

Methods: Levels of AST, ALT, and GGT were determined by enzymatic colorimetric assays. A multi-cohort Mexican-American ancestry GWAS was performed. SNPs were tested for association with liver outcomes by multivariable linear regression using an additive genetic model. Association analyses were carried out separately in each cohort followed by a non-parametric meta-analysis.

Results: In the *PNPLA3* gene, rs4823173 ($P=3.44\times10^{-10}$), rs2896019 ($P=7.29\times10^{-09}$), and rs2281135 ($P=8.73\times10^{-09}$), were significantly associated with AST levels. Although not genome-wide significant, these same SNPs were the top hits for ALT ($P=7.12\times10^{-08}$, $P=1.98\times10^{-07}$, and $P=1.81\times10^{-07}$, respectively). The strong correlation ($r^2=1.0$) for these SNPs indicates a single hit in the *PNPLA3* gene. No genome-wide significant associations were found for GGT.

Conclusion: *PNPLA3*, a locus previously identified with ALT, AST, and NAFLD in European and Japanese GWAS, is also associated with liver enzymes in Mexican-American ancestry populations.

Keywords

aspartate aminotransferase; alanine aminotransferase; gamma-glutamyl transferase; genetic variance; Mexican-American ancestry

Introduction

Liver enzymes are commonly used to assess liver function and disease. High concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transferase (GGT) are associated with various conditions, including non-alcoholic fatty liver disease (NAFLD), the most common cause of elevated liver enzymes in adults in the United States (1). NAFLD is often benign; however, it can lead to inflammation, fibrosis, and eventually, cirrhosis (2). NALFD is more common in Hispanic Americans than either African Americans or Caucasians (3, 4).

Plasma concentrations of liver enzymes are highly heritable (20-77%) (5), suggesting a genetic role that may help in interpretation of results and explain variation among individuals. Previous genome-wide association studies (GWAS) of liver enzymes in those of European (6, 7), Indian (7), and Japanese ancestry (8) identified candidate genes involved in various pathways, including glucose and lipid metabolism as well as inflammation and immunity. However, few studies have extensively examined the genetic contributions in Hispanics, a group at increased risk of NALFD (9), and no GWAS studies of liver enzymes or NAFLD have been conducted in individuals of Mexican-American ancestry.

The Genetics Underlying Diabetes in Hispanics (GUARDIAN) Consortium was formed to identify the genetic determinants of type 2 diabetes-related phenotypes in Hispanics (10), and is comprised of five family-based [BetaGene (11), Hypertension-Insulin Resistance Family study (HTN-IR) (12), Insulin Resistance Atherosclerosis Family Study (IRAS FS)

(13), Mexican-American Coronary Artery Disease study (MACAD) (14), and NIDDM-Atherosclerosis Study (NIDDM-Athero) (15)] and two non–family-based studies [IRAS (16) and Troglitazone in the Prevention of Diabetes study (TRIPOD) (17)]. The majority of these studies also included measures of liver function, with measures of AST, ALT and GGT. The objective of this study was to determine whether loci in known and novel genes were associated with variation in AST, ALT and GGT levels in Mexican-American ancestry populations in the GUARDIAN Consortium by conducting the first GWAS of liver enzymes in individuals of Mexican-American ancestry.

Methods

Study Population

All participants provided written informed consent, and the institutional review boards at the respective institutions approved the study. Five cohorts of self-reported individuals of Mexican-American ancestry, ascertained on varying conditions, had measures of liver enzymes and were used in these analyses. Cohort descriptions can be found in the Supplementary Materials.

Outcome Variables and Covariates

Concentrations in IU/L of ALT, AST, and GGT were determined by enzymatic colorimetric assays. Age and sex were self-reported. Body mass index (BMI) was calculated as weight (kg)/height (m^2).

Genotyping

All samples were genotyped on the Illumina HumanOmniExpress BeadChip, and alleles were called using GenomeStudio software (Illumina, San Diego, CA) (18, 19). Samples with call rates >0.98, single nucleotide polymorphisms (SNPs) with call rates >0.99, and minor allele frequency (MAF) >0.001 passed laboratory quality control (20), with 22,000 additional SNPs manually reviewed for clustering accuracy. Samples were removed from analysis if the overall call rate was <0.98, if the samples were genetic outliers for sex and admixture proportions, if the samples were monomorphic , or if there was inconsistent fingerprinting from existing SNP data (21).The primary inferential SNPs did not exhibit differential missingness by trait, had a SNP call rate >98%, and did not depart from Hardy-Weinberg equilibrium expectations. Pedigrees were examined for consistency of stated family structure. Each SNP was examined for Mendelian inconsistencies using PedCheck (Program for Detecting Marker Typing Incompatibilities in Pedigree Data,http://watson.hgen.pitt.edu/register/docs/pedcheck.html). and inconsistencies were converted to missing. A maximum of 659,222 SNPs were meta-analyzed.

Population Stratification

Population substructure was estimated using ADMIXTURE version 1.21 (http://www.genetics.ucla.edu/software/admixture) at each study site based on SNPs that passed quality control [n = 117,347 linkage disequilibrium (LD)–pruned SNPs]. Data from the HapMap Project, including Mexican ancestry, were used as reference populations (21).

Admixture proportions were included as covariates in the tests of association with liver enzymes.

Statistical Analysis

All cohorts had measures of AST and ALT, while only HTN-IR and IRAS FS had data available for GGT. To best approximate normality and homogeneity of variance in all cohorts, levels of AST, ALT and GGT were log-transformed. There are 3757 individuals for genotyping. Variance component analyses as implemented in the GWAF (Genome-Wide Association analyses with Family) (22) or SOLAR (Sequential Oligogenic Linkage Analysis Routines) (23) programs were used to test for association in family cohorts and linear regression analyses as implemented in QSNPGWA (http://github.com/guyrt/WFUBMC) were used to test for association in the non-family-based cohort. SNPs were tested for association with liver enzymes using an additive genetic model adjusted for age, sex, BMI, admixture proportions, family structure, and recruitment center (for multi-center studies). Conditional analyses were performed for significant loci with multiple uncorrelated variants by including the most significant variant as an additional covariate. For significant uncorrelated loci, SNP-age and SNP-sex interactions were examined.

Association analyses were carried out separately in each cohort followed by a non-parametric meta-analysis as implemented in METAL (http://www.sph.umich.edu/csg/abecasis/metal). For each liver enzyme outcome, a weighted, fixed-effects meta-analysis was calculated, weighting by cohort sample size due to different study design and ascertainment criteria, and correcting for genomic control inflation factors. A MAF >0.01 filter resulted in 659,222 SNPs for AST, 659,211 SNPs for ALT, and 655,023 SNPs for GGT. Genome-wide significance was determined at $P<1\times10^{-8}$, allowing for a Bonferroni adjustment for 10^6 SNPs tested and three liver enzyme outcomes.

Known variants (n=31) from previous liver enzyme and NAFLD GWAS that were available in the GUARDIAN Consortium genotype data were examined for association with AST, ALT and GGT. Associations were reported for enzyme specific variants (e.g. AST associations for reported AST variants), and associations with all liver enzymes were reported for previously reported NAFLD/NASH variants. If reported variants in the same gene had a LD > 0.80 in the HapMap Project Mexican population, then only the variant with the highest p-value was reported. Significance for the known variants was determined at $P<0.05$. We did not correct for multiple testing due to a priori hypotheses based on well-known associations.

Results

Characteristics of the study participants are shown in Table 1. Mean age ranged from 35-41 years, the majority was female, and mean BMI ranged between 28-31 kg/m^2. Mean levels of AST, ALT and GGT were within normal ranges (10-40 IU/L, 7-56 IU/L, and 0-45 IU/L, respectively.

The P-P plot of P values (Supplementary Figure S1) from the meta-analysis did not suggest any systematic biases, such as those related to population stratification. We identified three

SNPs, within the *PNPLA3* (patatin-like phospholipase domain containing 3) locus on chromosome 22, attaining genome-wide significance ($P < 1 \times 10^{-8}$) with AST levels (Table 2, Figure 1A, Supplementary Table S1) with a positive effect direction. These same three SNPs, rs4823173, rs2896019, and rs2281135, were also the top signals for ALT levels (Table 2, Figure 2B, Supplementary Table S2) in the same positive effect direction, approaching genome-wide significance ($P<7.12 \times 10^{-8}$). There were no genome-wide significant associations found with GGT levels (Table 2, Figure 2C, Supplementary Table S3), with the top variants representing *ZNF804A* (Zinc protein finger 804A) and *USP24P1* (ubiquitin specific peptidase 24 pseudogene 1). There were no significant rs482173xage (p=0.60 and p=0.47) or rs482173xsex interactions (p=0.54 and p=0.53) for AST and ALT, respectively.

There was perfect LD between rs4823173, rs2896019, and rs2281135 (r^2=1.0 for all correlations) in the HapMap Project Mexican population; combined with the regional plot using the 1000 genomes AMR population, this indicates a single hit on the *PNPLA3* gene (Figure 2). For the significant hit in the *PNPLA3* gene, the OmniExpress imputed data from the *PNPLA3* gene region on Chromosome 22 was pulled as it included rs738409, the most commonly reported significant SNP in the literature. To assess the evidence for multiple independent association signals within *PNPLA3* for AST levels, association with rs4823173 (the most significant SNP in the meta-analysis) were tested after adjusting for rs738409. After adjusting for rs738409 along with the standard covariates, rs4823173 was no longer statistically significant (p=0.32). The loss of significance of rs4823173 after adjustment for rs738409, the top literature SNP, was consistent with the LD among the SNPs in this population (r^2=0.84) and does not provide evidence for multiple association signals.

Thirty one variants reported in other liver enzyme or NAFLD/NASH GWAS in European (6, 7, 24-28), Indian(7, 28), Japanese(8, 29), and Korean (30) populations (Table 3) were examined. Beyond the *PNPLA3* gene, several SNPs associated with NAFLD showed significant associations with liver enzymes in the Mexican-American ancestry populations. rs3761472 in *SAMM50* was significantly associated with AST, ALT and GGT ($P=9\times10^{-4}$, 3×10^{-4}, and 0.01, respectively), and variants in *PARVB* were significantly associated with AST ($P=3.0\times10^{-4}$) and GGT (P=0.02) levels. For previous enzyme specific variants, prior ALT-associated variants rs11597086 in *CHUK* (P=0.03) and rs11597390 in *CPN1* (P=0.02) were significantly associated with ALT. In addition, several prior GGT-associated variants were associated with GGT levels in individuals of Mexican-American ancestry: 7310409 in *HNF1A* (P=0.02), rs4820599 in *GGT1* (P=0.04), rs4547811 in *ZNF827* (P=0.03), rs944002 in *C14orf73* ($P=1.0\times10^{-4}$), and rs339969 and rs340005 in *RORA* (P=0.02 for both). Aside from the variants in *PNPLA3* and *SAMM50*, no prior AST variants were significantly associated with AST in individuals of Mexican-American ancestry.

Discussion

We conducted the first GWAS of liver enzymes in individuals of Mexican-American ancestry, a population at increased risk of NAFLD. This study provided evidence that *PNPLA3* is associated with liver function in Mexican-Americans. We report genotyped data and therefore may limit our power to detect associations not included in our genotyping

array by not imputing. However, while previous candidate gene studies in Hispanics have reported associations between variants in *PNPLA3* and increased AST and ALT levels (31-35), we report a genome-wide significant association ($P < 1 \times 10^{-8}$) with AST and near significant association with ALT. There was high LD between rs4823173 with rs738409 in this population (r^2=0.84), the most commonly reported *PNPLA3* SNP in the literature, indicating that there is likely one association signal between *PNPLA3* and levels of AST and ALT. These results demonstrate that *PNPLA3*, a locus previously identified by GWAS for ALT and AST in European(6, 7), Indian(7), and Japanese ancestry (8), is associated with liver function in Hispanics as well.

The *PNPLA3* gene is located on chromosome 22 and has been implicated in genetic studies of liver function in both GWAS (6-8, 24, 26-30) and candidate gene (33, 36-40) studies across several ancestries. The PNPLA3 protein is expressed in both adipocytes and hepatocytes (41). PNPLA3 mediates triacylglycerol hydrolysis in adipocytes (42), and may be involved in energy storage and usage.

We confirmed several previously reported NAFLD SNPs and their associations with AST, ALT, and GGT. Variants in *SAMM50*, which is in the same genetic region as *PNPLA3* and is involved in the biogenesis of mitochondria, and *PARVB*, involved in lipid accumulation and fibrosis, were previously associated with NAFLD/NASH and were associated with AST, ALT and/or GGT in our cohort of individuals of Mexican-American ancestry. Recently, in a candidate gene study in a Mexican population with NAFLD, variants in *SAMM50* have been associated with increased with AST levels (35) Interestingly, using the browsers set up by the Genotype-Tissue Expression (GTEx) project (43), we found that our associated intronic variant rs4823173, which is in strong LD with PNPLA3 rs738409, is located near an exon, and SAMM50 expression, not PNPLA3, is affected by rs4821373in subcutaneous adipose tissue. These pathways could indicate that fatty liver is still ongoing and possibly progressing in this largely overweight/obese population with increasing accumulation of adipose tissue. In addition, we confirmed several prior enzyme-specific associations in our populations. For ALT, associations were found for variants in or near genes involved in inflammation and possibly modulation of insulin resistance through NF-kB (*CPN1-CHUK* gene cluster). For GGT, associations in or near genes involved in glutathione metabolism (*GGT1*), inflammation (*HNF1A, RORA*) and lipid metabolism (*HNF1A*), and several other genes with cardiombetabolic associations but with currently unknown functionality (*ZNF827, C14orf73*). Interestingly, we did not find any other significant associations with AST levels, indicating that *PNPLA3, SAMM50* and *PARVB*, all previously associated with NAFLD, may be the largest contributors to AST levels of the variants studied in individuals of Mexican-American ancestry.

Similar to other studies (36, 37), we report a higher minor allele frequency in individuals of Mexican-American ancestry compared to individuals of European, African American, or Asian ancestry. The similar effect size reported across ancestries (36) but the higher affect allele frequency may explain, in part, the higher AST and ALT levels in Hispanics (4, 44), as well as Hispanics greater risk of NAFLD (3, 4, 44, 45). One limitation to our study is that we only have liver density measures in one cohort (IRAS FS) so we could not extend our GWAS to include NAFLD as an outcome. However, the IRAS FS has previously reported

highly significant associations between rs738409 in *PNPLA3* and NAFLD (36, 40). To the extent that our associations with higher AST or ALT levels could be indicative of pre-clinical liver inflammation in Hispanics, our findings suggest that the presence of the *PNPLA3* risk alleles could indicate increased risk of NAFLD within this population.

Several studies have reported sex and age differences in the association of *PNPLA3* variants and liver enzymes, where younger females had an association with higher ALT and males had an association with higher AST levels (32, 38). We did not see any significant sex or age interactions with rs4823173. This could be due to differences in ancestry, or the younger age of our population compared to the other studies, as the age effect was seen mostly in women over 50 years of age. In addition, our population was majority female, so differences by sex may have been harder to discern.

We did not see any genome-wide significant associations with GGT. This is most likely due to the smaller sample size and resulting decreased power. When comparing our results to previously published loci, we found an association between rs944002 in *C14orf73* (7, 25) for GGT ($P=5.83 \times 10^{-5}$). *C14orf73*, also known as *EXOC3L4* (exocyst complex component 3 like 4), is expressed in the liver, and could indicate another genetic link to variation in liver enzymes.

In conclusion, we found that SNPs in the *PNPLA3* gene, rs4823173, rs2896019, and rs2281135 were significantly associated with AST levels. Although they did not reach the threshold for significance in this study, these same three SNPs in the *PNPLA3* gene were the top hits with ALT. The strong LD for these SNPs, as well as with rs738409, indicates a single hit in the *PNPLA3* gene. These results demonstrate that *PNPLA3*, a locus previously identified with ALT, AST, and NAFLD/NASH in other ancestries, is associated with liver function in Hispanics as well.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

The authors would like to thank the participants of BetaGene, the Hypertension-Insulin Resistance Family Study (HTN-IR), The Insulin Resistance and Atherosclerosis Study Family Study (IRAS FS), Mexican-American Coronary Artery Disease (MACAD) Study, and Troglitazone in the Prevention of Diabetes (TRIPOD).

Financial Support:

This research was supported by the GUARDIAN Study DK-085175 from the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and from the following grants: HL-047887 (IRAS), HL-047889 (IRAS), HL-047890 (IRAS), HL-47902 (IRAS), HL-060944 (IRAS-FS), HL-061019 (IRASFS), DK-061628 (BetaGene), American Diabetes Association Distinguished Clinical Scientist Award (BetaGene), HL-088457 (MACAD), HL-0697974 (HTN-IR), HL-055798 (NIDDM-Athero).

References

1. Angulo P. Nonalcoholic fatty liver disease. The New England journal of medicine. 2002;346(16): 1221–31. Epub 2002/04/19. doi: 10.1056/NEJMra011775. PubMed PMID: 11961152. [PubMed: 11961152]

2. Adams LA, Lymp JF, St Sauver J, Sanderson SO, Lindor KD, Feldstein A, et al. The natural history of nonalcoholic fatty liver disease: a population-based cohort study. Gastroenterology. 2005;129(1): 113–21. Epub 2005/07/14. PubMed PMID: 16012941. [PubMed: 16012941]

3. Browning JD, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, et al. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. Hepatology (Baltimore, Md). 2004;40(6):1387–95. Epub 2004/11/27. doi: 10.1002/hep.20466. PubMed PMID: 15565570.

4. Clark JM. The epidemiology of nonalcoholic fatty liver disease in adults. Journal of clinical gastroenterology. 2006;40 Suppl 1:S5–10. Epub 2006/03/17. doi: 10.1097/01.mcg.0000168638.84840.ff. PubMed PMID: 16540768. [PubMed: 16540768]

5. Rahmioglu N, Andrew T, Cherkas L, Surdulescu G, Swaminathan R, Spector T, et al. Epidemiology and genetic epidemiology of the liver function test proteins. PloS one. 2009;4(2):e4435. Epub 2009/02/12. doi: 10.1371/journal.pone.0004435. PubMed PMID: 19209234; PubMed Central PMCID: PMC2636884. [PubMed: 19209234]

6. Chalasani N, Guo X, Loomba R, Goodarzi MO, Haritunians T, Kwon S, et al. Genome-wide association study identifies variants associated with histologic features of nonalcoholic Fatty liver disease. Gastroenterology. 2010;139(5):1567–76, 76 e1-6. Epub 2010/08/17. doi: 10.1053/j.gastro.2010.07.057. PubMed PMID: 20708005; PubMed Central PMCID: PMC2967576. [PubMed: 20708005]

7. Chambers JC, Zhang W, Sehmi J, Li X, Wass MN, Van der Harst P, et al. Genome-wide association study identifies loci influencing concentrations of liver enzymes in plasma. Nature genetics. 2011;43(11):1131–8. Epub 2011/10/18. doi: 10.1038/ng.970. PubMed PMID: 22001757; PubMed Central PMCID: PMC3482372. [PubMed: 22001757]

8. Kitamoto T, Kitamoto A, Yoneda M, Hyogo H, Ochi H, Nakamura T, et al. Genome-wide scan revealed that polymorphisms in the PNPLA3, SAMM50, and PARVB genes are associated with development and progression of nonalcoholic fatty liver disease in Japan. Human genetics. 2013;132(7):783–92. Epub 2013/03/29. doi: 10.1007/s00439-013-1294-3. PubMed PMID: 23535911. [PubMed: 23535911]

9. Lazo M, Hernaez R, Eberhardt MS, Bonekamp S, Kamel I, Guallar E, et al. Prevalence of nonalcoholic fatty liver disease in the United States: the Third National Health and Nutrition Examination Survey, 1988-1994. American journal of epidemiology. 2013;178(1):38–45. Epub 2013/05/25. doi: 10.1093/aje/kws448. PubMed PMID: 23703888; PubMed Central PMCID: PMC3698993. [PubMed: 23703888]

10. Goodarzi MO, Langefeld CD, Xiang AH, Chen YD, Guo X, Hanley AJ, et al. Insulin sensitivity and insulin clearance are heritable and have strong genetic correlation in Mexican Americans. Obesity (Silver Spring, Md). 2014;22(4):1157–64. Epub 2013/10/15. doi: 10.1002/oby.20639. PubMed PMID: 24124113; PubMed Central PMCID: PMC3968231.

11. Watanabe RM, Allayee H, Xiang AH, Trigo E, Hartiala J, Lawrence JM, et al. Transcription factor 7-like 2 (TCF7L2) is associated with gestational diabetes mellitus and interacts with adiposity to alter insulin secretion in Mexican Americans. Diabetes. 2007;56(5):1481–5. Epub 2007/02/24. doi: 10.2337/db06-1682. PubMed PMID: 17317761; PubMed Central PMCID: PMC2925638. [PubMed: 17317761]

12. Xiang AH, Azen SP, Raffel LJ, Tan S, Cheng LS, Diaz J, et al. Evidence for joint genetic control of insulin sensitivity and systolic blood pressure in hispanic families with a hypertensive proband. Circulation. 2001;103(1):78–83. Epub 2001/01/04. PubMed PMID: 11136689. [PubMed: 11136689]

13. Henkin L, Bergman RN, Bowden DW, Ellsworth DL, Haffner SM, Langefeld CD, et al. Genetic epidemiology of insulin resistance and visceral adiposity. The IRAS Family Study design and methods. Annals of epidemiology. 2003;13(4):211–7. Epub 2003/04/10. PubMed PMID: 12684185. [PubMed: 12684185]

14. Goodarzi MO, Guo X, Taylor KD, Quinones MJ, Samayoa C, Yang H, et al. Determination and use of haplotypes: ethnic comparison and association of the lipoprotein lipase gene and coronary artery disease in Mexican-Americans. Genetics in medicine : official journal of the American College of Medical Genetics. 2003;5(4):322–7. Epub 2003/07/17. doi: 10.1097/01.gim.0000076971.55421.ad. PubMed PMID: 12865761. [PubMed: 12865761]

15. Wang Y, Kandeel F, Taylor KD, Hernandez D, Saad MF, Nadler J, et al. Insulin and blood pressure are linked to the LDL receptor-related protein locus on chromosome 12q. Diabetes. 2000;49:A204–A. PubMed PMID: WOS:000087005601841.

16. Wagenknecht LE, Mayer EJ, Rewers M, Haffner S, Selby J, Borok GM, et al. The insulin resistance atherosclerosis study (IRAS) objectives, design, and recruitment results. Annals of epidemiology. 1995;5(6):464–72. Epub 1995/11/01. PubMed PMID: 8680609. [PubMed: 8680609]

17. Buchanan TA, Xiang AH, Peters RK, Kjos SL, Marroquin A, Goico J, et al. Preservation of pancreatic beta-cell function and prevention of type 2 diabetes by pharmacological treatment of insulin resistance in high-risk hispanic women. Diabetes. 2002;51(9):2796–803. Epub 2002/08/28. PubMed PMID: 12196473. [PubMed: 12196473]

18. Gunderson KL, Steemers FJ, Ren H, Ng P, Zhou L, Tsan C, et al. Whole-genome genotyping. Methods in enzymology. 2006;410:359–76. Epub 2006/08/30. doi: 10.1016/s0076-6879(06)10017-8. PubMed PMID: 16938560. [PubMed: 16938560]

19. Shen R, Fan JB, Campbell D, Chang W, Chen J, Doucet D, et al. High-throughput SNP genotyping on universal bead arrays. Mutation research. 2005;573(1-2):70–82. Epub 2005/04/15. doi: 10.1016/j.mrfmmm.2004.07.022. PubMed PMID: 15829238. [PubMed: 15829238]

20. Grove ML, Yu B, Cochran BJ, Haritunians T, Bis JC, Taylor KD, et al. Best practices and joint calling of the HumanExome BeadChip: the CHARGE Consortium. PloS one. 2013;8(7):e68095. Epub 2013/07/23. doi: 10.1371/journal.pone.0068095. PubMed PMID: 23874508; PubMed Central PMCID: PMC3709915. [PubMed: 23874508]

21. Palmer ND, Goodarzi MO, Langefeld CD, Wang N, Guo X, Taylor KD, et al. Genetic Variants Associated With Quantitative Glucose Homeostasis Traits Translate to Type 2 Diabetes in Mexican Americans: The GUARDIAN (Genetics Underlying Diabetes in Hispanics) Consortium. Diabetes. 2015;64(5):1853–66. Epub 2014/12/20. doi: 10.2337/db14-0732. PubMed PMID: 25524916; PubMed Central PMCID: PMC4407862. [PubMed: 25524916]

22. Chen MH, Yang Q. GWAF: an R package for genome-wide association analyses with family data. Bioinformatics (Oxford, England). 2010;26(4):580–1. Epub 2009/12/31. doi: 10.1093/bioinformatics/btp710. PubMed PMID: 20040588; PubMed Central PMCID: PMC2852219.

23. Almasy L, Blangero J. Multipoint quantitative-trait linkage analysis in general pedigrees. American journal of human genetics. 1998;62(5):1198–211. Epub 1998/05/23. doi: 10.1086/301844. PubMed PMID: 9545414; PubMed Central PMCID: PMC1377101. [PubMed: 9545414]

24. Adams LA, White SW, Marsh JA, Lye SJ, Connor KL, Maganga R, et al. Association between liver-specific gene polymorphisms and their expression levels with nonalcoholic fatty liver disease. Hepatology (Baltimore, Md). 2013;57(2):590–600. Epub 2012/12/06. doi: 10.1002/hep.26184. PubMed PMID: 23213074.

25. Middelberg RP, Benyamin B, de Moor MH, Warrington NM, Gordon S, Henders AK, et al. Loci affecting gamma-glutamyl transferase in adults and adolescents show age x SNP interaction and cardiometabolic disease associations. Human molecular genetics. 2012;21(2):446–55. Epub 2011/10/20. doi: 10.1093/hmg/ddr478. PubMed PMID: 22010049; PubMed Central PMCID: PMC3276286. [PubMed: 22010049]

26. Shen H, Damcott C, Shuldiner SR, Chai S, Yang R, Hu H, et al. Genome-wide association study identifies genetic variants in GOT1 determining serum aspartate aminotransferase levels. Journal of human genetics. 2011;56(11):801–5. Epub 2011/09/09. doi: 10.1038/jhg.2011.105. PubMed PMID: 21900944; PubMed Central PMCID: PMC3608855. [PubMed: 21900944]

27. Speliotes EK, Yerges-Armstrong LM, Wu J, Hernaez R, Kim LJ, Palmer CD, et al. Genome-wide association analysis identifies variants associated with nonalcoholic fatty liver disease that have distinct effects on metabolic traits. PLoS genetics. 2011;7(3):e1001324. Epub 2011/03/23. doi: 10.1371/journal.pgen.1001324. PubMed PMID: 21423719; PubMed Central PMCID: PMC3053321. [PubMed: 21423719]

28. Yuan X, Waterworth D, Perry JR, Lim N, Song K, Chambers JC, et al. Population-based genome-wide association studies reveal six loci influencing plasma levels of liver enzymes. American journal of human genetics. 2008;83(4):520–8. Epub 2008/10/23. doi: 10.1016/j.ajhg.2008.09.012. PubMed PMID: 18940312; PubMed Central PMCID: PMC2561937. [PubMed: 18940312]

29. Kawaguchi T, Sumida Y, Umemura A, Matsuo K, Takahashi M, Takamura T, et al. Genetic polymorphisms of the human PNPLA3 gene are strongly associated with severity of non-alcoholic fatty liver disease in Japanese. PloS one. 2012;7(6):e38322. Epub 2012/06/22. doi: 10.1371/journal.pone.0038322. PubMed PMID: 22719876; PubMed Central PMCID: PMC3375283. [PubMed: 22719876]
30. Park TJ, Hwang JY, Go MJ, Lee HJ, Jang HB, Choi Y, et al. Genome-wide association study of liver enzymes in korean children. Genomics & informatics. 2013;11(3):149–54. Epub 2013/10/15. doi: 10.5808/gi.2013.11.3.149. PubMed PMID: 24124411; PubMed Central PMCID: PMC3794088. [PubMed: 24124411]
31. Flores YN, Velazquez-Cruz R, Ramirez P, Banuelos M, Zhang ZF, Yee HF Jr., et al. Association between PNPLA3 (rs738409), LYPLAL1 (rs12137855), PPP1R3B (rs4240624), GCKR (rs780094), and elevated transaminase levels in overweight/obese Mexican adults. Molecular biology reports. 2016;43(12):1359–69. Epub 2016/10/19. doi: 10.1007/s11033-016-4058-z. PubMed PMID: 27752939; PubMed Central PMCID: PMCPMC5106313. [PubMed: 27752939]
32. Li Q, Qu HQ, Rentfro AR, Grove ML, Mirza S, Lu Y, et al. PNPLA3 polymorphisms and liver aminotransferase levels in a Mexican American population. Clinical and investigative medicine Medecine clinique et experimentale. 2012;35(4):E237–45. Epub 2012/08/07. PubMed PMID: 22863562; PubMed Central PMCID: PMC3441048. [PubMed: 22863562]
33. Larrieta-Carrasco E, Leon-Mimila P, Villarreal-Molina T, Villamil-Ramirez H, Romero-Hidalgo S, Jacobo-Albavera L, et al. Association of the I148M/PNPLA3 variant with elevated alanine transaminase levels in normal-weight and overweight/obese Mexican children. Gene. 2013;520(2):185–8. Epub 2013/03/21. doi: 10.1016/j.gene.2013.03.038. PubMed PMID: 23510779. [PubMed: 23510779]
34. Larrieta-Carrasco E, Acuna-Alonzo V, Velazquez-Cruz R, Barquera-Lozano R, Leon-Mimila P, Villamil-Ramirez H, et al. PNPLA3 I148M polymorphism is associated with elevated alanine transaminase levels in Mexican Indigenous and Mestizo populations. Molecular biology reports. 2014;41(7):4705–11. Epub 2014/04/03. doi: 10.1007/s11033-014-3341-0. PubMed PMID: 24691744. [PubMed: 24691744]
35. Larrieta-Carrasco E, Flores YN, Macias-Kauffer LR, Ramirez-Palacios P, Quiterio M, Ramirez-Salazar EG, et al. Genetic variants in COL13A1, ADIPOQ and SAMM50, in addition to the PNPLA3 gene, confer susceptibility to elevated transaminase levels in an admixed Mexican population. Experimental and molecular pathology. 2018;104(1):50–8. Epub 2018/01/09. doi: 10.1016/j.yexmp.2018.01.001. PubMed PMID: 29307798. [PubMed: 29307798]
36. Palmer ND, Musani SK, Yerges-Armstrong LM, Feitosa MF, Bielak LF, Hernaez R, et al. Characterization of European ancestry nonalcoholic fatty liver disease-associated variants in individuals of African and Hispanic descent. Hepatology (Baltimore, Md). 2013;58(3):966–75. Epub 2013/04/09. doi: 10.1002/hep.26440. PubMed PMID: 23564467; PubMed Central PMCID: PMC3782998.
37. 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. Nature genetics. 2008;40(12):1461–5. Epub 2008/09/30. doi: 10.1038/ng.257. PubMed PMID: 18820647; PubMed Central PMCID: PMC2597056. [PubMed: 18820647]
38. 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 (Baltimore, Md). 2011;53(6):1883–94. Epub 2011/03/08. doi: 10.1002/hep.24283. PubMed PMID: 21381068.
39. Speliotes EK, Butler JL, Palmer CD, Voight BF, Hirschhorn JN. PNPLA3 variants specifically confer increased risk for histologic nonalcoholic fatty liver disease but not metabolic disease. Hepatology (Baltimore, Md). 2010;52(3):904–12. Epub 2010/07/22. doi: 10.1002/hep.23768. PubMed PMID: 20648472; PubMed Central PMCID: PMC3070300.
40. Wagenknecht LE, Palmer ND, Bowden DW, Rotter JI, Norris JM, Ziegler J, et al. Association of PNPLA3 with non-alcoholic fatty liver disease in a minority cohort: the Insulin Resistance Atherosclerosis Family Study. Liver international : official journal of the International Association for the Study of the Liver. 2011;31(3):412–6. Epub 2011/02/02. doi: 10.1111/j.

1478-3231.2010.02444.x. PubMed PMID: 21281435; PubMed Central PMCID: PMC3703938. [PubMed: 21281435]

41. Kotronen A, Johansson LE, Johansson LM, Roos C, Westerbacka J, Hamsten A, et al. A common variant in PNPLA3, which encodes adiponutrin, is associated with liver fat content in humans. Diabetologia. 2009;52(6):1056–60. Epub 2009/02/19. doi: 10.1007/s00125-009-1285-z. PubMed PMID: 19224197. [PubMed: 19224197]
42. Sookoian S, Pirola CJ. PNPLA3, the triacylglycerol synthesis/hydrolysis/storage dilemma, and nonalcoholic fatty liver disease. World journal of gastroenterology : WJG. 2012;18(42):6018–26. Epub 2012/11/17. doi: 10.3748/wjg.v18.i42.6018. PubMed PMID: 23155331; PubMed Central PMCID: PMC3496879. [PubMed: 23155331]
43. The Genotype-Tissue Expression (GTEx) project. Nature genetics. 2013;45(6):580–5. Epub 2013/05/30. doi: 10.1038/ng.2653. PubMed PMID: 23715323; PubMed Central PMCID: PMC4010069. [PubMed: 23715323]
44. Flores YN, Yee HF Jr., Leng M, Escarce JJ, Bastani R, Salmeron J, et al. Risk factors for chronic liver disease in Blacks, Mexican Americans, and Whites in the United States: results from NHANES IV, 1999-2004. The American journal of gastroenterology. 2008;103(9):2231–8. Epub 2008/08/02. doi: 10.1111/j.1572-0241.2008.02022.x. PubMed PMID: 18671818; PubMed Central PMCID: PMC4462194. [PubMed: 18671818]
45. Hernaez R, McLean J, Lazo M, Brancati FL, Hirschhorn JN, Borecki IB, et al. Association between variants in or near PNPLA3, GCKR, and PPP1R3B with ultrasound-defined steatosis based on data from the third National Health and Nutrition Examination Survey. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2013;11(9):1183–90 e2. Epub 2013/02/19. doi: 10.1016/j.cgh.2013.02.011. PubMed PMID: 23416328. [PubMed: 23416328]

Study Importance:

What is already known about this subject?

- Previous genome-wide association studies of liver enzymes in those of European, Indian, and Japanese ancestry identified candidate genes involved in various pathways, including glucose and lipid metabolism as well as inflammation and immunity.

What does this study add?

- Few studies have extensively examined the genetic contributions of liver enzymes in Hispanics, a group at increased risk of non-alcoholic fatty liver disease.
- This is the first GWAS of liver enzymes or NAFLD in individuals of Mexican-American ancestry.

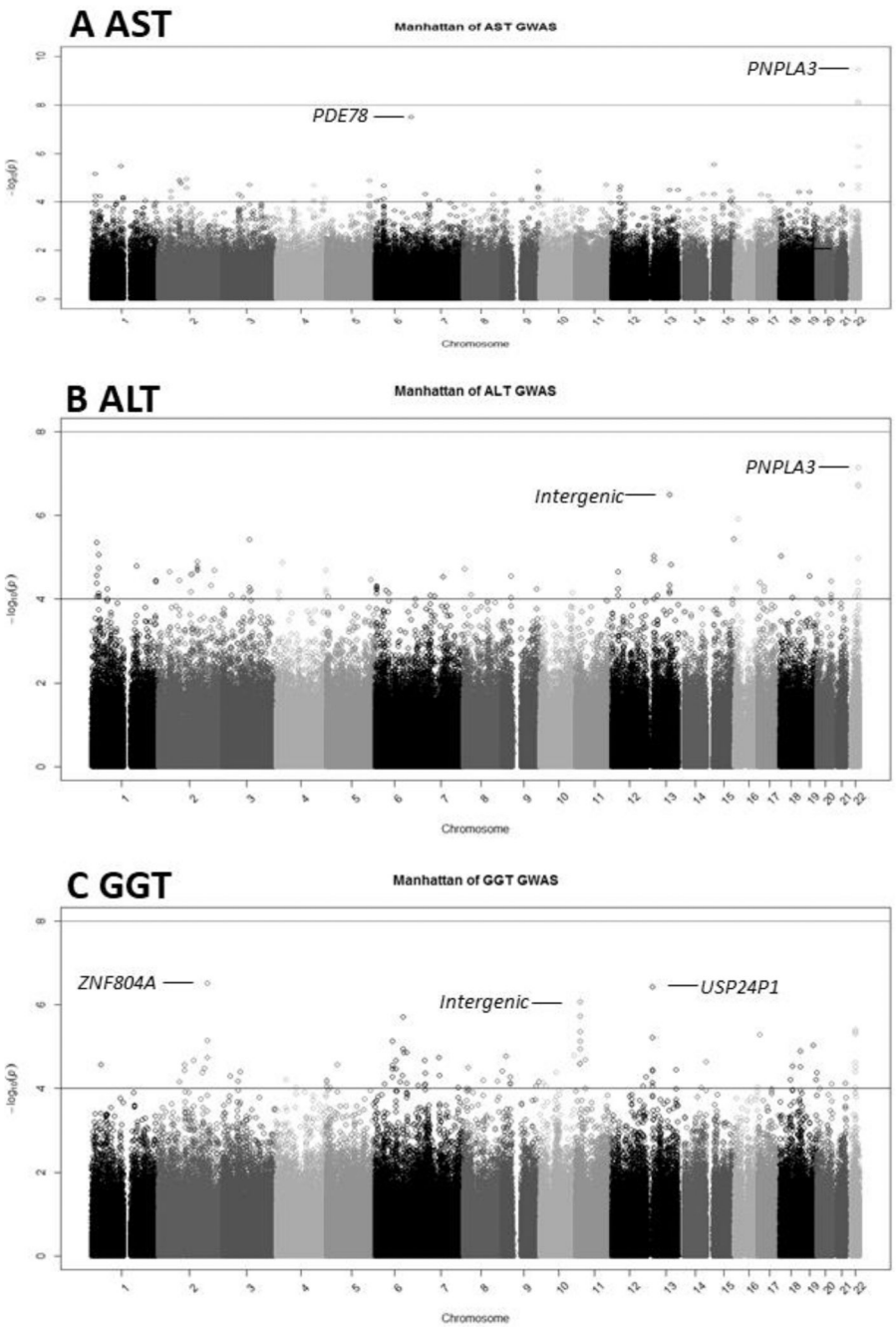

Figure 1.

Genome-wide Manhattan plots for the GUARDIAN Consortium meta-analysis. SNPs above the red line were genome-wide significant at $P < 1 \times 10^{-8}$. A. Results for AST of 659,222 SNPs indicate a genome-wide significant loci for *PNPLA3* ($P=3.44 \times 10^{-10}$). B. Results for ALT of 659,211 SNPs. C. Results for GGT of 655,023 SNPs.

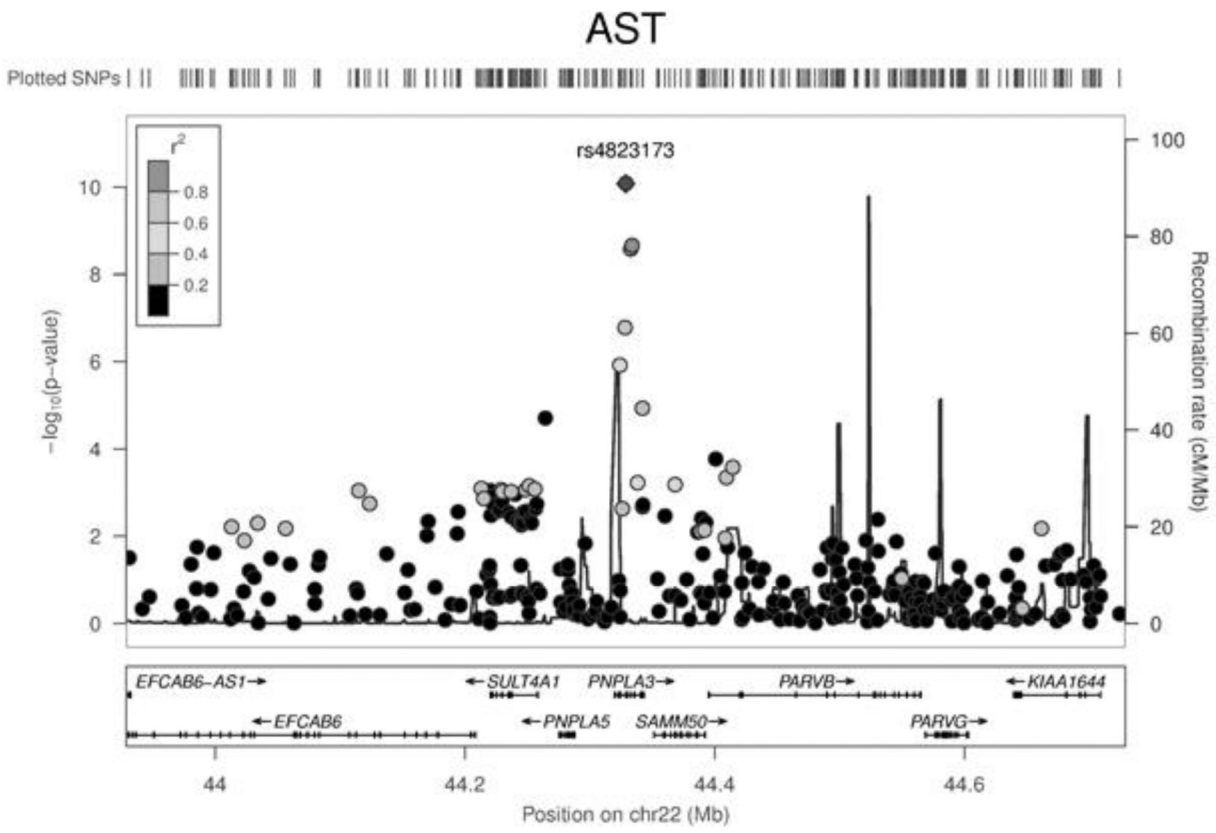

Figure 2.

Regional plot of the *PNPLA3* locus in the GUARDIAN Consortium meta-analysis for AST. Genotyped SNPs are plotted with their meta-analysis p-values ($-\log_{10}$) according to their chromosomal position (hg19). The purple diamond represents rs4823173, the top variant in the meta-analysis for AST; red circles represent rs2896019 and rs2281135 ($r^2 \geq 0.80$).

Table 1.

Characteristics of the GUARDIAN Consortium meta-analysis cohorts.

	BetaGene	HTN-IR	IRAS FS	MACAD	TRIPOD
Sample Size	1195	689	1034	714	125
Age	34.69 ± 7.92	37.28 ± 14.13	40.60 ± 13.7	34.49 ± 8.86	34.72 ± 6.26
Female (%)	71.72	58.90	58.90	56.74	100.00
BMI (kg/m^2)	29.58 ± 6.09	28.73 ± 5.45	28.30 ± 5.80	28.92 ± 5.11	30.50 ± 5.43
AST (IU/L)	23.21 ± 11.60	24.57 ± 13.09	19.10 ± 8.90	24.86 ± 10.92	18.98 ± 8.10
ALT (IU/L)	36.83 ± 24.56	35.19 ± 23.88	11.30 ± 9.90	28.86 ± 20.65	26.21 ± 16.40
GGT (IU/L)	NA	31 ± 25.51	39.40 ± 43.70	NA	NA

Data are mean ± SD unless otherwise indicated. NA, not applicable. BMI, body mass index. AST, aspartate aminotransferase. ALT, alanine aminotransferase. GGT, gamma-glutamyl transferase. Data on GGT was not available in BetaGene, TRIPOD nor MACAD.

Table 2.

Top hits in the GUARDIAN Consortium liver enzyme meta-analysis by trait ($P < 1 \times 10^{-7}$).

SNP	Chromosome	Position[1]	Gene	Alleles[2]	MAF	Trait	P-value
rs4823173	22	44328730	*PNPLA3*	A/G	0.42	AST	3.44×10^{-10}
rs2896019	22	44333694	*PNPLA3*	G/T	0.43	AST	7.29×10^{-9}
rs2281135	22	44332570	*PNPLA3*	A/G	0.43	AST	8.73×10^{-9}
rs178571	6	136346130	*PDE7B*	T/C	0.03	AST	3.21×10^{-8}
rs1883350	22	44328043	*PNPLA3*	T/C	0.49	AST	5.22×10^{-7}
rs4823173	22	44328730	*PNPLA3*	A/G	0.42	ALT	7.12×10^{-8}
rs2281135	22	44332570	*PNPLA3*	A/G	0.43	ALT	1.81×10^{-7}
rs2896019	22	44333694	*PNPLA3*	G/T	0.43	ALT	1.98×10^{-7}
rs9601485	13	81475442	*intergenic*	T/C	0.12	ALT	3.17×10^{-7}
rs10497655	2	185462041	*ZNF804A*	C/T	0.30	GGT	3.10×10^{-7}
rs9511099	13	19591944	*USP24P1*	G/T	0.21	GGT	3.72×10^{-7}
rs10458877	11	15941099	*intergenic*	T/C	0.13	GGT	8.59×10^{-7}

SNP, single nucleotide polymorphism. MAF, minor allele frequency. AST, aspartate aminotransferase. ALT, alanine aminotransferase. GGT, gamma-glutamyl transferase.

For BetaGene, there are n=1191 for AST and n=1193 for ALT, respectively; for IRAS FS, there are n=925 n=878, and n=888 samples with AST, ALT, and GGT respectively; sample size for HTN is n=688 for ALT; and sample size for ALT is n=711 in MACAD.

1. Build hg19.

2. Minor/Major allele.

Table 3.

GUARDIAN Consortium meta-analysis P values for regions reported in other liver enzyme or NAFLD/NASH GWAS. P value is listed for the GUARDIAN Consortium meta-analysis.

Gene	SNP	Previously Associated GWAS Traits	AST GUARDIAN Consortium P-value	ALT GUARDIAN Consortium P-value	GGT GUARDIAN Consortium P-value
PNPLA3	rs2896019 rs381062	AST [9,30], ALT[9,10,30],NAFLD/NASH[9,29,31]	P=7.29×10^{-9} P=0.78	P=1.98×10^{-7} P=0.62	P=0.008 P=0.89
SAMM50	rs738491 rs3761472	ALT[30] NAFLD/NASH[29]	P=0.11 P=9×10^{-4}	P=0.08 P=3×10^{-4}	P=0.37 P=0.01
CHUK	rs11597086	ALT[30]		P=0.03	
CPN1	rs11597390	ALT[9,30]		P=0.02	
ST6GALNAC3	rs4949718	ALT[32]		P=0.81	
CELF2	rs596406	AST[28],ALT[32]	P=0.92	P=0.59	
GOT1	rs17109512	AST[28]	P=0.28		
ST6GALNAC3	rs4949718	AST[28]	P=0.64		
ZP4	rs2499604	AST[26]	P=0.64		
PZP	rs6487679	AST[26]	P=0.45		
HNF1A	rs1169313 rs1169288 rs7310409	GGT[9,27,30]			P=0.10 P=0.29 P=0.02
GGT1	rs4820599 rs5751901	GGT [9,27,30]			P=0.04 P=0.09
C2ofr16	rs1260326	GGT[9]			P=0.23
ZNF827	rs4547811	GGT[9]			P=0.03
ITGA1	rs4074793	GGT[9]			P=0.93
MLXIPL	rs17145750	GGT[9]			P=0.81
C14orf73	rs944002	GGT[9,27]			P=1.0×10^{-4}
RORA	rs339969 rs340005	GGT[9,27]			P=0.02 P=0.02
CD276	rs8038465	GGT[9]			P=0.59
FUT2	rs516246	GGT[9]			P=0.74
FDFT1	rs2645424	NAFLD/NASH[26]	P=0.09	P=0.77	P=0.70
PARVB	rs5764455 rs6006611	NAFLD/NASH[29]	P=0.72 P=3.0×10^{-4}	P=0.26 P=0.08	P=0.02 P=0.75
SLC38A8	rs11864146	NAFLD/NASH[26]	P=0.77	P=0.80	P=0.72
GCKR	rs780094	NAFLD/NASH[29]	P=0.33	P=0.39	P=0.34