Hepatitis B virus infection in Taiwan: The role of NTCP rs2296651 variant in relation to sex

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INTRODUCTION

Taiwan is one of the endemic areas of hepatitis B virus (HBV) infection, which is a global health issue. Several routes of transmission have been reported, but most people in Taiwan are believed to have acquired the virus early in life. Genetic variation, environmental factors and genome variability are some of the multiple factors that influence chronic HBV infection. Genetic variation in the HBV receptor gene (NTCP) was significantly associated with a decreased risk of HBV infection in Taiwanese women.

Some of the several susceptibility loci that have been strongly associated with HBV-related diseases in different populations include HLA-DPB1, sodium taurocholate cotransporting polypeptide (NTCP), SOCS1, CIITA, TGF-β1, LTβR, PRKAA1, MIF, HLA-DP, HLA-DQ and PTPN22. Host genetics significantly contributes to the clinical outcome of HBV infection. NTCP plays an important role in the enterohepatic circulation of bile acids and also serves as a receptor for hepatitis B and D viruses. However, a previous study has reported that common variants in NTCP might not

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Summary
Sodium taurocholate cotransporting polypeptide (NTCP) is a functional receptor for hepatitis B virus (HBV) infection. NTCP rs2296651 is believed to be an Asian-specific variant responsible for HBV susceptibility. We investigated the relationship between rs2296651 and HBV infection in Taiwan based on stratification by gender and menopausal status. We recruited 10,017 Taiwan Biobank participants aged 30-70 years with complete genetic data and sociodemographic information. Gender-stratified multivariate logistic regression models were used to determine the relationship between NTCP variant and HBV infection. Among individuals with HBV infection, the genotype frequencies of GG, AG and AA in women were 0.85, 0.15 and 0 while those in men were 0.82, 0.18 and 0, respectively. The multivariate-adjusted odds ratios (OR) of HBV infection were 0.77 (95% CI 0.59-0.10) in women and 0.98 (95% CI 0.79-1.20) in men. The adjusted OR was 0.87 (CI 0.63-1.19) in premenopausal and 0.59 (0.36-0.97) in postmenopausal women. We found that genetic variation in the HBV receptor gene (NTCP) was significantly associated with a decreased risk of HBV infection in Taiwanese women.

KEYWORDS
hepatitis B virus, menopause, polymorphism, single nucleotide polymorphism
influence the expression level of SLC10A1 at transcriptional regulation, hence may not be associated with HBV susceptibility.\textsuperscript{10} NTCP variant (rs2296651) has been found to be absent among Moroccans regardless of chronic HBV infection status.\textsuperscript{11} Similarly, no association was observed between rs2296651 and HBV chronicity in a study that recruited Tibetan and Uygur HBV-infected patients.\textsuperscript{12}

In East Asia, women are said to be responsible for the majority of chronic HBV.\textsuperscript{13} NTCP rs2296651 has been reported as an Asian-specific variant responsible for HBV susceptibility.\textsuperscript{8} However, the associations of this variant with HBV-related diseases have not been extensively studied in Taiwan. In this study, we assessed the relationship between NTCP gene variant rs2296651 and HBV based on sex and menopausal status.

2  |  MATERIALS AND METHODS

2.1  |  Data source

Data were obtained from Taiwan Biobank, a population cohort recruited across Taiwan from 2008 to 2016. It contains physical, genetic, biological, as well as questionnaire measures of sociodemographic characteristics. Currently, it contains data from approximately 74,397 residents aged 30-70 years. All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Institutional Review Board of Chung Shan Medical University.

2.2  |  Study participants

Initial recruitment included 10,755 Taiwan Biobank participants. After excluding persons with incomplete and missing information (n = 738), 5121 men and 4896 pre- and postmenopausal Taiwanese women were included in the study. HBV infection was determined based on a positive hepatitis B surface antigen (HBsAg). Hepatitis B surface antibody (HBsAb)-positive individuals were excluded. This was necessary to avoid the possibility of enrolling persons naturally immune to HBV or those with resolved infections. All control individuals were confirmed negative for HBsAg. Participants were separated into HBV positive and negative individuals. Women were further stratified into 2 subgroups: premenopausal and postmenopausal individuals. Women were defined as premenopausal if they had a regular menstrual pattern. Postmenopausal women were defined as women with natural menopause (no regular menstrual pattern) whose current age was ≥1 year than their age of menopause. This information was obtained through the use of questionnaires within the Biobank. Women were defined as smokers if they have been smoking regularly during the last 6 months. The study protocol was approved by the Institutional Review Board of Chung Shan Medical University. A written informed consent was signed by each participant prior to data collection.

2.3  |  Genetic variant selection

A thorough review of past literature was made and NTCP rs2296651, an Asian-specific variant which has been associated with HBV infection was selected. SNP genotyping was carried out using the custom Taiwan Biobank chips and run on the Axiom™ Genome-Wide Array Plate System (Affymetrix, Santa Clara, CA, USA).

2.4  |  Statistical analyses

The characteristics of study participants were analysed according to gender and menopausal status. The Student’s t test was used to compare the continuous variables between pre- and postmenopausal women. Multivariate logistic regression models were used to determine the relationship between NTCP rs2296651 and HBV infection. Confounding variables included age, smoking, physical exercise, body mass index (BMI), hepatitis C virus (HCV), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C). PLINK 1.09 beta software version and the SAS 9.3 software (SAS Institute, Cary, NC, USA) were used for analyses. Hardy-Weinberg equilibrium (HWE) test was performed for the rs2296651 SNP.

3  |  RESULTS

Baseline characteristics of the 10,017 participants in our study are shown in Table 1. The samples included 4896 Taiwanese women (HBV positive, 481 and HBV negative, 4415 individuals) and 5121 men (HBV positive, 708 and HBV negative, 4413 individuals). There were significant differences in the number of HBV-positive and HBV-negative male (P = .0310) and female (P = .0290) patients who were carriers of rs2296651. Among the HBV-positive individuals, the genotype frequencies of GG, AG and AA in women were 0.85, 0.15 and 0.00 while those in men were 0.82, 0.18 and 0.00, respectively. The multivariate-adjusted odds ratios (OR) of HBV were 0.77 (95% CI 0.59-0.10) in women and 0.98 (95% CI 0.79-1.20) in men (Table 2). After stratification based on menopausal status, the multivariate-adjusted ORs for HBV infection were 0.87 (CI 0.63-1.19) in premenopausal, and 0.59 (0.36-0.97) in postmenopausal women as shown in Table 3.

4  |  DISCUSSION

We have investigated for the first time, the relationship between NTCP rs2296651 and HBV infection in Taiwan based on sex and menopausal status. The variant was found to significantly reduce the risk of HBV in Taiwanese women. There were no significant results in men. In addition, we found that the AA genotype of rs2296651 was absent in both HBV-positive men and women, unlike the HBV-negative individuals. This aligns with findings reported among the Han Chinese but differs from those observed among the Taiwanese
TABLE 1 Baseline characteristics of study participants

|                  | Men                        | Women                      |
|------------------|----------------------------|----------------------------|
|                  | HBV                        | HBV                        |
|                  | Positive       | Negative       | P-value | Positive       | Negative       | P-value |
| rs2296651        | 581 (82.06)   | 3613 (81.87)   | .0310   | 410 (85.24)   | 3606 (81.68)   | .0290   |
| GG               | 127 (17.94)   | 758 (17.18)    | .0310   | 71 (14.76)    | 766 (17.35)    | .0290   |
| AG               | 0 (0.00)      | 42 (0.95)      | .0310   | 0 (0.00)      | 43 (0.97)      | .0290   |
| Age (y)          | 47.43 ± 9.95  | 48.93 ± 11.26  | .0003   | 55.22 ± 9.97  | 47.54 ± 10.75  | <.0001  |
| Age at menarche (y) |               | 13.52 ± 1.50   | .8910   | 13.51 ± 1.55  | .8910   |
| Smoking (N, %)   | 168 (23.73)   | 925 (20.96)    | .0950   | 14 (2.91)     | 106 (2.40)     | .4920   |
| Physical exercise (N, %) | 540 (76.27) | 3488 (79.04)   | .0710   | 467 (97.09)   | 4309 (97.60)   | .4920   |
| BMI (kg/m²)      | 24.94 ± 3.42  | 25.29 ± 3.38   | .0100   | 23.38 ± 3.63  | 23.43 ± 3.58   | .7760   |
| HCV (N, %)       | 20 (2.82)     | 122 (2.76)     | .0710   | 11 (2.29)     | 123 (2.79)     | .5240   |
| TC (mg/dL)       | 186.6 ± 32.95 | 193.50 ± 34.79 | <.0001  | 188.10 ± 33.34 | 194.20 ± 35.97 | <.0001  |
| TG (mg/dL)       | 116.6 ± 70.88 | 138.20 ± 106.00| <.0001  | 88.18 ± 49.08 | 100 ± 67.87    | <.0001  |
| HDL-C (mg/dL)    | 48.44 ± 10.64 | 48.38 ± 11.26  | .9000   | 59.12 ± 14.06 | 58.15 ± 13.16  | .1520   |
| LDL-C (mg/dL)    | 120.10 ± 30.56| 123.70 ± 31.63 | .0100   | 114 ± 29.42   | 119.40 ± 31.96 | .0000   |

BMI, body mass index; HBV, hepatitis B virus infection; HCV, hepatitis C virus infection; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride.

All variables are presented as mean ± SD (continuous variables) or numbers (%).

TABLE 2 Multivariate-adjusted OR of HBV infection among male and female Taiwanese individuals

|                  | Men                      | Women                     |
|------------------|--------------------------|---------------------------|
|                  | HBV                      | HBV                       |
|                  | OR 95% CI                 | OR 95% CI                 |
| rs2296651        | 0.98 0.79-1.20           | 0.77 0.59-0.10            |
| Age              | 0.99 0.98-1.01           | 0.99 0.98-1.01            |
| Age at menarche  | - 1.04 0.97-1.11         | - 1.04 0.97-1.11          |
| Smoking          | 1.23 1.01-1.49           | 1.22 0.69-2.15            |
| Physical exercise| 0.92 0.77-1.10           | 0.85 0.68-1.05            |
| BMI              | 0.98 0.96-1.01           | 1.03 1.00-1.06            |
| HCV              | 0.95 0.59-1.55           | 0.84 0.44-1.57            |
| TC               | 1.00 0.99-1.01           | 1.00 0.99-1.01            |
| TG               | 1.00 0.99-1.01           | 1.00 0.99-1.01            |
| HDL-C            | 0.99 0.98-1.00           | 1.00 0.98-1.02            |
| LDL-C            | 1.00 0.99-1.01           | 0.99 0.98-1.01            |

BMI, body mass index; CI, confidence interval; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; OR, odds ratio; TC, total cholesterol; TG, triglyceride.

Odds ratios for rs2296651 were determined from the dominant model (that is, NTCP rs2296651 AG+AA; Reference, GG genotype).

TABLE 3 Association of NTCP rs2296651 with HBV infection among Taiwanese women based on menopausal status

|                  | Premenopause | Postmenopause |
|------------------|--------------|---------------|
|                  | OR 95% CI    | OR 95% CI     |
| rs2296651        | 0.87 0.63-1.19 | 0.59 0.36-0.97 |
| Age              | 1.00 0.98-1.02 | 0.99 0.96-1.02 |
| Age at menarche  | 1.08 0.99-1.17 | 0.99 0.89-1.10 |
| Smoking          | 1.06 0.54-2.07 | 1.92 0.65-5.67 |
| Physical exercise| 0.66 0.49-0.88 | 1.22 0.87-1.73 |
| BMI              | 1.03 1.00-1.07 | 1.02 0.97-1.08 |
| HCV              | 1.35 0.56-3.24 | 0.55 0.22-1.40 |
| TC               | 1.01 0.99-1.03 | 0.99 0.97-1.01 |
| TG               | 1.00 0.99-1.01 | 1.00 0.99-1.00 |
| HDL-C            | 0.99 0.97-1.02 | 1.00 0.98-1.03 |
| LDL-C            | 0.99 0.97-1.00 | 1.00 0.98-1.02 |

BMI, body mass index; CI, confidence interval; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; OR, odds ratio; TC, total cholesterol; TG, triglyceride.

Odds ratios for rs2296651 were determined from the dominant model (that is, NTCP rs2296651 AG+AA; Reference, GG genotype).
where the AA genotype was found to be significantly and negatively associated with chronic HBV infection. However, in their study, individuals were not stratified based on sex. In a study to assess the relationship between NTCP gene variant and chronic HBV infection, the frequency of A allele of rs2296651 in healthy Han Chinese (12.6%) was observed to be higher compared to the reported frequency in Koreans (3.1%) and the Chinese-Americans (7.5%).

Our study is consistent with some of the previous publications which found that subjects with rs2296651 polymorphism were not susceptible to chronic HBV infection. The mutant allele (SNP rs2296651) is believed to have a deleterious effect on the transport of bile acids and would also reduce the chance of HBV entering hepatocytes. We found that the association was significant in Taiwanese women. The male counterparts had a slightly higher but protective OR even though the association was not significant. This difference may be linked to hormone variations. Oestrogen is said to play an important role in the protection and defence of hepatic cells against the development and progression of chronic liver disease such as HBV infections. This in part may account for the lower odds of HBV as recorded among the Taiwanese women. Another study conducted on rats has also reported that oestrogen regulates NTCP genes. It has been speculated that heterozygous individuals are more susceptible to HBV infection. However, because there are no reports about the association of NTCP gene variants and HBV infection in Taiwan, more investigations would be needed to confirm this speculation among Taiwanese individuals.

It is worth stating that we included only individuals that were tested positive for HBsAg. HBsAb-positive individuals were excluded because of a possible history of vaccination. We noticed that HBV-positive individuals had no homozygous mutant gene type (AA genotype) in the present study. This serves as a limitation of the study. Furthermore, stratification by menopausal status showed significant results only in postmenopausal women. Considering that in postmenopausal women, there is a lower production of estradiol and a reduced response to its actions, one would have rather expected a more significant result in premenopausal and not postmenopausal women as observed in this study. However, higher levels of estradiol have also been reported in menopausal infected HCV-infected women. It is not clear whether this difference is due to hormone variations. Nonetheless, the possibility of potential contributors of other unknown factors cannot be ruled out. Based on a previous study, many genes are expected to show changes in expression with age although only a subset of the genes contributes to individual variation in transcriptional response to ageing. However, it remains to be investigated whether the NTCP gene is related to age in order to make a valid conclusion regarding the relationship between genotype and survival. NTCP is one of the HBV-associated genes that has not been extensively studied in Taiwan.

In conclusion, we suggest that the rs2296651 variant in this receptor gene might decrease susceptibility to HBV infection in Taiwanese women. However, more investigations remain necessary before reaching definitive conclusions in this regard.

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CONFLICT OF INTEREST
All authors declare there are no competing financial interests in relation to this work.

AUTHOR CONTRIBUTIONS
ONN, YPL and DMT conceived and designed the study. CTL, ONN and TD acquired data and performed the literature search. WHL, SYH, CTL and YPL were responsible for statistical design and the analysis plan. SYH, WHL, LW and MFW generated the data. ONN wrote the manuscript. TD, WL and DMT made a critical revision of the manuscript for important intellectual content. All authors revised the manuscript critically and approved the final version.

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