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

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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 association with ALD.

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.

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**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. 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, 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.

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), *membrane bound O-acyltransferase domain (MBOAT7)* containing 7-transmembrane channel-like 4, and the nonsynonymous variant of *superoxide dismutase 2*, in addition to several microRNAs (miRs), such as miR-155, miR-34a, miR-122, and miR-21.

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 consumed, 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.

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. 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). 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.

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.
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.\textsuperscript{[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 $\pm 10$ 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 ($5 \times 10^{-8} \leq p < 1 \times 10^{-5}$) (Supporting Digital Content 2). The most significant SNP was rs2006227 (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 \textit{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 \textit{ZNF827} region of chromosome 4). The top GWAS SNP in the \textit{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, $p = 3.23 \times 10^{-18}$; 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 \textit{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), and 85 GWAS SNPs were located in the same locus (i.e., the \textit{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 rs4835265 (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 \textit{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 \textit{ZNF827} region of chromosome 4). The top GWAS SNP in the \textit{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, $p = 3.23 \times 10^{-18}$; Supporting Digital Content 3). A comparison between the ND and LD results, including rs4835265, is given in Table 2.

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 \textit{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 \textit{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 \textit{ZNF827} region of chromosome 4). The top GWAS SNP in the \textit{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, $p = 3.23 \times 10^{-18}$; Supporting Digital Content 3). A comparison between the ND and LD results, including rs4835265, is given in Table 2.
| 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/m²)              | 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 (10³ count/µL)       | 5.55 ± 1.50          | 5.81 ± 1.63   | <0.0001 | 5.73 ± 1.50          | 6.20 ± 1.73      | <0.0001 | 5.93 ± 1.60         | 6.32 ± 1.66      | <0.0001 |
| RBC (10³ 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      | 0.01  |
| 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.
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).

**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.
| SNP     | Chr | BP       | A1 | Present Study | EAS   | EUR   | AMR   | Feature               | Candidate Gene | OR (95% CI)          | P         |
|---------|-----|----------|----|---------------|-------|-------|-------|------------------------|----------------|----------------------|-----------|
| 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 pair; 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.
1P 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 HNF4α and TGFβ1. ALD development is characterized by down-regulation of liver-enriched transcription factor, which is most represented by HNF4α. Alcohol drinking up-regulates TGFβ1, which inhibits HNF4α and HNF4α-dependent genes.[28] HNF1A can promote HNF4α expression by suppressing TGFβ1.

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

| 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       | 3.32E-08 | 1.018       | 9.10E-01 |
| rs1183910 | 12  | 121420807 | A     | 0.4725        | 0.3810 | 0.3300 | 0.3300 | intron_variant       | HNF1A          | 0.754       | 9.33E-08 | 0.700       | 2.93E-02 |
| rs2006227 | 22  | 24995756  | A     | 0.3466        | 0.3750 | 0.3678 | 0.4481 | upstream_gene_variant | GGT1           | 1.578       | 1.80E-18 | 1.196       | 2.77E-01 |
| LD       |     |           |       |               |     |     |     |                     |                |             |       |             |       |
| rs4835265 | 4   | 146821410 | A     | 0.4189        | 0.4067 | 0.1610 | 0.3631 | intron_variant       | ZNF827         | 1.259       | 1.93E-08 | 1.225       | 1.26E-01 |
| rs1183910 | 12  | 121420807 | A     | 0.4725        | 0.3810 | 0.3300 | 0.3300 | intron_variant       | HNF1A          | 0.752       | 5.49E-12 | 0.702       | 7.18E-03 |
| rs2006227 | 22  | 24995756  | A     | 0.3466        | 0.3750 | 0.3678 | 0.4481 | upstream_gene_variant | GGT1           | 1.578       | 5.92E-28 | 1.589       | 1.24E-06 |
| HD       |     |           |       |               |     |     |     |                     |                |             |       |             |       |
| rs4835265 | 4   | 146821410 | A     | 0.4189        | 0.4067 | 0.1610 | 0.3631 | intron_variant       | ZNF827         | 1.103       | 6.38E-02 | 1.087       | 3.95E-01 |
| rs1183910 | 12  | 121420807 | A     | 0.4725        | 0.3810 | 0.3300 | 0.3300 | intron_variant       | HNF1A          | 0.910       | 6.62E-02 | 0.921       | 3.82E-01 |
| rs2006227 | 22  | 24995756  | A     | 0.3466        | 0.3750 | 0.3678 | 0.4481 | upstream_gene_variant | GGT1           | 1.591       | 2.73E-17 | 1.670       | 2.76E-04 |

Abbreviations: AMR, American; BP, base pair; Chr, chromosome; EAS, East Asian; EUR, European; MAF, minor allele frequency.
associated with hepatocarcinogenesis.\textsuperscript{[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\textsuperscript{[31]} and is related to an innate immune system pathway.\textsuperscript{[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.\textsuperscript{[33]} This alteration is reversible because the level of DNA methylation induced by alcohol decreases after reducing drinking.\textsuperscript{[34]}

A remarkable finding of this study is that genes like \textit{PNPLA3} and \textit{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 \textit{PNPLA3}, we expanded a regional plot in chromosome 22. On analysis, minor allele rs738409 on \textit{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 \textit{PNPLA3} may be involved in the phenotype expression of ALD but were not absolutely certain. When we reviewed a meta-analysis, the involvement of \textit{PNPLA3} showed weak \( P \) values.\textsuperscript{[6]} We also analyzed the role played by \textit{MBOAT7} in the development of ALD using the same method as that used for \textit{PNPLA3}, and found that \textit{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 \textit{PNPLA3} and \textit{MBOAT7} in this study, in contrast to the findings of previous studies in which \textit{PNPLA3} and \textit{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.\textsuperscript{[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

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig2}
\caption{Forest plot comparing the risk for ALD of representative SNPs of GGT1, ZNF827 and HNF1A for all drinker groups.}
\end{figure}
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;

**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)
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.

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SUPPORTING INFORMATION
Additional supporting information may be found in the online version of the article at the publisher’s website.

Supplementary Material

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