Association of Genetic Polymorphisms With Hepatitis C Virus-related Liver Cirrhosis in Japan

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Abstract. Background/Aim: Hepatitis C virus (HCV) infection is an important health problem in the direct-acting antivirals-era. HCV causes life-threatening diseases, such as cirrhosis and hepatocellular carcinoma. Our aim was to examine whether certain single-nucleotide polymorphisms (SNPs) are associated with the prevalence of HCV infections progressing to cirrhosis in the Japanese population by a genome-wide association study-based approach. Materials and Methods: We used DNA extracted from blood specimens of Japanese subjects with the establishment of the BioBank Japan project. Results: We observed statistically significant differences in the frequency of 4 SNPs (rs1989972, rs2293766, rs1877033 and rs4805439) between anti-HCV-positive cirrhotic patients and controls. Conclusion: Four SNPs are associated with susceptibility to cirrhosis among HCV-infected Japanese subjects, while further studies with cohorts other than those sourced from BioBank Japan, must be conducted.

Hepatitis C virus (HCV) infection is an important health problem in Japan and the United States, although direct-acting antivirals (DAAs) against HCV have been introduced (1, 2). HCV infection causes acute and chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC) for hepatic manifestations and cryoglobulinemia, lymphoma, insulin resistance, type 2 diabetes and chronic kidney diseases for extrahepatic manifestations (3, 4). HCC occurs in ~7% of patients with cirrhosis per year, although HCC occurs in less than 1% of patients with non-advanced liver fibrosis per year (3).

Patients infected with HCV do not always develop cirrhosis (5). It is well known that the frequencies of cirrhosis and HCC are different among different races (6, 7). These facts suggest that certain single-nucleotide polymorphisms (SNPs) affect the development of hepatic fibrosis in patients with HCV infection. In the United States, subjects with chronic hepatitis C carrying the DEAD box polypeptide 5 (DDX5) minor allele or DDX5- DNA polymerase gamma 2, accessory subunit (POLG2) haplotypes are at increased risk of developing advanced fibrosis, whereas those carrying the carnitine palmitoyltransferase 1A (CPT1A) minor allele are at decreased risk (8).

It has been reported that compared to a major, wild-type (WT) CC allele, the minor allele rs4986791 in the TLR4 gene encoding the T399I change, confers protection against fibrosis progression, along with another highly cosegregated SNP (rs4986790) located at codon position 299 (p.D299G) (9), although the TLR4 SNPs (rs4986790 and rs4986791) are uniformly distributed in Japanese patients (10).

In the present study, we examined whether certain SNPs are associated with the prevalence of HCV infections progressing to cirrhosis in the Japanese population by a genome-wide association study (GWAS)-based approach. We used DNA extracted from blood specimens of Japanese subjects with the establishment of the BioBank Japan project that stores and maintains a number of annotated liver disease cases (11). We observed that four SNPs are associated with susceptibility to cirrhosis among HCV infected Japanese subjects.

Materials and Methods

Subjects, DNA preparation and SNP genotyping. Study 1. We selected patients with cirrhosis (n=195; male/female: 107/88; age, 65±9 years) and control subjects who did not have liver diseases (n=1,553; male/female: 889/664; age, 61±18 years). The HCV infection status of patients with cirrhosis was: 80, positive; 25, negative; and 90, unknown. Control subjects were also enrolled as patients with diseases other than liver diseases in BioBank Japan (11). Patients who had cirrhosis were also excluded from these controls. A GWAS was performed with high-density oligonucleotide arrays (Perlegen Sciences, Santa Clara, CA, USA) for SNP genotyping.
Study 2. We selected patients with cirrhosis (n=753; male/female: 431/322; age, 63±10.7 years) and control subjects who did not have liver diseases (n=1,358; male/female: 738/620; age, 59±13.5 years). The HCV infection status of patients with cirrhosis was: 95, positive; 110, negative; and 548, unknown. Control subjects were enrolled as patients with diseases other than liver diseases in BioBank Japan (11). Patients who had cirrhosis were also excluded from these controls. A GWAS was performed with a GeneChip SNP array (Affymetrix, Santa Clara, CA, USA) for SNP genotyping.

Study 3. We performed 29 SNP analyses of anti-HCV-positive patients with cirrhosis (n=80; male/female: 45/35; age, 65±11 years). DNA samples were genotyped with a TaqMan SNP genotyping assay (Applied Biosystems Inc, Foster City, CA, USA) using an ABI 7500 Fast real-time PCR system, according to the manufacturer's recommended protocols (10). The PCR was performed as follows: 95°C for 10 min, followed by 55 cycles of 95°C for 15 sec and 60°C for 1 min. The subjects did not have HCC at blood sample collection. SNP alleles of Japanese healthy subjects (HapMap-JPT) were used as controls from the International HapMap Project (https://hapmap.ncbi.nlm.nih.gov/index.html) (12).

BioBank Japan was launched in 2003, establishing a large Japanese patient-oriented biobank to contribute to the implementation of personalized medicine (11). All patients participating in the present study provided written informed consent and the study protocol was approved by the ethics committees of RIKEN Yokohama Institute and of each participating institution. The study protocol for study 3 was also approved by the Ethics Committee of Nihon University School of Medicine (i-1) and conformed to the ethical guidelines of the Declaration of Helsinki.

Statistical analysis. Statistical analyses were performed using a Fisher's exact test. p<0.05 was considered as a statistically significant difference. Statistical analysis was performed with SPSS v.15 (SPSS Inc, Chicago, IL, USA). We analyzed the differences between the case and control groups in terms of the distribution of genotypes with an allelic model by using a Cox's proposal hazards model analysis.

Results

Candidate SNPs associated with cirrhosis selected by the GWAS. A GWAS was performed with high-density oligonucleotide arrays (Perlegen Sciences, Santa Clara, CA, USA), resulting in the selection of 233,820 SNPs for...
genotyping, at the first stage. A GWAS was performed with a GeneChip SNP array (Affymetrix, Santa Clara, CA, USA) including 233,820 SNPs, resulting in the selection of 2,670 SNPs for genotyping, at the second stage. Finally, we selected 29 highly ranked SNPs as candidates associated with cirrhosis (Table I).

Association of SNPs with HCV infection and cirrhosis in Japanese subjects. Table II shows the frequencies of these SNPs in 80 anti-HCV-positive patients with cirrhosis. We observed the statistically significant differences in the frequencies of 4 SNPs (rs1989972, rs2293766, rs1877033 and rs4805439) between anti-HCV-positive cirrhotic patients and controls (Tables II-VI). Two SNPs (rs1989972 and rs2293766) were located within an intron of transforming growth factor beta induced (TGFBI) and zonadhesin (ZAN), respectively. The other 2 SNPs (rs1877033 and rs4805439) were located outside the protein coding regions. Together, we identified four loci conferring susceptibility to cirrhosis among HCV-infected subjects.

Discussion

In the present study, we used the collection of GWAS data and DNA from HCV-infected cirrhotic individuals and controls from BioBank Japan (11) and used the data of SNP alleles of Japanese healthy subjects (HapMap-JPT) as a control from the International HapMap Project (12). We identified the involvement of four loci in conferring susceptibility to cirrhosis among HCV-infected subjects.

TGFBI is one of the useful markers to diagnose hepatitis B virus (HBV)-related cirrhosis (13). Plasma proteome profiling revealed a strong association between TGFBI, dipeptidyl peptidase 4 (DPP4), alanyl aminopeptidase, membrane (ANPEP), polymeric immunoglobulin receptor (PIGR) and apolipoprotein E (APOE) and nonalcoholic fatty liver disease.

### Table II. Frequencies of single-nucleotide polymorphism (SNP) rs1989972 between anti-hepatitis C virus (HCV)-positive subjects with liver cirrhosis (LC) and controls.

| Allele | LC  | Control | OR  | 95% CI       | p-Value |
|--------|-----|---------|-----|--------------|---------|
| C/C    | 33  | 22      | –   | –            | 0.048   |
| C/A    | 35  | 54      |     |              |         |
| A/A    | 12  | 10      |     |              |         |
| C/C    | 33  | 22      | 2.04| 1.06-3.94    | 0.047   |
| A/A    | 12  | 10      | 0.75| 0.3-1.84     | 0.648   |
| Others | 47  | 64      |     |              |         |
| Others | 68  | 76      |     |              |         |
| Allele C | 104 | 98      | 1.33| 0.86-2.07    | 0.264   |
| Allele A | 59  | 74      |     |              |         |

OR, Odds ratio; 95% CI, 95% confidence interval; *p*<0.05, statistically significant difference by Fisher’s exact test.

### Table V. Frequencies of single-nucleotide polymorphism (SNP) rs1877033 between anti-hepatitis C virus (HCV)-positive subjects with liver cirrhosis (LC) and controls.

| Allele | LC  | Control | OR  | 95% CI       | p-Value |
|--------|-----|---------|-----|--------------|---------|
| T/C    | 33  | 25      | –   | –            | 0.085   |
| T/C    | 28  | 43      |     |              |         |
| C/C    | 13  | 18      |     |              |         |
| T/T    | 35  | 25      | 2.08| 1.09-3.98    | 0.034   |
| Others | 41  | 61      |     |              |         |
| Others | 63  | 68      |     |              |         |
| Allele T | 98  | 93      | 1.54| 0.99-2.41    | 0.149   |
| Allele C | 54  | 79      |     |              |         |

OR, Odds ratio; 95% CI, 95% confidence interval; *p*<0.05, statistically significant difference by Fisher’s exact test.

### Table VI. Frequencies of single-nucleotide polymorphism (SNP) rs4805439 between anti-hepatitis C virus (HCV)-positive subjects with liver cirrhosis (LC) and controls.

| Allele | LC  | Control | OR  | 95% CI       | p-Value |
|--------|-----|---------|-----|--------------|---------|
| G/G    | 53  | 24      | –   | –            | 0.052   |
| G/A    | 17  | 15      |     |              |         |
| A/A    | 2   | 5       |     |              |         |
| G/G    | 53  | 24      | 2.32| 1.05-5.13    | 0.044   |
| Others | 19  | 20      |     |              |         |
| Others | 70  | 39      | 3.59| 0.63-20.5    | 0.103   |
| Allele G | 123 | 63      | 2.32| 1.21-4.47    | 0.017   |
| Allele A | 21  | 25      |     |              |         |

OR, Odds ratio; 95% CI, 95% confidence interval; *p*<0.05, statistically significant difference by Fisher’s exact test.
Fibroblast growth factor 19 (FGF-19) treatment increased the expression of proteins known to drive proliferation [TGFBI, vascular cell adhesion molecule-1 (VCAM-1), Annexin A2 and high density lipoprotein binding protein (HDLBP)] (15). It is well known that FGF is also a potent angiogenic molecule involved in HCC progression (17). Recently, Kim et al. reported that FGF-2 and its receptor SNPs are associated with the survival of patients with HBV-related HCC (18). Although rs1989972 is located within an intron of TGFBI, we observed a weak association between rs1989972 C/C and patients with HCV infection and cirrhosis (Table III).

ZAN is a mosaic-type protein that localizes to the apical head of spermatozoa (19). The association between ZAN and hepatic fibrosis is unclear. Although rs2293766 is located within the ZAN-coding region, we observed that an association between rs2293766 T/T and patients with HCV infection and cirrhosis (Table IV).

Two SNPs (rs1877033 and rs4805439) were located outside the protein-coding regions, and these SNPs are associated with patients with HCV infection and cirrhosis (Table V and VI). It has been reported that noncoding RNAs (such as microRNAs and long noncoding RNAs) are involved in hepatic fibrosis (20, 21). Further studies are needed.

Although treatment with DAAs can reduce Model for End-Stage Liver Disease (MELD) and Child-Pugh-Turcotte (CPT) scores in patients with HCV infection and decompensated cirrhosis, many HCV-infected patients with decompensated cirrhosis still die or require liver transplantation (22). It is important to predict reversible or irreversible hepatic fibrosis in HCV-infected patients with cirrhosis. SNPs are thought to be non-modifiable factors that affect disease activity or the efficacy of treatment (23, 24).

BioBank Japan project is a large patient-oriented cohort. DNA, serum samples and clinical information which will be used for further studies (25). In the present study, we demonstrated an overview of the patients with susceptibility to HCV-related cirrhosis in this project. However, there are some limitations of the present study. The study is not a prospective study, and we should also perform a study of cohorts other than those sourced from BioBank Japan. In conclusion, we elucidated that four SNPs are associated with susceptibility to cirrhosis among HCV-infected Japanese subjects.

Conflicts of Interest

The Authors declare no conflicts of interest with regards to the present study.

Authors\' Contributions

S.K., A.T. and M.M. conceptualized the study, collected data, carried out the analysis. S.K., A.T., T.I., T.K. and M.M. drafted the initial manuscript and revised the manuscript. All Authors approved the final manuscript.

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