A Non-Synonymous Variant rs12614 of Complement Factor B Associated with Risk of Chronic Hepatitis B in a Korean Population

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Abstract

Background: Hepatitis B is known to cause several forms of liver diseases including chronic hepatitis B (CHB), and hepatocellular carcinoma. Previous genome-wide association study of CHB risk has demonstrated that rs12614 of complement factor B (CFB) was significantly associated with CHB risk. In this study, fine-mapping study of previously reported GWAS single nucleotide polymorphism (SNP, CFB rs12614) was performed to validate genetic effect of rs12614 on CHB susceptibility and identify possible additional causal variants around rs12614 in a Korean population. This association study was conducted in order to identify genetic effects of CFB single nucleotide polymorphisms (SNPs) and to identify additional independent CHB susceptible causal markers within a Korean population.

Methods: A total of 10 CFB genetic polymorphisms were selected and genotyped in 1,716 study subjects comprised of 955 CHB patients and 761 population controls.

Results: A non-synonymous variant, rs12614 (Arg32Trp) in exon2 of CFB, had significant associations with risk of CHB (odds ratio = 0.43, P = 5.91×10^-10). Additional linkage disequilibrium and conditional analysis confirmed that rs12614 had independent genetic effect on CHB susceptibility with previously identified CHB markers. The genetic risk scores (GRSs) were calculated and the CHB patients had higher GRSs than the population controls. Moreover, OR was found to increase significantly with cumulative GRS.

Conclusions: rs12614 showed significant genetic effect on CHB risk within the Korean population. As such rs12614 may be used as a possible causal genetic variant for CHB susceptibility.

Background

Hepatitis B, caused by the hepatitis B virus (HBV) infection, is known to cause several liver diseases including chronic hepatitis B (CHB), cirrhosis, and hepatocellular carcinoma (HCC) [1, 2]. According to the 2015 WHO report, HBV infection affects 3.5 % of the world population (257 million individuals) and is especially prevalent in Asian populations [3]. Several genome-wide association studies (GWASs) on CHB risk have been conducted on Asian populations and have found that CHB risk associated loci are typically located in human leukocyte antigen (HLA) regions, such as HLA-DR and HLA-DQ [4-7], in Chinese, Japanese, and Korean populations. According to our previous GWASs, several genes in HLA regions, such as transcription factor 19 (TFCF19), and valyl-tRNA synthetase 2 (VARS2)-surfactant associated 2 (SFTA2), euchromatin histone-lysine-methyltransferase2 (EHMT2), showed significant genetic effects on CHB susceptibility in a Korean population [5-9]. Interestingly, genetic variants of CFB, found on the nearby gene of EHMT2, have strong association with CHB susceptibility in Chinese studies [10-12].

CFB located on HLA genomic region is essential for regulating T-cell mediated innate immunity in the complement system [13-15]. A number of studies have demonstrated that CFB genetic variants are also associated with several diseases related to innate immune responses, anterior uveitis and Vogt-Koyanagi-Harada disease [16, 17]. This study conducted association analysis between CFB SNPs and CHB susceptibility to validate genetic effect of rs12614 and identify possible additional causal variants around rs12614 in a Korean population by fine-mapping of CFB region. Furthermore, the genetic risk scores (GRSs) of all known CHB risk makers were calculated to investigate the cumulative genetic effects of CHB susceptibility in individuals.

Methods

Study subjects

A total of 1,716 subjects (955 cases and 761 controls) were recruited for the study. CHB patients (n = 955) were obtained from the outpatient clinic of the Liver Unit and the Center for Health Promotion at Seoul National University Hospital, Ajou University Medical Center (Suwon, Korea), and Ulsan University Hospital (Ulsan, Korea). Among the CHB patients, 296 cases were also diagnosed with HCC. The population control (PC) samples (n = 761) were provided by Korea BioBank, the Center for Genome Science, the National Institute of Health, and Korea Centers for Disease Control and Prevention. For the CHB patients, seropositivity of the hepatitis B surface (HBsAg; Enzygnost® HBsAg 5.0; Dade Behring, Marburg, Germany) over a 6-month period was used for inclusion criterion to diagnose individuals with chronic HBV infection (Supplementary Table 1). HBsAg detection is determined using the Enzygnost® HBsAg 5.0 Kit assay. A blood sample of 100μL is added to a microplate well coated with sheep polyclonal antibodies against HBsAg, and the plate is then loaded into an assay processor, which performs all steps automatically, such as incubation, binding of the antigen, and the washing processes. For the controls, individuals were used for population controls whose response to an HBV infection is unknown and some of control group still have a chance of progression to CHB and/or HCC when exposed to HBV, though individuals with HBsAg (-) and anti-HBc (+) (spontaneously cleared via viral infection) are the best disease controls. Diagnosis of HCC was based on imaging findings of nodules that were larger than 1 cm, showing intense arterial uptake, followed by washout of contrast in the venous-delayed phases, in a 4-phase multi-detector CT scan or dynamic contrast enhanced MRI and/or biopsy [18]. The study protocol conformed to the Declaration of Helsinki. The study was approved by the institutional review board of Seoul National University Hospital, Ajou University Medical Center, and Ulsan University Hospital. All the subjects participating in the study provided written informed consent.

SNP genotyping

Following criteria were adopted for SNP selection: 1) Candidate SNPs of the genomic region around CFB (CFB gene with 2kb upstream (to include promoter region) and 500bp downstream regions; Chr6: 31,911,721-31,920,361) were extracted from 1000 genomes Japanese and Han Chinese data,
and minor allele frequency (MAF) and linkage disequilibrium (LD) status of the extracted SNPs were calculated. 2) Using NCBI dbSNP, investigate the functional location of the SNPs (upstream variant (2kb), 5′-prime UTR variant, missense, synonymous-codon, intron variant, downstream variant (500bp), 3′-prime UTR variant) extracted in 1). 3) Based on the 1) and 2), among the SNPs with high LD ($r^2 > 0.98$), SNP with relatively frequent (MAF > 5%) and functional effect based on the position was selected. And promoter region and non-synonymous SNPs with low frequency (MAF ≤ 5%) are additionally selected. As results, 5 tagging SNPs (rs1048709, rs537160, rs541862, rs4151657 and rs2072633) were selected along with 5 non-synonymous SNPs (rs4151667, rs12614, rs641153, rs117314762 and rs45484591). A total of 10 SNPs were genotyped in 955 CHB patients and 761 healthy controls. Genotyping reactions were performed by using BioMark HD system (Fluidigm 192.24 SNPtype™, San Francisco, CA, USA). The primer pools were designed for Specific target amplifications, Allele-specific and Locus-specific primers to detect candidate SNPs, and all the primers for 10 investigated SNPs in this study were designed and provided by the manufacturer of Fluidigm system (Fluidigm Corp., San Francisco, CA, USA). The additional workflow was followed by the manufacturer’s instructions for using the Integrated IFC Controller RX, FC1 Cycler, and EP1 Reader. Signal intensities for genotyping calling were scanned using the EP1 data collection and SNP Genotyping analysis software (Fluidigm Corp., San Francisco, CA, USA). The locations of the investigated SNPs are shown in Supplementary Figure 1A.

**Statistical analysis**

LD was obtained using Haploview v4.2 software downloaded from the Broad Institute (http://www.broadinstitute.org/mpg/haploview), with examination of Lewontin’s D’ ($|D|$) and the LD coefficient $r^2$ between all pairs of bi-allelic loci [19]. Logistic regression models were used to compare genotype distributions, including MAF and Hardy-Weinberg Equilibrium (HWE), among CHB patients and controls, and to calculate odds ratios (ORs), 95% confidence intervals, and corresponding $P$-values adjusted for age (continuous value) and sex (male = 0, female = 1) as covariates using SAS, version 9.4 (SAS Inc., Cary, NC, USA). In corrections for multiple comparisions, Bonferroni correction for multiple testing was applied. In order to examine whether the new association signal of investigated SNP is independent or affected by already known CHB susceptible loci, conditional logistic regression analyses were performed. Allele test based on the allele distribution of each SNP was also performed to assess the detailed genetic effects. Ten previously reported CHB susceptible loci in a Korean population (rs9277535 of HLA-DPB1, rs3077 of HLA-DPA1, rs2856718 of HLA-DQB1; rs7453920 of HLA-DQB2; rs1419881 of TCF19, rs1265163 of OCT4; rs652888 and rs35875104 of EHMT2; rs9394021 and rs2517459 of VARS2-SFTA) [5-9] were used for the conditional analysis and allele test. Based on the results from allele test, GRSs were calculated by multiplying the number of minor alleles by effect size (OR) of the SNP. Then, the combined genetic effects for each individual were calculated as the sum of the GRSs. Calculating the distribution of cumulative GRSs, ORs, 95% confidence intervals, and corresponding $P$-values between patients and controls were also performed.

**Results**

**Genotyping of CFB genetic variants**

A total of 10 CFB SNPs were selected and genotyped in 1,716 Korean subjects, comprised of 955 CHB patients and 761 population controls (Supplementary Table 1). Patients were divided into two subgroups, 659 HCC (-) CHB cases and 296 HCC (+) CHB cases. A gene map and LD among investigated SNPs are shown in Supplementary Figure 1A and 1B. Detailed information on the investigated SNPs, such as chromosome, position, allele, genotype distribution, heterozygosity, and HWE $P$, are presented in Supplementary Table 2.

**Association of CFB genetic polymorphisms with CHB risk**

In order to investigate the association between CFB genetic polymorphisms and risk of CHB, logistic regression analysis under an additive model was conducted. Analysis results indicated that rs12614 was significantly associated with risk of CHB even after applying Bonferroni correction for multiple testing ($OR = 0.43$, $P = 5.91 \times 10^{-10}$, $P_{corr} = 2.36 \times 10^{-6}$; Table 1). In order to validate the genetic effects of rs12614, association analysis was conducted using the training and test sets from the subjects in this study (Supplementary Table 3). Additional subgroup analysis was performed to investigate the association between CFB SNPs and CHB-related HCC progression. Again, analysis results found that, rs12614 had significant associations with risk of CHB in both the HCC (-) CHB and the HCC (+) CHB subgroups ($P = 6.60 \times 10^{-8}$ and $3.10 \times 10^{-6}$, respectively) even after Bonferroni correction was applied for multiple testing ($P_{corr} = 2.64 \times 10^{-6}$ and $1.24 \times 10^{-4}$, respectively). However, rs12614 did not show significant genetic effect on CHB-related HCC progression ($P > 0.05$).

**Table 1.** Association of CFB genetic polymorphisms with the risk of CHB and HCC
Table 3

In order to examine the detailed genetic effects of all 11 CHB susceptible loci including rs12614 (rs12614 of CFB; rs9277535 of HLA-DPB1; rs3077 of HLA-DPA1; rs2856718 of HLA-DQB1; rs7453920 of HLA-DQB2; rs1419881 of TCF19; rs265163 of OCT4; rs652888 and rs35875104 of EHMT2; rs9394021 and rs2517459 of VARS2-SFTA) in a Korean population, an allele test was conducted for each SNP. The GRSs of the genotypes were calculated using the ORs from allele test (Table 3).

Table 3. Determination of Genetic Risk Score based on Allele test of CHB susceptible loci
The complement system is composed of over 30 plasma proteins and is activated by microbes or antibodies which attached to microbes or other antigens. The complement activations occur through three pathways: the classical pathway, the lectin pathway and the alternate pathway. According to a GWAS conducted on a Chinese population, a CFB genetic variant had significant association with risk of CHB [10]. In this study, we aimed to 1) validation of GWAS association signal SNP (CFB rs12614) on CHB susceptibility in a Korean population, and 2) identification of possible additional causal variants around GWAS association signal SNP on CHB susceptibility in a Korean population by fine-mapping of CFB region.

To elucidate the cumulative genetic effects of all 11 CHB loci in the study subjects, the cumulative GRSs were evaluated. The cumulative GRSs ranged from 5.24 (most protected group) to 17.38 (most susceptible group), and CHB patients showed significantly higher cumulative GRSs than did the healthy control subjects (Supplementary Table 4 and Figure 1A). It was found that as cumulative GRSs increased, ORs significantly increased as well. In particular, individuals with GRSs of less than 7 showed an OR of 0.17 (log_{10} OR = -0.77), while individuals with GRSs of over 14 showed an OR of 3.42 (log_{10} OR = 0.53) (Figure 1B).

**Discussion**

Hepatitis B, an infectious disease with a high rate of incidence in Asian populations [1], is a major cause of CHB, liver failure, liver cirrhosis, and HCC development, which often result in death [20, 21]. Although the mechanisms underlying the different clinical results of HBV infection have not been fully understood, previous studies have linked a diverse range of factors such as viral strain, gender, age of infection, host immune system, and genetic information of the host, with risk of CHB [22]. When viral infection occurs, several immune-related genes are activated, leading to disease outbreak. According to a GWAS conducted on a Chinese population, a CFB genetic variant had significant association with risk of CHB [10]. In this study, we aimed to 1) validation of GWAS association signal SNP (CFB rs12614) on CHB susceptibility in a Korean population, and 2) identification of possible additional causal variants around GWAS association signal SNP on CHB susceptibility in a Korean population by fine-mapping of CFB region.

The complement system is composed of over 30 plasma proteins and is activated by microbes or antibodies which attached to microbes or other antigens [23, 24]. This system is an innate immune system that helps operate rapid responses against pathogenic invasions by opsonizing or recruiting inflammatory cells or pathogen lysis [25]. The complement activations occur through three pathways: the classical pathway, the lectin pathway and the alternate pathway.
alternative pathway. These pathways are worked through a cascade of enzymes reaction [24, 26]. CFB is essential to activate the complement system, particularly the alternative pathway that is against microbe invasion which includes viruses [27].

Previous Chinese studies have identified CFB genetic variants which have genetic effect on CHB risk. The most significant association was identified at rs12614 of CFB (P = 1.28×10^{-34} - 4.0×10^{-3}) [10, 11]. In this study, rs12614 showed the same direction of genetic effect as found in previous Chinese studies. In order to validate the associations, we have conducted the validation analysis of CFB genetic variant, rs12614, using random sampling of the training and test sets from the subjects. As result, all training sets showed significant results and although not all test sets showed significant results due to small sample sizes in test sets, the trends of effects were the same directions (Supplementary Table 3). Moreover, CFBrs12614 was significantly associated with risk of CHB in the HCC (-) CHB and the HCC (+) CHB groups (P = 6.60×10^{-6} and 3.10×10^{-6}, respectively). However, there was no significant genetic effect on CHB-related HCC progression. Additionally, the rs12614 C>T T allele was more frequently observed in the PC group than the CHB patients with a significance (OR = 0.43, P_{corr} = 2.36×10^{-5}). Considering that individuals with the non-synonymous variant (rs12614 T allele) had significantly higher CFB expression than those with the rs12614 C allele in the Chinese study, it can be seen that the rs12614 may affect immune response by influencing the complement system when viral infection occurs [10].

The rs12614 which is located on coding region of CFB, C to T allele change causes the amino acid change, arginine to tryptophan. To predict the effects of the rs12614 amino acid change, we conducted in silico analysis using the PolyPhen-2 program (http://genetics.bwh.harvard.edu/ph2/index.shtml) [28]. The results demonstrated that this amino acid change is predicted to be probably damaging that means this substitution might be damaging with high confidence (Supplementary Figure 3A, [28]). Amino acid alignment from the program, arginine at position 32 is highly conserved among species (Supplementary Figure 3B). Additionally, protein structure prediction was performed using CFSSP: Chou & Fasman Secondary Structure Prediction Server (http://www.biogem.org/tool/chou-fasman/index.php) [29]. Changes in protein secondary structure of rs12614 region, from coil structure to helix structure, by amino acid change from arginine to tryptophan were predicted. (Supplementary Figure 4). Protein function is closely related to the structure so that amino acids residue substitution can modify functional sites or protein interactions. And also disease-causing substitutions are more likely to occur at positions that is conserved throughout evolution [30], the rs12614 C to T allele substitution may affect CFB functions. Because the alternative pathway is important to against pathogen invasion, an amino acid change in CFB important in the alternative pathway may affect the immune system to against hepatitis B virus invasion.

Some individuals are more susceptible to diseases while others are less susceptible. Identification of the genetic background is key to understand differences in individuals’ disease susceptibility, and that can potentially lead to the targeting of preventive measures at those who are at greatest risk [31]. The results of the conditional analysis conducted on the 10 previously identified markers indicated that rs12614 can be used as a novel causal variant of CHB susceptibility. To elucidate its cumulative genetic effects, we used odd ratios of rs12614 and previously identified 10 CHB markers. Consequently, CHB group showed higher GRSs than the PC group and the higher genetic risk scores range indicated higher odds ratios. These implies CHB patients are more likely to have higher scores than controls.

There is a sampling limitation in this study. While the ideal subjects for the control groups would be the people who are HBsAg (-) and anti-Hbc (+) (spontaneously cleared), we used population controls with unknown responses to HBV infection. And some individuals in the control group still have a chance of progression to CHB when exposed to HBV. Although using the population controls in a case-control study may reduce statistical power, it is useful when it is difficult to obtain a sufficient number of disease controls.

Conclusions

A non-synonymous variant, rs12614 (Arg32Trp) of CFB was found to have significant associations with risk of CHB in a Korean population. Moreover, genetic effect of rs12614 on CHB risk was independent of all known CHB risk loci, and rs12614 can be used as possible causal variant of CHB susceptibility. Therefore, the results from this study may help in understanding and predicting genetic susceptibility to CHB in a Korean population.

Declarations

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Authors’ contributions

Conceptualization: HDS, YJK; methodology: HDS, YJK; investigation: JYS, JGS; formal analysis: JYS, JGS, BJY, SN, HSC, LHK, JOK; drafting of the manuscript: JYS, JGS; reviewing and editing the manuscript: BJY, HDS, YJK; all authors read and approved the final version of the manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Ethics approval and consent of participate

The study was approved by the institutional review board of Seoul National University Hospital, Ajou University Medical Center, and Ulsan University Hospital and performed in accordance with the principles expressed in the Declaration of Helsinki. All the subjects participating in the study provided written informed consent.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Abbreviations

CHB chronic hepatitis B
CFB complement factor B
SNP single nucleotide polymorphism;
GRSs genetic risk scores
HBV hepatitis B virus
HCC hepatocellular carcinoma
GWASs genome-wide association studies
HLA human leukocyte antigen
TCF19 transcription factor 19
VARS2-SFTA2 valyl-tRNA synthetase 2-surfactant associated 2
EHMT2 euchromatin histone-lysine-methyltransferase 2
PC population control
MAF minor allele frequency
LD linkage disequilibrium
HWE Hardy-Weinberg Equilibrium
ORs odds ratios

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