Received: 28 December 2020 | Revised: 2 August 2021 | Accepted: 3 August 2021

DOI: 10.1002/hep.32115

ORIGINAL ARTICLE

Genome-wide association of individual vulnerability with alcohol-associated liver disease: A Korean genome and epidemiology study

Kwang Yoon Kim[1] | **Jung Oh Kim**[2] | **Young-Sang Kim**[3] | **Ja-Eun Choi**[2] | **Jae-Min Park**[4] | **Kunhee Han**[5] | **Da-Hyun Park**[2] | **Yon Chul Park**[6,7] | **Bom Taeck Kim**[1] | **Kyung-Won Hong**[2]

[1]Department of Family Practice & Community Health, Ajou University School of Medicine, Suwon, Republic of Korea

[2]Healthcare R&D Division, Theragen Bio Co. Ltd., Suwon, Republic of Korea

[3]Department of Family Medicine, CHA Bundang Medical Center, CHA University, Seongnam, Republic of Korea

[4]Department of Family Medicine, Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, Republic of Korea

[5]Department of Family Medicine, Seonam Hospital, Seoul, Republic of Korea

[6]Department of Family Medicine, Wonju Severance Christian Hospital, Wonju, Republic of Korea

[7]Department of Medical Education, Yonsei University Wonju College of Medicine, Wonju, Republic of Korea

Correspondence
Kyung-Won Hong, Healthcare R&D Division, Theragen Bio Co., Ltd., Suwon, Gyeonggi-do 16229, Republic of Korea.
Email: kyungwon.hong@theragenbio.com

Bom Taeck Kim, Department of Family Practice & Community Health, Ajou University School of Medicine, Suwon, Gyeonggi-do 16499, Republic of Korea.
Email: lovesong@ajou.ac.kr

Funding information
Supported by the National Biobank of Korea, the Centers for Disease Control and Prevention, Republic of Korea (2019-059), and the Ministry of Trade, Industry and Energy of Korea (20002781)

Abstract

Background and aims: The quantity of alcohol leading to alcohol-associated liver disease (ALD) varies individually. Genetic backgrounds contributing to the divergence in individual susceptibility to alcohol-induced liver damage have not been elucidated in detail.

Approach and results: Based on the Korean Genome and Epidemiology Study Health Examination (KoGES_HEXA) cohort data, 21,919 participants (40-79 years old) were included and divided into cases and controls based on the ALD diagnostic criteria proposed by the American College of Gastroenterology. Data generated by a genome wide-association study were analyzed using logistic regression to assess the risk of ALD development in nondrinkers, light drinkers, and heavy drinkers. We detected three loci, *gamma-glutamyltransferase 1* (*GGT1*), *zinc protein finger 827* (*ZNF827*) and *HNF1 homeobox A* (*HNF1A*), which were significantly associated with ALD risk. The *GGT1* rs2006227 minor allele was strongly associated with all groups. Among the minor alleles of single nucleotide polymorphisms (SNPs) in *HNF1A*, rs1183910 had the strongest

Abbreviations: ALD, alcohol-associated liver disease; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GAS2, growth arrest specific 2; GGT1, gamma-glutamyltransferase 1; GTEx, genotype-tissue expression; GWAS, genome-wide association study; HD, heavy drinker; HNF1A, HNF1 homeobox A; KoGES, Korean Genome and Epidemiology Study; LD, light drinker; MBOAT7, membrane bound O-acyltransferase domain; miR, microRNA; ND, nondrinker; PNPLA3, patatin-like phospholipase domain-containing 3; SNP, single-nucleotide polymorphism; SVIP, small VCP interacting protein; ZNF827, zinc protein finger 827; γ-GTP, gamma-glutamyl transpeptidase.

Kwang Yoon Kim and Jung Oh Kim contributed equally to this work.

SEE EDITORIAL ON PAGE 246

association with a protective effect from ALD in light drinkers. However, this association was not observed in heavy drinkers. Five SNPs on chromosome 11 showed suggestive significance in protective effects against ALD.

Conclusions: SNPs, including *HNF1A* rs1183910 minor allele, are the most promising genetic candidates for protection against ALD. The expression of genes contributing to ALD development may be altered by the amount of alcohol consumed.

INTRODUCTION

Excessive alcohol consumption is known to cause liver damage. According to a study conducted in the United States, 48% of liver cirrhosis cases were attributed to alcohol consumption.[1] However, the development of alcohol-associated liver disease (ALD) is not always proportional to the quantity of alcohol consumed. Although the mechanisms prompting alcohol-induced liver damage have been investigated in previous studies,[2,3] the reasons underpinning the differences in individual vulnerability to alcohol-induced liver damage have not been clearly elucidated. Individual susceptibility to alcohol has been hypothesized to arise as a result of variances in heredity, sex, diet, and coinciding medical conditions.[4] Recently, numerous studies have identified several significant genetic factors related to ALD development, including variants of the following genes: *patatin-like phospholipase domain-containing 3* (*PNPLA3*),[5-7] *membrane bound O-acyltransferase domain* (*MBOAT7*) containing *7-transmembrane channel-like 4*,[8,9] and the nonsynonymous variant of *superoxide dismutase 2*,[8,10] in addition to several microRNAs (miRs), such as miR-155, miR-34a, miR-122, miR-212, and miR-21.[11] Although these studies have attempted to determine the genetic backgrounds responsible for conferring ALD susceptibility, they did not consider gene–alcohol interaction. To clarify the effect of genetic variances on individual vulnerability to ALD and to evaluate the changes in genetic expression as a function of the amount of alcohol consumption, we performed a genome-wide association study (GWAS) among groups of individuals with varying degree of alcohol consumption; for this, we selected participants based on the data generated from the Korean Genome and Epidemiology Study (KoGES) that involved a large cohort of Korean individuals from an urban area.

PATIENTS AND METHODS

Study participants

Data were obtained from the KoGES_HEXA study, including a community-dwellers cohort of men and women aged 40-79 years at the baseline, recruited from a national health examinee registry. Eligible participants were asked to volunteer through on-site invitations, letters, telephone calls, media campaigns, or community conferences. The responders were invited to visit the survey sites, including national and international medical schools, hospitals, and health institutions for an interview (a questionnaire administered by trained staff and physical examination). Information on past medical history, smoking history, alcohol consumption, and physical activity was collected during the health interview. All study participants of KoGES were of Korean ethnicity. The detailed history and profile of subjects are described elsewhere.[12]

In total, 58,701 participants with genome-wide single nucleotide polymorphism (SNP) genotype data were included in the city-based cohort of KoGES. Participants with missing values in response to the alcohol intake questionnaire, total bilirubin levels, blood urea nitrogen levels, serum creatinine levels, and white blood cell counts were excluded from this study (n = 21,919). All participants were over 40 years old, and liver function tests were performed for each participant. ALD was defined as a ratio of aspartate aminotransferase (AST) to alanine aminotransferase (ALT) > 3 or ratio of gamma-glutamyl transpeptidase (γ-GTP) to ALT > 2.5, based on the clinical guidelines proposed by the American College of Gastroenterology.[13] Subjects who did not meet the ALD criteria were assigned as controls.

Genotyping and quality control

Genotype data were provided by the Center for Genome Science, Korea National Institute of Health. The genotype data were produced by the Korea Biobank Array (Affymetrix, Santa Clara, CA).[14] The experimental results were filtered using the following quality control criteria: call rate >97%, minor allele frequency >1%, and Hardy–Weinberg equilibrium test $p < 1 \times 10^{-5}$. After quality control procedures, the experimental genotypes were used as the genotype imputation data set against the 1000 Genome Phase 1 and 2 Asian panel. Finally, the GWAS included 7,975,321 SNPs from chromosomes 1 to 22.

Statistical analysis

Clinical characteristics of study participants according to phenotype were compared using two-tailed Student *t* tests for continuous variables and chi-squared tests for categorical variables. We allocated the participants into three groups (nondrinkers [NDs], light drinkers [LDs], and heavy drinkers [HDs]) based on significant alcohol consumption.[15,16] A GWAS of the risk of ALD in the three groups was performed using logistic regression, adjusting for age and sex as covariates, using PLINK version 1.09. ORs and 95% CIs were calculated. The threshold for significant associations was defined as $p < 5.00 \times 10^{-8}$. The top significant SNPs obtained through the analysis were classified into loci based on whether they satisfied the criteria of $D' > 0.8$ and $R^2 > 0.8$, and using the LDlink tool (https://ldlink.nci.nih.gov/?tab=home) to confirm the linkage disequilibrium with cluster SNPs at a range of ±10 kb.

RESULTS

Clinical characteristics

The clinical characteristics of subjects in the ND, LD, and HD groups are provided in Table 1 and Supporting Digital Content 1. The case group included 782 (4.1%), 1,379 (9.5%), and 1,185 (34.5%) patients in ND, LD, and HD, respectively.

Case–control GWAS of NDs

The results of a case–control GWAS of the NDs are shown in Figure 1A. In total, 55 SNPs showed genome-wide significance ($p < 5 \times 10^{-8}$), and 276 SNPs showed suggestive associations ($5 \times 10^{-8} \leq 55$; SNPs showing genome-wide significance $p < 1 \times 10^{-5}$) (Supporting Digital Content 2). The most significant SNP was rs2006227 (OR = 1.578, CI = 1.425-1.748, $p = 1.80 \times 10^{-18}$) (Supporting Digital Content 2), and 45 SNPs were located in the same locus (i.e., gamma-glutamyltransferase 1 [*GGT1*; GeneID 2678, GenBank NC_000022.11, region 24503750 to 24628996]). The top SNP in the second strongest GWAS gene was rs4835265 (OR = 1.332, CI = 1.203-1.474, $p = 3.32 \times 10^{-8}$), and eight GWAS SNPs were located in the same locus (i.e., *zinc protein finger 827* (*ZNF827*; GeneID 152485, GenBank NC_000004.12, region 145757627 to 145938823). Among the suggestive SNPs, rs1183910 (OR = 0.757, CI = 0.683-0.840, $p = 9.33 \times 10^{-8}$; Supporting Digital Content 2) and those in the *HNF1 homeobox A* (*HNF1A*; GeneID 6927, GenBank NC_000012.12, region 120977683 to 121002512) showed a prominent association. Genomic context and *P* values for the most associated SNP at each of the three loci are given in Table 2.

Case–control GWAS of LDs

The results of a case–control GWAS of the LD are shown in Figure 1B. In total, 143 GWAS SNPs and 469 suggestive SNPs were detected. The most significant SNP was rs2330805 (OR = 1.592, CI = 1.468–1.727, $p = 3.84 \times 10^{-29}$) (Supporting Digital Content 3), and 85 GWAS SNPs were located in the same locus (i.e., the *GGT1* region of chromosome 22). The top GWAS SNP in the ND group GWAS, rs2006227, was also significant in the LD group (OR = 1.578, CI = 1.454–1.712, $p = 5.62 \times 10^{-28}$) (Supporting Digital Content 3). For a comparison between the results of the ND and LD groups, we included rs2006227 in Table 2. The top SNP in the second strongest GWAS SNP locus was rs1183910 (OR = 0.752, CI = 0.693–0.815, $p = 5.49 \times 10^{-12}$) (Supporting Digital Content 3), and 30 GWAS SNPs were located in the same locus (i.e., the *HNF1A* region of chromosome 12). The top SNP in the third strongest GWAS SNP locus was rs10024759 (OR = 1.273, CI = 1.175-1.380, $p = 3.44 \times 10^{-9}$) (Supplementary Digital Content 3), and 25 GWAS SNPs were located in the same locus (i.e., the *ZNF827* region of chromosome 4). The top GWAS SNP in the *ZNF827* region of the ND group GWAS was rs13147616, and the relationship between this SNP-like locus and LD rs13147616 was also significant in the GWAS of LDs (OR = 1.254, CI = 1.157-1.359, $p = 3.32 \times 10^{-8}$; Supporting Digital Content 3). A comparison between the ND and LD results, including rs4835265, is given in Table 2.

Case–control GWAS of HDs

Figure 1C shows the results of the case–control GWAS of the HDs. A total of 46 GWAS SNPs and 103 suggestive SNPs were detected (Supporting Digital Content 4). The most significant SNP was rs2006094 (OR = 1.593, CI = 1.431-1.774, $p = 2.10 \times 10^{-17}$) (Supporting Digital Content 4), and 45 GWAS SNPs were located in the same locus (i.e., the *GGT1* region of chromosome 22). The top GWAS SNP in the ND group GWAS, rs2006227, was also significant in the HD group (OR = 1.591, CI = 1.429-1.772, $p = 2.73 \times 10^{-17}$) (Supporting Digital Content 4). A comparison of GWAS results for the ND, LD, and HD groups, including rs2006227, is given in Table 2, which also includes the results for two SNPs (rs4835265 of *ZNF827* and rs1183910 of *HNF1A*) that were not significant in the HD group (Table 2). There were some suggestive SNPs on chromosomes 9, 11, and 19

TABLE 1 Baseline Characteristics of the Study Population Classified Into Three Groups (ND, LD, and HD) in the Case–Control Study

	ND (*n* = 18,840)			LD (*n* = 14,511)			HD (*n* = 3,431)		
Characteristics	**Control (*n* = 18,058)**	**Case (*n* = 782)**	***P***	**Control (*n* = 13,132)**	**Case (*n* = 1,379)**	***P***	**Control (*n* = 2,246)**	**Case (*n* = 1,185)**	***P***
Age (years, mean ± SD)	54.54 ± 7.82	55.72 ± 7.62	<0.0001	52.35 ± 8.07	54.18 ± 8.13	<0.0001	52.31 ± 8.14	53.18 ± 7.65	0.002
BMI (kg/m^2)	23.69 ± 2.98	24.17 ± 2.92	<0.0001	23.84 ± 2.79	24.40 ± 2.84	<0.0001	24.52 ± 2.81	24.75 ± 2.94	0.022
Waist (cm)	78.88 ± 8.56	80.47 ± 8.06	<0.0001	80.68 ± 8.89	84.32 ± 8.87	<0.0001	84.14 ± 8.88	86.30 ± 8.56	<0.0001
SBP (mmHg)	121.79 ± 15.09	122.97 ± 15.94	0.032	122.17 ± 14.58	126.16 ± 15.06	<0.0001	125.24 ± 14.05	129.72 ± 15.62	<0.0001
DBP (mmHg)	74.41 ± 9.63	75.24 ± 10.66	0.018	75.49 ± 9.76	78.00 ± 10.14	<0.0001	78.14 ± 9.66	81.10 ± 10.23	<0.0001
WBC (10^{3}count/μL)	5.55 ± 1.50	5.81 ± 1.63	<0.0001	5.7 3 ± 1.50	6.20 ± 1.73	<0.0001	5.93 ± 1.60	6.32 ± 1.66	<0.0001
RBC (10^{3}count/μL)	4.47 ± 0.37	4.49 ± 0.39	0.185	4.62 ± 0.42	4.76 ± 0.43	<0.0001	4.75 ± 0.40	4.80 ± 0.40	0.001
Platelet (103 count/μL)	257.44 ± 58.74	264.68 ± 72.64	0.001	252.42 ± 56.30	255.94 ± 67.75	0.030	245.63 ± 54.80	246.57 ± 58.44	0.639
FBS (mg/dL)	93.94 ± 18.68	97.94 ± 27.10	<0.0001	94.58 ± 17.52	101.34 ± 26.46	<0.0001	98.75 ± 22.12	105.57 ± 29.23	<0.0001
BUN (mg/dL)	14.41 ± 3.99	14.59 ± 4.40	0.224	14.36 ± 3.83	14.46 ± 3.66	0.374	14.79 ± 3.88	14.50 ± 3.97	0.04
Serum creatinine (mg/dL)	0.75 ± 0.20	0.77 ± 0.26	<0.0001	0.81 ± 0.18	0.88 ± 0.17	<0.0001	0.88 ± 0.17	0.89 ± 0.16	0.022
ALT (IU/L)	20.81 ± 14.53	21.59 ± 13.66	0.139	21.79 ± 14.95	22.58 ± 15.35	0.065	25.64 ± 19.49	26.36 ± 15.07	0.271
AST (IU/L)	22.83 ± 9.89	25.02 ± 15.11	<0.0001	22.88 ± 9.29	24.69 ± 11.65	<0.0001	25.96 ± 14.03	28.52 ± 15.50	<0.0001
γ-GTP (IU)	21.21 ± 14.13	77.31 ± 63.84	<0.0001	25.79 ± 17.65	88.64 ± 78.87	<0.0001	38.30 ± 26.99	121.80 ± 137.57	<0.0001
Total bilirubin (mg/dL)	0.69 ± 0.27	0.69 ± 0.28	0.948	0.76 ± 0.31	0.81 ± 0.34	<0.0001	0.82 ± 0.33	0.84 ± 0.33	0.182
ALP (IU/L)	178.27 ± 95.73	218.65 ± 123.05	<0.0001	170.21 ± 104.30	190.53 ± 98.46	<0.0001	168.51 ± 85.40	183.04 ± 95.69	<0.0001
Albumin (mg/dL)	4.62 ± 0.24	4.62 ± 0.28	0.992	4.65 ± 0.24	4.67 ± 0.25	<0.0001	4.67 ± 0.26	4.68 ± 0.27	0.347
Total cholesterol (mg/dL)	198.83 ± 36.00	205.99 ± 38.94	<0.0001	196.62 ± 35.16	201.42 ± 37.73	<0.0001	196.22 ± 35.10	200.94 ± 37.24	<0.0001
Triglyceride (mg/dL)	118.98 ± 76.06	136.15 ± 85.60	<0.0001	121.09 ± 82.12	170.03 ± 126.36	<0.0001	145.16 ± 108.88	199.57 ± 143.82	<0.0001
HDL-cholesterol (mg/dL)	54.52 ± 13.06	54.39 ± 13.99	0.786	55.02 ± 13.64	53.97 ± 13.96	0.007	55.12 ± 14.41	55.58 ± 13.94	0.364
Uric acid (mg/dL)	4.37 ± 1.09	4.63 ± 1.19	<0.0001	4.80 ± 1.28	5.50 ± 1.40	<0.0001	5.39 ± 1.34	5.84 ± 1.39	<0.0001
Calcium (mg/dL)	9.23 ± 0.41	9.29 ± 0.42	<0.0001	9.22 ± 0.41	9.29 ± 0.46	<0.0001	9.25 ± 0.41	9.30 ± 0.48	<0.0001

Note: ND, no drinking history; LD, alcohol consumption <210 g/week (men) or >140 g/week (women); HD, alcohol consumption >210 g/week (men) or >140 g/week (women).

Abbreviations: ALP, alkaline phosphatase; BMI, body mass index; BUN, blood urea nitrogen; DBP, diastolic blood pressure; FBS, fasting blood sugar; RBC, red blood cell; SBP, systolic blood pressure; WBC, white blood cell.

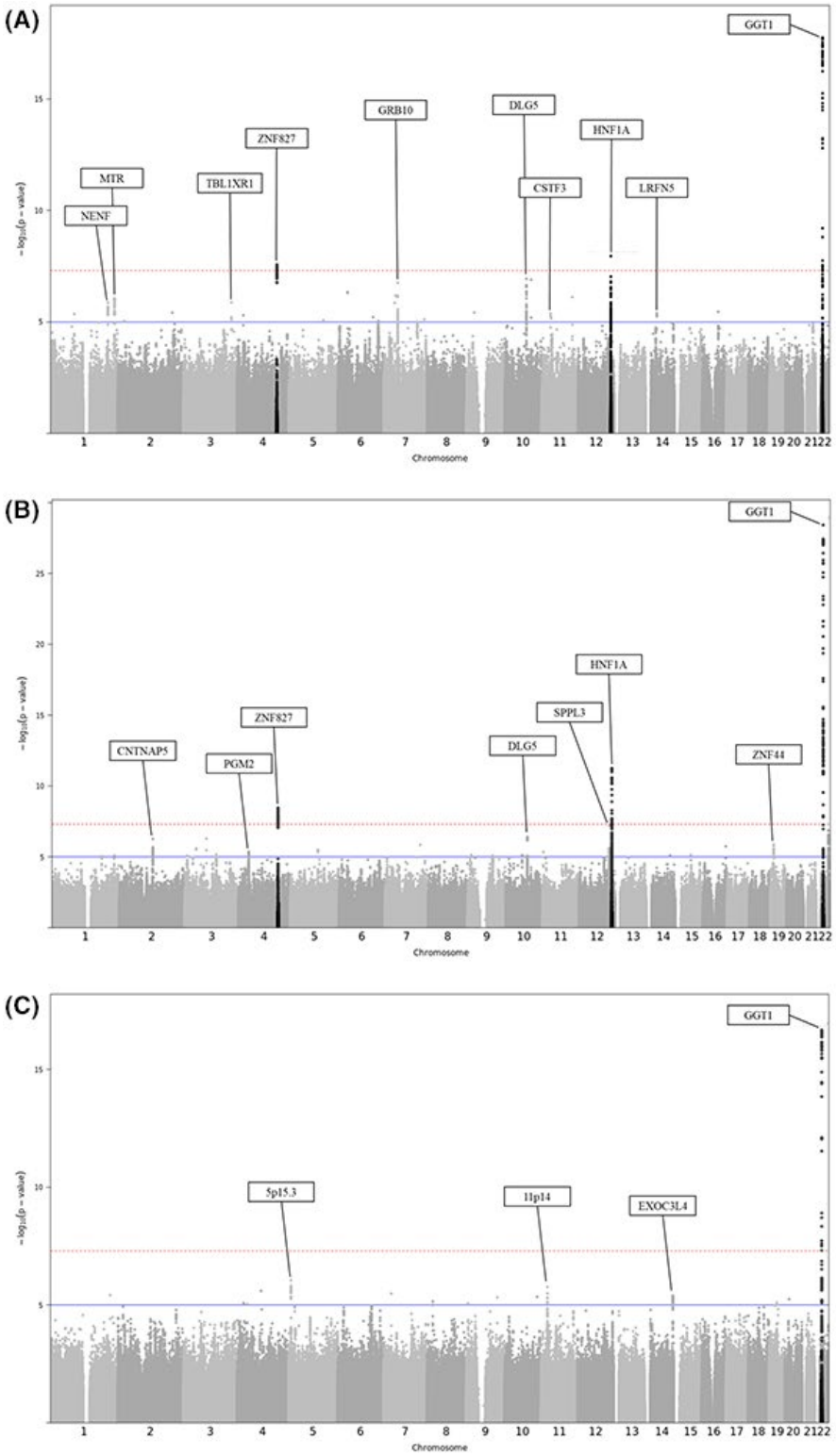

FIGURE 1 Manhattan plot by genetic locus for association with the risk of alcohol-associated liver disease in three groups (ND, LD, and HD). (A) In a GWAS of the ND, the strongest, significant signals were detected in three loci, and seven suggestive markers were detected. (B) In the LD group, three significant signals were detected in the same loci as those in the ND, and five suggestive loci were obtained. (C) In the HD, one significant locus and three suggestive loci were detected. Loci with significant associations are highlighted by black dots

that showed protective effects only in the HD group, including rs117035473 (OR = 0.534, CI = 0.405-0.704, $p = 8.76 \times 10^{-6}$), rs11027261 (OR = 0.785, CI = 0.709-0.869, $p = 3.25 \times 10^{-6}$), and rs59057498 (OR = 0.767, CI = 0.683-0.862, $p = 7.87 \times 10^{-6}$) (Supporting Digital Content 4).

TABLE 2 Genetic Loci and Candidate Genes Associated With ALD Risk in NDs, LDs, and HDs

SNP	Chr	BP	A1	MAF				Feature	Candidate Gene	OR (95% CI)	*P*
				Present Study	EAS	EUR	AMR				
ND											
rs2006227	22	24995756	A	0.3466	0.3750	0.3678	0.4481	upstream_gene_variant	*GGT1*	1.578 (1.425-1.748)	1.80E-18
rs1183910	12	121420807	A	0.4725	0.3810	0.3330	0.3300	intron_variant	*HNF1A*	0.754 (0.679-0.836)	9.33E-08
rs4835265	4	146821410	A	0.4189	0.4067	0.1610	0.3631	intron_variant	*ZNF827*	1.332 (1.203-1.474)	3.32E-08
LD											
rs2006227	22	24995756	A	0.3466	0.3750	0.3678	0.4481	upstream_gene_variant	*GGT1*	1.578 (1.454-1.712)	5.92E-28
rs1183910	12	121420807	A	0.4725	0.3810	0.3330	0.3300	intron_variant	*HNF1A*	0.752 (0.693-0.815)	5.49E-12
rs4835265	4	146821410	A	0.4189	0.4067	0.1610	0.3631	intron_variant	*ZNF827*	1.259 (1.162-1.364)	1.93E-08
HD											
rs2006227	22	24995756	A	0.3466	0.3750	0.3678	0.4481	upstream_gene_variant	*GGT1*	1.591 (1.429-1.772)	2.73E-17
rs1183910	12	121420807	A	0.4725	0.3810	0.3330	0.3300	intron_variant	*HNF1A*	0.910 (0.823-1.006)	6.62E-02
rs4835265	4	146821410	A	0.4189	0.4067	0.1610	0.3631	intron_variant	*ZNF827*	1.103 (0.994-1.222)	6.38E-02

Note: ND, no drinking history; LD, alcohol consumption <210 g/week (men) or >140 g/week (women); HD, alcohol consumption >210 g/week (men) or >140 g/week (women).

Abbreviation: AMR, American; BP, base paire; Chr, chromosome; EAS, East Asian; EUR, European; MAF, minor allele frequency.

*The case group included participants with AST:ALT > 3 or γ-GTP:ALT > 2.5 in a liver function test.

†*P* values were calculated using logistic regression.

Replication test in another published cohort

We conducted a replication analysis in another gene cohort from Korea, the Ansan-Ansung cohort, which is one of Korea's representative genome cohorts. The results of the present study are consistent with those observed in other cohorts (Table 3).

Expression of each genetic variant in various tissues using the genotype-tissue expression database

Supporting Digital Content 5 presents the results of supplementary analyses using data hosted on the genotype-tissue expression (GTEx) database. The expression of each genetic variant in various tissues has been provided. Findings based on the data from the GTEx database were similar to the results from our study, the exception being the results for *ZNF827*.

DISCUSSION

In the present study, we identified three significant loci (*GGT1*, *ZNF827*, and *HNF1A*) associated with ALD in NDs and LDs, but only *GGT1* was associated with ALD in HDs. The effect sizes of the SNPs reported in this study are depicted in Figure 2 as forest plots for each drinker group. Individuals harboring the *GGT1* rs2006227 minor allele were found to be at a higher risk of developing ALD than those harboring the major alleles in all groups (Figure 3A,B). Similar results were observed in the analysis of the *ZNF827* rs4835265 minor allele on the liver enzymes in NDs and LDs (Figure 3C,D). Furthermore, the *HNF1A* rs1183910 minor allele has protective effects on liver function; LD showed a significant decrease (Figure 3E,F). These findings support that each of the individuals has a unique genetic susceptibility to ALD and suggest that their genetic expressions may be altered by the quantity of alcohol consumed. We also identified some SNPs on chromosomes 9, 11, and 19 in HDs, which may suggest a potential role in the protective mechanism against ALD.

GGT1 encodes gamma-glutamyltransferase 1, a plasma biomarker for liver dysfunction or excessive alcohol consumption or both.[17] *GGT1* defends cells against oxidative stress by providing cysteine to maintain adequate intracellular glutathione levels in liver cells.[18] Because of its anti-oxidant property, *GGT1* has been found to be closely related to oxidative stress-related diseases, especially metabolic syndrome, cardiovascular diseases, diabetes, and ALD.[19-21] Our results revealed that the minor frequency alleles of GWAS SNPs in the *GGT1* region are consistently associated with a 1.5-fold or higher risk of liver enzyme elevation compared to the major alleles; this finding is consistent with previous findings in other populations.[22] The *GGT1* rs2006227 minor allele was most strongly associated with liver function deterioration in all drinking groups. This result suggests that the population with the *GGT1* rs2006227 minor allele is more vulnerable to liver disease, regardless of alcohol consumption.

The direct relationship between *ZNF827* and ALD has not been definitively explained. *ZNF827* provides a platform for telomere recombination in response to telomere-specific DNA damage, which may facilitate the repair of damaged liver cells.[23] There is also evidence for oxidative stress originating from ethanol consumption evoking telomere damage in hepatocytes, the process of which could induce liver cell damage.[24,25]

In the present study, the *HNF1A* rs1183910 minor allele and other SNPs in *HNF1A* demonstrated a significant protective effect against alcohol-induced liver toxicity in the LD group, which was not detected in the HD group. *HNF1A* has hepatoprotective effects due to its roles in the inflammatory pathways of liver damage. *HNF1A* regulates acute phase reactants such as C-reactive protein (CRP), fibrinogen, and IL-1A.[26] A recent study revealed that the *HNF1A* rs1183910 minor allele is related to a 13.9% reduction in CRP.[27] In addition, the hepatoprotective function of *HNF1A* may be explained in the context of its relationship with *HNF4*α and *TGF*β*1*. ALD development is characterized by down-regulation of liver-enriched transcription factor, which is most represented by *HNF4*α. Alcohol drinking up-regulates *TGF*β*1*, which inhibits *HNF4*α and *HNF4*α-dependent genes.[28] *HNF1A* can promote *HNF4*α expression by suppressing *TGF*β*1*.

Additionally, we identified potential SNPs that were not statistically significant but may play a protective role in the HD group. Six SNPs located in the intergenic regions of chromosome 11, two SNPs on chromosome 19, and one SNP on chromosome 9 demonstrated a protective effect in hepatocytes against alcohol in the HD group (Supporting Digital Content 4). In particular, several suggestive SNPs on chromosome 11 formed a cluster. The suggestive SNPs were located in an intergenic region; however, the ±1 Mbp region around the SNPs included three functional genes, *GAS2* (*growth arrest specific 2*), *SVIP* (*small VCP interacting protein*), and *CCDC179 (coiled-coil domain-containing protein 179*), as well as 13 SNPs in the GWAS catalogue. The function of *CCDC179* is difficult to evaluate with respect to alcohol consumption or ALD because of the lack of relevant research. However, *GAS2* and *SVIP* are predominantly expressed in the liver. In particular, *GAS2* is involved in p53/*extracellular signal-regulated kinase* signaling,[29,30] affecting the cell cycle, and is

TABLE 3 Replication analysis in other gene cohort in Korea, the Ansan-Ansung cohort, Korea's representative genome cohorts

				MAF						City Cohort		Ansan-Ansung Cohort	
SNP	Chr	BP	A1	Present Study	EAS	EUR	AMR	Feature	Candidate Gene	OR (95% CI)	*P*	OR (95% CI)	*P*
ND													
rs4835265	4	146821410	A	0.4189	0.4067	0.1610	0.3631	intron_variant	*ZNF827*	1.332 (1.203-1.474)	3.32.E-08	1.018 (0.741-1.400)	9.10.E-01
rs1183910	12	121420807	A	0.4725	0.3810	0.3330	0.3300	intron_variant	*HNF1A*	0.754 (0.679-0.836)	9.33.E-08	0.700 (0.508-0.965)	2.93.E-02
rs2006227	22	24995756	A	0.3466	0.3750	0.3678	0.4481	upstream_ gene_variant	*GGT1*	1.578 (1.425-1.748)	1.80.E-18	1.196 (0.867-1.650)	2.77.E-01
LD													
rs4835265	4	146821410	A	0.4189	0.4067	0.1610	0.3631	intron_variant	*ZNF827*	1.259 (1.162-1.364)	1.93.E-08	1.225 (0.945-1.589)	1.26.E-01
rs1183910	12	121420807	A	0.4725	0.3810	0.3330	0.3300	intron_variant	*HNF1A*	0.752 (0.693-0.815)	5.49.E-12	0.702 (0.542-0.909)	7.18.E-03
rs2006227	22	24995756	A	0.3466	0.3750	0.3678	0.4481	upstream_ gene_variant	*GGT1*	1.578 (1.454-1.712)	5.92.E-28	1.589 (1.318-1.917)	1.24.E-06
HD													
rs4835265	4	146821410	A	0.4189	0.4067	0.1610	0.3631	intron_variant	*ZNF827*	1.103 (0.994-1.222)	6.38.E-02	1.087 (0.902-1.310)	3.95.E-01
rs1183910	12	121420807	A	0.4725	0.3810	0.3330	0.3300	intron_variant	*HNF1A*	0.910 (0.823-1.006)	6.62.E-02	0.921 (0.761-1.114)	3.82.E-01
rs2006227	22	24995756	A	0.3466	0.3750	0.3678	0.4481	upstream_ gene_variant	*GGT1*	1.591 (1.429-1.772)	2.73.E-17	1.670 (1.267-2.202)	2.76.E-04

Abbreviations: AMR, American; BP, base paire; Chr, chromosome; EAS, East Asian; EUR, European; MAF, minor allele frequency.

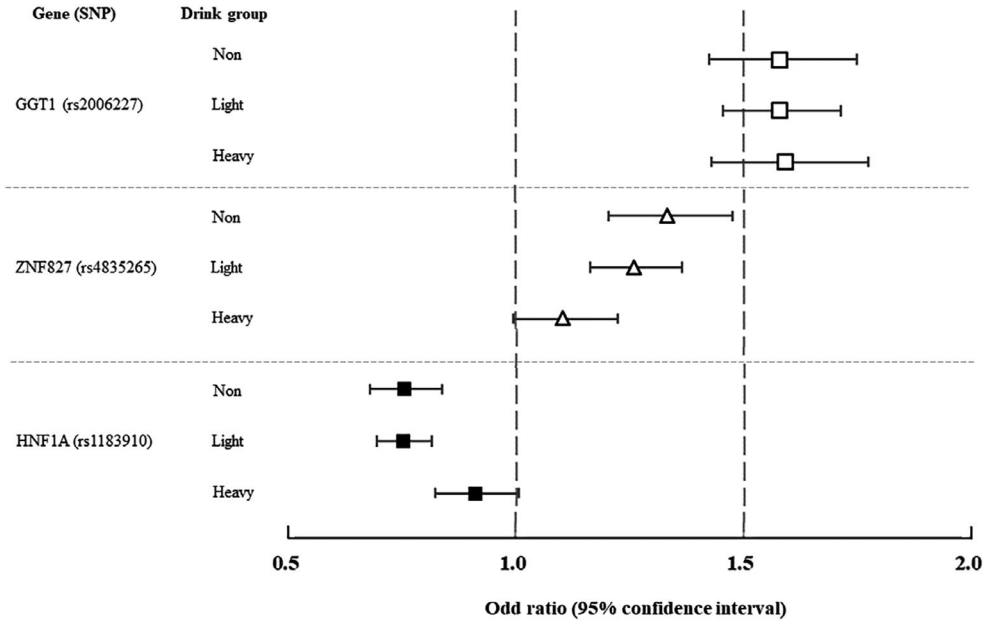

FIGURE 2 Forest plot comparing the risk for ALD of representative SNPs of GGT1, ZNF827 and HNF1A for all drinker groups

associated with hepatocarcinogenesis.[30] These properties of *GAS2*, which functions in apoptosis and is expressed in the liver, may contribute to the mechanism underlying liver damage caused by alcohol consumption and genetic variation. *SVIP* is responsible for proteolysis in the endoplasmic reticulum[31] and is related to an innate immune system pathway.[32]

One of the main findings of this study is that the amount of alcohol consumption can shift genetic susceptibility. Alcohol consumption makes alteration of DNA methylation and alcohol-induced DNA methylation more robust in HDs.[33] This alteration is reversible because the level of DNA methylation induced by alcohol decreases after reducing drinking.[34]

A remarkable finding of this study is that genes like *PNPLA3* and *MBOAT7* that have been previously associated with the development of ALD are not the most important risk factors. We checked whether a linkage disequilibrium relationship exists between *GGT1* and *PNPLA3* but found no such relationship (see Supporting Digital Content 6). To demonstrate the detailed association between ALD and *PNPLA3*, we expanded a regional plot in chromosome 22. On analysis, minor allele rs738409 on *PNPLA3* appeared significant in the LD group (OR = 0.8713, CI = 0.7401–0.8713, $p = 1.39 \times 10^{-7}$) (see Supporting Digital Content 7). We thought that it is very likely that *PNPLA3* may be involved in the phenotype expression of ALD but were not absolutely certain. When we reviewed a meta-analysis, the involvement of *PNPLA3* showed weak *P* values.[6] We also analyzed the role played by *MBOAT7* in the development of ALD using the same method as that used for *PNPLA3*, and found that *MBOAT7* rs8736 was not significantly associated with ALD development (see Supporting Digital Content 8). Difference in study design and methodology may contribute to these differences. Although GWAS has been previously conducted on controls and patients with alcohol-associated liver cirrhosis, we conducted it in controls and patients with ALD who were stratified based on the amount of alcohol consumption. Our findings show that genetic vulnerability toward ALD development can vary with the amount of alcohol consumed. Furthermore, difference in methodology may be the reason why a weak association was identified between ALD development and *PNPLA3* and *MBOAT7* in this study, in contrast to the findings of previous studies in which *PNPLA3* and *MBOAT7* were identified as the most promising candidate genes responsible for ALD development. Tian et al. conducted the most representative PNPLA3 GWAS study and used a broader criteria for defining and classifying ALD compared with that used in our study.[7]

Although GWAS is a useful approach to investigate the genetic variation contributing to the development of ALD, our study is limited in that we did not conduct experimental validation that demonstrates gene expression in hepatocytes. We attempted to develop an in vitro model similar to our research model. However, we faced difficulty in simulating chronic alcohol exposure with hepatocytes in vitro during a time frame similar to that of our study observations; it was also difficult to obtain hepatic tissue samples through biopsy from the vast number of study subjects (58,701). Therefore, we adopted another methodology that involved the use of the GTEx database instead of functional studies based on the use of human hepatocytes. Findings based on the data from the GTEx database were similar to the

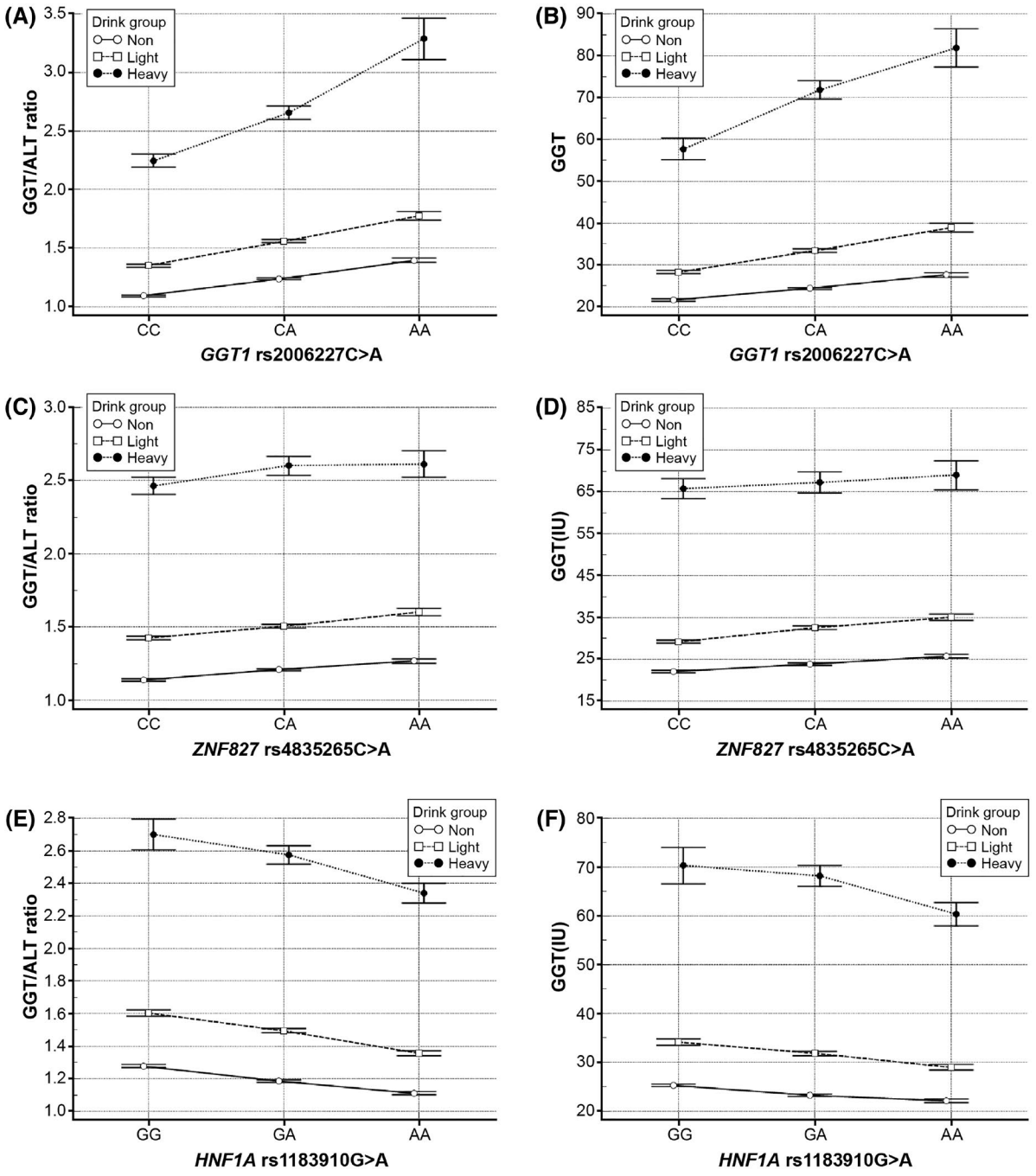

FIGURE 3 Changes in liver enzymes related to ALD compared with different allele types. (A) The minor type of rs2006227(AA) in GGT1 shows the highest GGT/ALT ratio compared to the major allele types (CC or CA) (B) The minor type of rs2006227(AA) in GGT1 shows the highest GGT value compared to the major types of alleles (CC or CA) (C) The minor type of rs4385265(AA) in ZNF827 shows the highest GGT/ALT ratio compared to the major allele types (CC or CA) (D) The minor type of rs4385265(AA) in ZNF827 shows the highest GGT value compared to the major allele types (CC or CA) (E) The minor type of rs1183910(AA) in HNF1A shows the highest GGT/ALT ratio compared to the major allele types (GG or GA) (F) The minor type of rs1183910(AA) in HNF1A shows the highest GGT value compared to the major allele types (GG or GA)

results described in our study. However, GTEx may not be completely able to explain our results. The absence of functional research results can be pointed out as an important limitation of our study, and future functional studies using in vivo or in vitro models will be needed to compensate for this limitation.

Overall, using a large-scale population, we showed that during ALD development, the alterations in gene expression are a function of the amount of alcohol consumed. The *HNF1A* rs1183910 minor allele exhibited a protective effect against ALD in the background of low levels of alcohol consumption;

however, this effect does not extend to individuals with excessive alcohol consumption. We found that *HNF1A* regulates the inflammatory cascade, and the *HNF4α* and *TGFβ1* pathways can provide a critical insight into the contribution of gene expression to ALD. SNPs including the *HNF1A* rs1183910 minor allele were the most promising genetic candidates for conferring protection against ALD. In conclusion, the alteration in the expression of genes contributing to ALD development may be a function of the amount of alcohol consumed.

CONFLICT OF INTEREST

Nothing to report.

ETHICS STATEMENT

The research was conducted in accordance with the principles described in the Declaration of Helsinki. The Institutional Review Board of Theragen Etex Bio Institute approved this study (internal review board No.: 700062-20190819-GP-006-02), and all participants provided written informed consent.

AUTHOR CONTRIBUTIONS

Kyung-Won Hong, Bom Taeck Kim, and Kwang Yoon Kim contributed to the research design. Jung Oh Kim, Ja-Eun Choi, and Da-Hyun Park contributed to the statistical analysis. Kwang Yoon Kim, Jung Oh Kim, and Kyung-Won Hong contributed to the genomic interpretation. Kwang Yoon Kim, Bom Taeck Kim, Young-Sang Kim, Kunhee Han, and Yon Chul Park contributed to the clinical interpretation. Kwang Yoon Kim, Jung Oh Kim, Kyung-Won Hong, and Bom Taeck Kim contributed to the manuscript draft. Bom Taeck Kim, Kyung-Won Hong, Young-Sang Kim, Kunhee Han, and Jae-Min Park contributed to the manuscript review.

ORCID

Kwang Yoon Kim https://orcid.org/0000-0001-9844-9540
Young-Sang Kim https://orcid.org/0000-0002-7397-5410
Jae-Min Park https://orcid.org/0000-0001-8873-8832
Kunhee Han https://orcid.org/0000-0001-8307-7692
Yon Chul Park https://orcid.org/0000-0002-1787-0884
Bom Taeck Kim https://orcid.org/0000-0002-0395-0410
Kyung-Won Hong https://orcid.org/0000-0002-3137-4609

REFERENCES

1. Yoon YH, Chen CM. Surveillance Report #105. Liver cirrhosis mortality in the United States: national, state, and regional trends, 2000-13. National Institute on Alcohol Abuse and Alcoholism, 2016. [Cited 2021 Oct 24]. Available from: https://pubs.niaaa.nih.gov/publications/surveillance105/Cirr13.pdf.
2. Lucey MR, Mathurin P, Morgan TR. Alcoholic hepatitis. N Engl J Med. 2009;360:2758–69.
3. Stickel F, Datz C, Hampe J, Bataller R. Pathophysiology and management of alcoholic liver disease: Update 2016. Gut Liv. 2017;11:173–88.
4. Maher JJ. Exploring alcohol's effects on liver function. Alcohol Health Res World. 1997;21:5–12.
5. Buch S, Stickel F, Trépo E, Way M, Herrmann A, Nischalke HD, et al. A genome-wide association study confirms PNPLA3 and identifies TM6SF2 and MBOAT7 as risk loci for alcohol-related cirrhosis. Nat Genet. 2015;47:1443–8.
6. Stickel F, Hampe J, Trépo E, Datz C, Romeo S. PNPLA3 genetic variation in alcoholic steatosis and liver disease progression. Hepatobiliary Surg Nutr. 2015;4:152–60.
7. Tian C, Stokowski RP, Kershenobich D, Ballinger DG, Hinds DA, et al. Variant in PNPLA3 is associated with alcoholic liver disease. Nat Genet. 2010;42:21–3.
8. Meroni M, Longo M, Rametta R, Dongiovanni P. Genetic and epigenetic modifiers of alcoholic liver disease. Int J Mol Sci. 2018;19:3857.
9. Mancina RM, Dongiovanni P, Petta S, Pingitore P, Meroni M, Rametta R, et al. The MBOAT7-TMC4 variant rs641738 increases risk of nonalcoholic fatty liver disease in individuals of European descent. Gastroenterology. 2016;150:1219–30.
10. Stewart SF, Leathart JB, Chen Y, Daly AK, Rolla R, Vay D, et al. Valine-alanine manganese superoxide dismutase polymorphism is not associated with alcohol-induced oxidative stress or liver fibrosis. Hepatology. 2002;36:1355–60.
11. Xu T, Li LI, Hu H-Q, Meng X-M, Huang C, Zhang L, et al. MicroRNAs in alcoholic liver disease: recent advances and future applications. J Cell Physiol. 2018;234:382–94.
12. Kim Y, Han BG, KoGES Group. Cohort profile: The Korean genome and epidemiology study (KoGES) consortium. Int J Epidemiol. 2017;46:1–10.
13. Kwo PY, Cohen SM, Lim JK. ACG clinical guideline: evaluation of abnormal liver chemistries. Am J Gastroenterol. 2017;112:18–35.
14. Moon S, Kim YJ, Han S, Hwang MY, Shin DM, Park MY, et al. The Korea biobank array: design and identification of coding variants associated with blood biochemical traits. Sci Rep. 2019;9:1382.
15. The Korean Association for the Study of the Liver. KASL clinical practice guidelines: management of nonalcoholic fatty liver disease. Clin Mol Hepatol. 2013;19:325–48.
16. Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, et al. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology and the American Gastroenterological Association. Hepatology. 2012;55:2005–23.
17. Xu X, Qin L, Tian YE, Wang M, Li G, Du Y, et al. Family-based analysis of GGT1 and HNF1A gene polymorphisms in patients with polycystic ovary syndrome. Reprod BioMed Online. 2018;36:11–9.
18. Hanigan MH, Ricketts WA. Extracellular glutathione is a source of cysteine for cells that express gamma-glutamyl transpeptidase. Biochemistry. 1993;32:6302–6.
19. Lee DS, Evans JC, Robins SJ, Wilson PW, Albano I, Fox CS, et al. Gamma glutamyl transferase and metabolic syndrome, cardiovascular disease, and mortality risk: the Framingham heart study. Arterioscler Thromb Vasc Biol. 2007;27:127–33.
20. Sabanayagam C, Shankar A, Li J, Pollard C, Ducatman A. Serum gamma-glutamyl transferase level and diabetes mellitus among US adults. Eur J Epidemiol. 2009;24:369–73.
21. Teschke R, Rauen J, Neuefeind M, Petrides AS, Strohmeyer G. Alcoholic liver disease associated with increased gamma-glutamyltransferase activities in serum and liver. Adv Exp Med Biol. 1980;132:647–54.

22. 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. Nat Genet. 2011;43:1131–8.
23. Pickett HA, Reddel RR. Molecular mechanisms of activity and derepression of alternative lengthening of telomeres. Nat Struct Mol Biol. 2015;22:875–80.
24. Barnard A, Moch A, Saab S. Relationship between telomere maintenance and liver disease. Gut Liv. 2019;13:11–15.
25. Harpaz T, Abumock H, Beery E, Edel Y, Lahav M, Rozovski U, et al. The effect of ethanol on telomere dynamics and regulation in human cells. Cells. 2018;7:169.
26. Armendariz AD, Krauss RM. Hepatic nuclear factor 1-a: inflammation, genetics, and atherosclerosis. Curr Opin Lipidol. 2009;20:106–11.
27. Elliott P, Chambers JC, Zhang W, et al. Genetic loci associated with C-reactive protein levels and risk of coronary heart disease. J Am Med Assoc. 2009;302:37–48.
28. Argemi J, Latasa MU, Atkinson SR, Blokhin IO, Massey V, Gue JP, et al. Defective HNF4alpha-dependent gene expression as a driver of hepatocellular failure in alcoholic hepatitis. Nat Commun. 2019;10:3126.
29. Benetti R, Del Sal G, Monte M, Paroni G, Brancolini C, Schneider C. The death substrate GAS2 binds m-calpain and increases susceptibility to p53-dependent apoptosis. EMBO J. 2001;20:2702–14.
30. Zhu R, Mok MTS, Kang W, Lau SSK, Yip W-K, Chen Y, et al. Truncated HBx-dependent silencing of GAS2 promotes hepatocarcinogenesis through deregulation of cell cycle, senescence and p53-mediated apoptosis. J Pathol. 2015;237:38–49.
31. Li X, Tran KM, Aziz KE, Sorokin AV, Chen J, Wang W. Defining the protein–protein interaction network of the human protein tyrosine phosphatase family. Mol Cell Proteomics. 2016;15:3030–44.
32. Buschow SI, Van Balkom BWM, Aalberts M, Heck AJ, Wauben M, Stoorvogel W. MHC class II-associated proteins in B-cell exosomes and potential functional implications for exosome biogenesis. Immunol Cell Biol. 2010;88:851–6.
33. Dugué P-A, Wilson R, Lehne B, Jayasekara H, Wang X, Jung C-H, et al. Alcohol consumption is associated with widespread changes in blood DNA methylation: analysis of cross-sectional and longitudinal data. Addict Biol. 2019;26:e12855.
34. Tammen SA, Park LK, Dolnikowski GG, Ausman LM, Friso S, Choi SW. Hepatic DNA hydroxymethylation is site-specifically altered by chronic alcohol consumption and aging. Eur J Nutr. 2017;56:535–44.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.
Supplementary Material

How to cite this article: Kim KY, Kim JO, Kim Y-S, Choi J-E, Park J-M, Han K, et al. Genome-wide association of individual vulnerability with alcohol-associated liver disease: A Korean genome and epidemiology study. Hepatology. 2022;75:391–402. doi:10.1002/hep.32115