Hepatitis B infection is causally associated with extrahepatic cancers: A Mendelian randomization study

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Summary

Background Evidence from observational studies suggests that chronic hepatitis B virus (HBV) infection is associated with extrahepatic cancers. However, the causal association between chronic HBV infection and extrahepatic cancers remains to be determined.

Methods We performed two-sample Mendelian randomization (MR) to investigate whether chronic HBV infection is causally associated with extrahepatic cancers. We identified four independent genetic variants strongly associated (P-value $< 5 \times 10^{-8}$) with the exposure, chronic HBV infection in 1371 cases and 2938 controls of East Asian ancestry in Korea, which were used as instrumental variables. Genome-wide association summary level data for outcome variables, that included cancer of the biliary tract, cervix, colorectum, endometrium, esophagus, gastric, hepatocellular carcinoma, lung, ovary and pancreas were obtained from Biobank Japan.

Findings Using the multivariable inverse variance weighted method, we found genetic liability to chronic HBV infection causally associated with extrahepatic cancers including cervical cancer (odds ratio [OR] = 1.57, 95% confidence interval [CI] = 1.29−1.91, P-value = 0.0001) and gastric cancer (OR = 1.12, 95% CI = 1.05−1.19, P-value = 0.0001). Moreover, chronic HBV infection (OR = 1.20, 95% CI = 1.07−1.34, P-value = 0.0021) was causally associated with hepatocellular carcinoma, supporting a well-established association between chronic HBV infection and hepatocellular carcinoma.

Interpretation Our MR analysis revealed that chronic HBV infection is causally associated with extrahepatic cancers including cervical and gastric cancers.

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Introduction

About 360 million people worldwide are chronically infected with hepatitis B virus (HBV) infection.[1,2] Evidence from observational studies indicates that chronic

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Research in context

Evidence before this study

Epidemiological studies, suggests that chronic HBV infection is associated with extrahepatic cancers. However, uncertainty exists whether these associations are causal, as much of the current evidence originates from observational studies, which are prone to confounding and reverse causation.

Added value of this study

Using multivariate Mendelian randomization, we revealed that chronic HBV infection is causally associated with extrahepatic cancers including cervical cancer and gastric cancer in individuals of East Asian ancestry. Our analysis further found chronic HBV infection causally associated with hepatocellular carcinoma, supporting evidence from previous observational studies that chronic HBV infection is the risk factor for hepatocellular carcinoma.

Implications of all the available evidence

By establishing a causal link between chronic HBV infections with extrahepatic cancers, our findings will provide a basis for screening patients with chronic HBV infection for some site-specific extrahepatic cancers to prevent cancer development.

HBV infection is the risk factor for hepatocellular carcinoma.[3–5] Further evidence suggests that chronic HBV infection is associated with extrahepatic cancers including cancer of the pancreas, lung, colorectum, kidney, cervix, gastric, and thyroid gland.[6–9] Moreover, our previous analysis using data from Taiwan National Health Insurance Research Database (NHIRD) revealed that chronic HBV infection is associated with cancer of the liver, pancreas, kidney, colorectum, ovary, non-Hodgkin's lymphoma, gallbladder and extrahepatic bile duct.[10]

Although chronic HBV infection has been associated with extrahepatic cancers, uncertainties exist whether these associations are causal. Much of the current evidence originated from observational studies,[6–10] that are susceptible to residual confounding. Hence, these associations need to be investigated further using different approaches that are not prone to confounding. Mendelian randomization (MR) is an analytical approach that can improve causal inference by using genetic variants as instrumental variables to infer the causality of exposure to an outcome.[11] Unlike observational studies, MR analyses are not confounded by some unmeasured factors owing to the random independent segregation of alleles during meiosis.[12,13] Besides, MR analyses are not prone to reverse causation as genetic variants are fixed at birth and do not change over time.[14] This makes MR an ideal approach for inferring the causality of an exposure on an outcome.

Here we performed a two-sample MR to investigate the causal associations between chronic HBV infection and extrahepatic cancers using genetic variants strongly associated with chronic HBV infection as instrumental variables. We investigated whether genetic liability to chronic HBV infection is causally associated with biliary tract, cervical, colorectal, endometrial, esophageal, gastric, liver, lung, ovarian and pancreatic cancers.

Methods

Exposure data

Genetic variants strongly associated with chronic HBV infection were selected from a genome-wide association study (GWAS) of individuals of East Asian descent in Korea.[15] A flow chart of study and instrumental variable selection is presented in Fig. S1. Chronic HBV infection was defined as the seropositivity of hepatitis B surface antigen (HBsAg) for more than six months. The discovery GWAS comprised of 1371 chronic HBV patients and 2938 controls; adjusted for age, sex and population structure by including 10 principal components as covariates in the model. Four independent genetic variants strongly associated with chronic HBV infection from this GWAS were identified by selecting the single nucleotide polymorphisms (SNPs) with the lowest *P*-value (*P*-value $< 5 \times 10^{-8}$), in 500 kb window around a lead SNP that were also less correlated with other SNPs in this region ($r^2 < 0.01$) (Fig. S1). To avoid violating the third assumption of MR (horizontal pleiotropy), we used PhenoScanner to identify and remove any genetic variants that were directly associated with any site-specific cancers.[16] Interestingly, all our instrumental variables were not associated with any site-specific cancers. Moreover, the strength of our instrumental variables were measured using the F-statistics as described in detail by the previous study.[17] Detailed information for genetic variants used as instrumental variables in the current analysis is provided in Table 1.

Outcome data

GWAS summary level data for the associations between genetic variants and biliary tract cancer ($n = 339$), cervical cancer ($n = 605$), colorectal cancer ($n = 7062$), endometrial cancer ($n = 999$), esophageal cancer ($n = 1300$), gastric cancer ($n = 6563$), liver cancer ($n = 1866$), lung cancer ($n = 4050$), ovarian cancer ($n = 720$) and pancreatic cancer ($n = 442$) were obtained from a recent large GWAS of the East-Asians ancestry in Japan.[18] In these analyses, Ishigaki et al.[18] adjusted for covariates, which include age, sex, and top five principal components. All participants included in the analysis were of East-Asian

SNP	CHR	BP	A1	A2	MAF	Beta	SE	F-statistics	Nearest genes	*P*-value
rs9277535	6	33,054,861	A	G	0.388	0.635	0.026	1020	*HLA-DPB1*	3.74×10^{-40}
rs7453920	6	32,730,012	A	G	0.127	0.693	0.036	513	*HLA-DQB2*	6.71×10^{-26}
rs1419881	6	31,130,593	G	A	0.433	0.315	0.038	220	*TCF19*	1.26×10^{-13}
rs9268634	6	32,406,530	G	A	0.481	0.435	0.053	449	*HLA-DRA*	2.87×10^{-08}

Table 1: **Instrumental variables used in the Mendelian randomization of chronic HBV infection with various site-specific cancers.**
HBV; hepatitis B virus, SNP; single nucleotide polymorphisms. CHR: chromosome. BP; base pair position. A1; effect allele. A2; other alleles. MAF; minor allele frequency. SE; standard error.

descent recruited from 12 medical institutions throughout Japan into the Biobank Japan (BBJ), which has over 47 diseases and phenotypes including 13 site-specific cancers.[19]

Ethics statement

Our study used publicly available GWAS summary statistics data from the Biobank Japan, which obtained informed consent from all participating studies by following the protocols approved by their respective institutional review boards.[18] No separate ethical approval was required for this study.

Statistical analysis

Our MR analyses were performed using the inverse-variance weighted (IVW) method, which provides accurate estimates when there is no heterogeneity and directional pleiotropy between the exposure and outcome variable.[20] The heterogeneity of causal association between chronic HBV infection and extrahepatic cancers were investigated by estimating Cochran's Q statistics and I^2 statistics assuming a fixed-effect model.[21] We performed sensitivity analyses to check and correct for pleiotropy in the causal estimates.[20] We defined the presence of heterogeneity if the Q statistics were significant at a *P*-value $\leq$ 0.05 and consequently, we used a random-effect IVW method in our analysis.[22] We assessed the presence of horizontal pleiotropy using MR-Egger regression based on its intercept terms and Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO) .[23,24] When the MR-Egger intercept deviates from zero or its *P*-value is statistically significant at *P*-value $\leq$ 0.05 it indicates the presence of horizontal pleiotropy[25] and a different MR method was used to report the results. In this analysis, we used the weighted median method in the presence of heterogeneity and horizontal pleiotropy. The weighted median methods can give valid estimates under the presence of horizontal pleiotropy when up to 50% of the instruments variables are valid.[20] MR-PRESSO was used to detect and remove outlier instrumental variables.[24]

To avoid our results from being confounded, we performed a multivariable IVW (MIVW) method adjusting for chronic hepatitis C virus (HCV) infection and cigarette smoking. Our previous analysis found chronic HCV to be associated with site-specific cancers including liver, ovary, non-Hodgkin's lymphoma, gallbladder and extrahepatic bile duct.[10] Cigarette smoking was adjusted as one of the risk factors for some site-specific cancers. Genetic variants strongly associated with cigarette smoking and chronic HCV infection were obtained from the East Asian ancestry population in BBJ and included in the multivariable MR analysis.[26] For multiple testing, we used the Bonferroni method[27] and causal associations were considered to be statistically

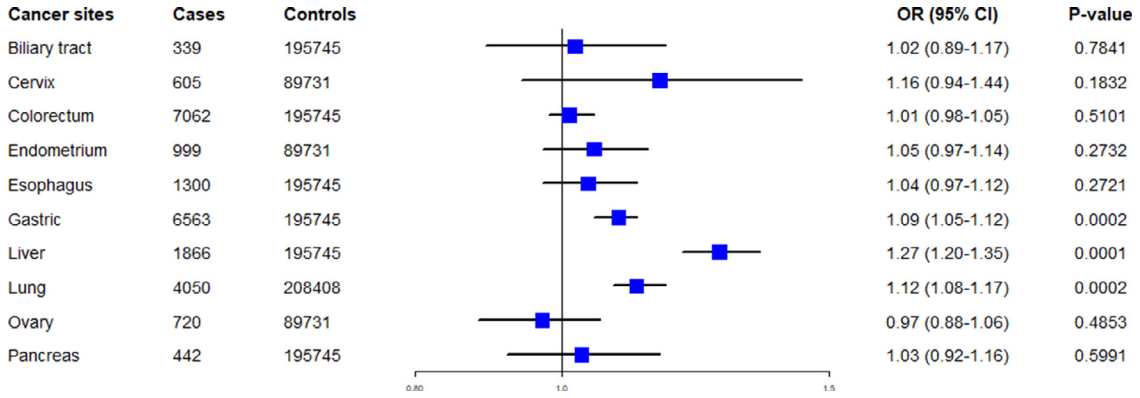

Fig. 1. The causal association between chronic HBV infection with extrahepatic cancer in individuals of East-Asian ancestry in Japan using the inverse variance weighted (IVW) method. OR; odds ratio, CI; confidence interval, IVW *P*-value < 0.005 was defined as statistically significant after Bonferroni multiple corrections.

significant when a *P*-value < 0.005 (0.05/10 outcomes). All statistical analyses were performed using the Mendelian Randomization[28] and Two-sample MR[29] packages using the R programming language.

Role of the funding source

The funders did not have any role in the analysis and interpretation of the data; writing of the manuscript; or in the decision to submit the paper for publication.

Results

We identified four independent significant SNPs strongly associated with chronic HBV infection from individuals of East Asian ancestry in Korea (Table 1). These genetic variants had an F-statistics of more than 220, thus indicating that our instrumental variables were strongly associated with chronic HBV infection. Moreover, our instrumental variables were not directly associated with any site-specific cancers.

Chronic HBV infection MR estimates

We evaluated whether chronic HBV infection is causally associated with extrahepatic cancers in East-Asians descents. The IVW method was our primary MR method in the absence of horizontal pleiotropy and we found chronic HBV infection causally associated with hepatocellular carcinoma, odds ratio (OR) = 1.27 and 95% confidence interval [CI] = 1.20−1.35, *P*-value < 0.0001, IVW, Fig. 1). Additionally, we found chronic HBV infection causally associated with extrahepatic cancers including gastric cancer (OR = 1.09, 95% CI = 1.05−1.12, *P*-value < 0.0002, IVW) and lung cancer (OR = 1.12, 95% CI = 1.08−1.17, *P*-value < 0.0002, IVW) in individuals of East Asian ancestry in Japan. Moreover, we found genetic liability to chronic HBV infection positively associated with biliary tract cancer, cervical cancer, colorectal cancer, endometrial cancer, esophageal cancer, and pancreatic cancer although our results were not statistically significant (Fig. 1). Surprisingly, we found genetic liability to chronic HBV infections inversely associated with ovarian cancer (OR = 0.97, 95% CI = 0.88−1.06, *P*-value = 0.4853, IVW). However, this result was not statistically significant.

Multivariable MR estimates

We then performed a multivariable MR analysis adjusting for chronic HCV infection and cigarette smoking. We found genetic liability to chronic HBV infection causally associated with hepatocellular carcinoma (OR = 1.20, 95% CI = 1.07−1.34, *P*-value = 0.0021, MIVW) and extrahepatic cancers including cervical cancer (OR = 1.57, 95%CI = 1.29−1.91, *P*-value = 0.0001, MIVW) and gastric cancer (OR = 1.12, 95%CI = 1.05−1.19, *P*-value = 0.0001, MIVW, Fig. 2) in individuals of East Asian ancestry in Japan. Moreover, genetic liability to chronic HBV infections were positively associated with biliary tract cancer, colorectal cancer and lung cancer and inversely associated with ovarian and prostate cancers (Fig. 2). However, these results were not statistically significant.

Sensitivity analyses

We performed sensitivity tests using different MR approaches including MR-PRESSO, MR egger, simple median and weighted median methods. We found no evidence of horizontal pleiotropy for chronic HBV infection with all site-specific cancers with *P*-values > 0.05 for the MR-Egger regression intercept approach (Table

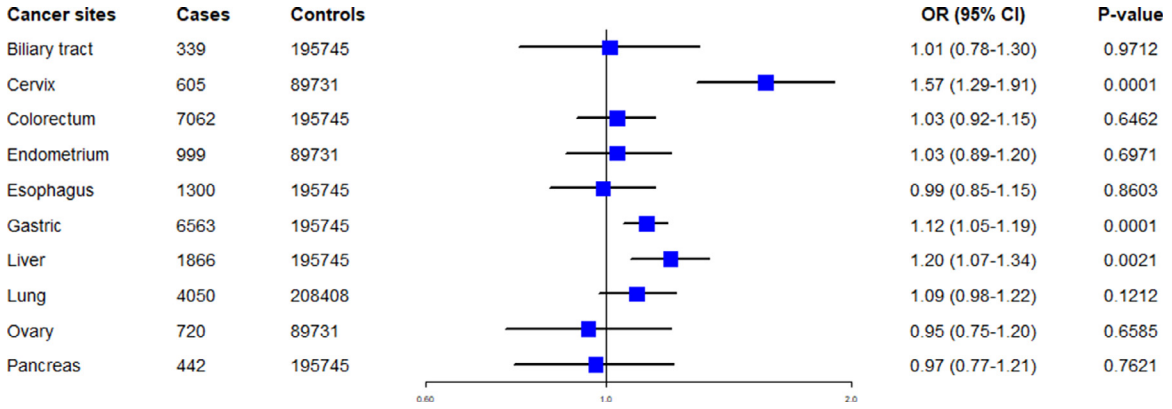

Fig. 2. The causal association between chronic HBV infection with extrahepatic cancer in individuals of East-Asian ancestry in Japan using the multivariate inverse variance weighted (MIVW) method. OR; odds ratio, CI; confidence interval, MIVW *P*-value < 0.005 was defined as statistically significant after Bonferroni multiple corrections.

S1). However, we found evidence for heterogeneity between genetic liability to chronic HBV infections with cervical cancer, I^2 = 76.9% and *P*-value for heterogeneity = 0.0047 (Fig. S2). Our sensitivity analysis revealed rs7453920 as the instrument variable driving the causal association between genetic liability to chronic HBV infection and cervical cancer (OR = 1.16, 95%CI = 0.94−1.44, *P*-value = 0.1832, MR egger, Fig. S2B). We then performed MR-PRESSO, which identify and remove outlier instrumental variables. Our outlier corrected method found genetic liability to chronic HBV infection not causally associated with cervical cancer (OR = 1.05, 95%CI = 0.97−1.13, *P*-value = 0.3673, MR-PRESSO). This association should be interpreted with caution as it was found in multivariable MR only. However, using simple median and weighted median MR methods (Fig. S3), our results for gastric, liver, and lung cancers remain statistically significant (Table S1), thus indicating that our results are statistically robust. Furthermore, we found genetic liability to chronic HBV infection to be positively associated with colorectal cancer (Table S1) using MR-Egger and MR-intercept. However, this finding was not statistically significant after Bonferroni corrections (*P*-value < 0.005).

Discussion

To the best of our knowledge, this is the first MR study set out to investigate whether chronic HBV infection is causally associated with extrahepatic cancers. We found genetic liability to chronic HBV infection causally associated with extrahepatic cancers including cervical cancer and gastric cancer in individuals of East Asian ancestry in Japan. Moreover, our genetic liability to chronic HBV infection was causally associated with hepatocellular carcinoma, supporting the overwhelming evidence from observational studies that chronic HBV infection is a risk factor for hepatocellular carcinoma.[3–6]

HBV is a hepatotropic virus strongly associated with hepatocellular carcinoma.[3–8] Our MR analysis has confirmed this association and indicates that chronic HBV infection is causally associated with hepatocellular carcinoma. Although HBV is a hepatotropic virus, some observational evidence showed that chronic HBV infections are also associated with other extrahepatic cancers including esophageal, endometrial, cervical, gastric, lung and colorectal cancers.[8–10,30–32] Given the limitations of observational epidemiological studies in establishing causal associations, we, therefore, performed a MR study to determine whether chronic HBV infection is causally associated with extrahepatic cancers. Our MR analysis revealed that genetically predicted chronic HBV infection is causally associated with extrahepatic cancers including cervical and gastric cancers, corroborating with previous observational studies.[6–10,30,31] However, the biological mechanism linking chronic HBV infection with extrahepatic cancers has not yet been fully illustrated. Still, it is thought that HBV protein X, a transcriptional coactivator, plays a crucial role in initiating tumorigenesis by modulating key regulators of the apoptosis, interfering with the DNA repair pathways and tumor suppressor genes.[29] Moreover, Song et al. observed a higher HBV protein X expression in the cancerous part of the tissue specimen than the healthy part of the same specimen, supporting the oncogenic role of HBV protein X.[7] Furthermore, some studies found HBV DNA in some extrahepatic tissues, including gastric, kidney, gallbladder and pancreas,[33,34] suggesting that HBV can initiate and promote tumorigenesis outside the liver.

Although our MR analysis failed to find any significant association between genetic liability to chronic HBV infection and with some extrahepatic cancers including biliary tract, esophageal, endometrial and colorectal cancers, our ORs for these associations were > 1 (Fig. 2), suggesting that chronic HBV infection may

be a risk factor for these site-specific cancers. Moreover, previous studies found chronic HBV infection associated with these extrahepatic cancers.[6–8,10,30] Surprisingly, we found genetic liability to chronic HBV infection inversely associated with ovarian and prostate cancers, contrary to our previous analysis in Han Chinese patients.[10] However, these associations were not statistically significant (Fig. 1) and future studies should validate these findings further.

Our IVW, simple median, and weighted median methods found genetic liability to chronic HBV infection significantly associated with gastric, liver and lung cancers (Table S1 and Fig. S3). However, our multivariate IVW method adjusting for chronic HCV infection and cigarette smoking found genetic liability to chronic HBV infection causally associated with cervical, gastric and hepatocellular carcinomas (Fig. 2), suggesting that the association between chronic HBV infection with cervical and lung cancers may have been confounded by chronic HCV infection and cigarettes smoking. Although MR analyses are not susceptible to confounding and reverse causation, the robustness of the results usually depends on the statistical approach used and whether risk factors strongly associated with the exposure or outcome variables were adequately adjusted.

Our study has several strengths including the use of the MR approach, which eliminate some confounders commonly observed in epidemiological studies. Moreover, we used multiple SNPs, which were strongly associated with chronic hepatitis HBV. Besides, we use a homogenous population that minimizes heterogeneity commonly observed when individuals of different ancestry populations are used in genetic studies. We further adjusted for cigarette smoking and chronic HCV infection, indicating our results are statistically robust. However, our study had limitations including small sample sizes on certain site-specific cancers including biliary tract cancer and pancreatic cancer. Our analyses included individuals from East Asia where there is a high prevalence of chronic HBV infection and therefore our results might not be generalizable to other ancestry populations. Moreover, our results may have been confounded by some residual population structures between Koreans and Japanese. Nevertheless, both the exposure and outcome data were adjusted for population structure by including 10 and five principal components, respectively; hence the impact of population structure confounding our results is minimal. In addition, our Cochran's Q test failed to find any obvious heterogeneity between the Koreans and Japanese. Interestingly, the three major East Asian populations (Han Chinese, Japanese and Koreans) share a clade, dietary intake pattern, lifestyle and cultural factors; hence the impact of population structure is minimal. In summary, our MR results support a well-established relationship between chronic HBV infection and hepatocellular carcinoma and suggest that chronic HBV infection may also be a risk factor for extrahepatic cancers including cervical and gastric cancers.

Contributors

Conceptualization, ABK; methodology, ABK, SF, CCY, TC; Verified the underlying data, ABK, TC; writing-original draft, ABK, SF, TC; writing-review and editing, ABK, SF, MGS, CCY, TC. All the authors participated in planning, execution, and analysis and have read and approved the final submitted version.

Data sharing statement

All data used in this study are available in the public repository.

Declaration of Interests

The authors declare no conflict of interest.

Acknowledgements

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ebiom.2022.104003.

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