

ORIGINAL ARTICLE

Associations between six dietary habits and risk of hepatocellular carcinoma: A Mendelian randomization study

Yunyang Deng¹  | Junjie Huang¹  | Martin C. S. Wong^{1,2,3} 

¹The Jockey Club School of Public Health and Primary Care, Faculty of Medicine, Chinese University of Hong Kong, Hong Kong SAR, China

²School of Public Health, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

³School of Public Health, Peking University, Beijing, China

Correspondence

Martin C. S. Wong, The Jockey Club School of Public Health and Primary Care, Faculty of Medicine, Chinese University of Hong Kong, Hong Kong SAR, 999077, China.

Email: wong_martin@cuhk.edu.hk

Abstract

Diet is reported to be associated with hepatocellular carcinoma (HCC), but whether there is a causal relationship remains unclear. This study aimed to explore the potential causal associations between dietary habits and HCC risk using Mendelian randomization in an East Asian population. From the BioBank Japan, we obtained summary-level genome-wide association studies data for the following six dietary habits: ever/never drinker ($n = 165,084$), alcohol consumption ($n = 58,610$), coffee consumption ($n = 152,634$), tea consumption ($n = 152,653$), milk consumption ($n = 152,965$), and yoghurt consumption ($n = 152,097$). We also obtained data on HCC (1866 cases and 195,745 controls). Single-nucleotide polymorphisms (SNPs) that were associated with exposures ($p < 5 \times 10^{-8}$) were selected as instrumental variables (IVs). Five, two, and six SNPs were identified for ever/never drinkers, alcohol consumption, and coffee consumption. One SNP was used for consumption of tea, milk, and yoghurt. The odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by inverse variance weighted (for an IV with more than one SNP) or Wald ratio (for an IV with one SNP). Ever/never drinkers (OR, 1.11; 95% CI, 1.05–1.18; $p < 0.001$) and alcohol consumption (OR, 1.57; 95% CI, 1.32–1.86; $p < 0.001$) were positively associated with HCC risk. Conversely, coffee consumption was inversely related to HCC risk (OR, 0.69; 95% CI, 0.53–0.90; $p = 0.007$). Similar inverse associations were observed for consumption of tea, milk, and yoghurt, with ORs (95% CIs) of 0.11 (0.05–0.26), 0.18 (0.09–0.34), and 0.18 (0.09–0.34), respectively (all $p < 0.001$). **Conclusion:** There are potential causal associations between six dietary habits and HCC risk. Our findings inform clinical practice by providing evidence on the impact of dietary habits on HCC.

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INTRODUCTION

Liver cancer is the sixth most common cause of cancer and ranks third for cancer mortality globally.^[1] In some Asian countries, such as Mongolia, Thailand, and Cambodia, liver cancer is the most commonly diagnosed cancer and the leading cause of cancer death.^[1] Owing to its poor prognosis at the time of diagnosis, liver cancer could impose a heavy global burden of disease.^[2] Hepatocellular carcinoma (HCC) is the most common subtype of liver cancer, comprising 75%–85% of cases.^[1] It is a multifactorial disease involving many potential etiological factors,^[3] implying the importance of risk factor identification to inform its prevention.^[2]

Over the past decades, many epidemiological studies have shown that dietary habits are associated with liver cancer risk. A meta-analysis of 16 cohort studies found that compared with nondrinkers, heavy alcohol drinkers (three or more drinks per day) had a 16% increased risk of liver cancer.^[4] Another meta-analysis of 26 studies showed that an additional intake of two cups of coffee per day was associated with a 35% lower HCC risk.^[5] Green tea consumption was inversely related to liver cancer risk in a meta-analysis of nine cohort articles.^[6] A meta-analysis of 15 studies also reported an inverse association between yogurt intake and liver cancer risk.^[7] Nevertheless, observational studies can be biased by confounders and reverse causality when it comes to causal inferences.^[8] The randomized controlled trial (RCT) also has its own limitations with respect to ethical concerns, time of observation, as well as resources and cost.^[9] Therefore, whether there are causal associations between dietary habits and HCC risk is unclear.

Mendelian randomization (MR) was introduced as a study design to infer causality.^[8] Most MR studies use genetic variants significantly associated with exposures as instrumental variables (IVs) to assess the associations between genetic-predicted exposures and outcomes.^[8] Owing to the fact that genetic variants are randomly inherited from parents to offspring at conception, they are less likely to be influenced by potential confounders and reverse causality.^[8] A study design based on MR allows investigation of many exposures that cannot be studied RCTs.^[9] However, to our knowledge, no MR studies have been conducted to explore the potential causal relationships between dietary habits and HCC risk.

The purpose of this study was to explore the potential causal associations between six dietary habits (ever/never drinkers, alcohol consumption, coffee consumption, tea consumption, milk consumption, and yoghurt consumption) and HCC risk based on MR analyses in an East Asian population.

MATERIALS AND METHODS

This MR study was reported according to the Strengthening the Reporting of Observational Studies in Epidemiology Using MR (STROBE-MR) guidelines.^[10]

Data sources and IV selection

This study was based on data from the BioBank Japan (BBJ). From 2003 to 2018, over 200,000 East Asian participants aged 20–89 years were recruited and followed up in 12 medical institutions consisting of 66 hospitals in the BBJ.^[11,12] Informed consents were collected from all subjects, and studies related to the BBJ were approved by the ethical committees of RIKEN Yokohama Institute and the Institute of Medical Science, University of Tokyo.^[11,12] All data for this study are at summary level and are publicly available at JENGER (<http://jenger.riken.jp/en/>) and the National Bioscience Database Center Human Database (<https://humandbs.biosciencedbc.jp/en/>) without access restriction.^[11,12]

For the exposure variables,^[11] genome-wide association studies (GWAS) were conducted from data in the BBJ to assess single-nucleotide polymorphisms (SNPs) associated with ever/never alcohol drinker ($n = 165,084$), alcohol consumption ($n = 58,610$), coffee consumption ($n = 152,634$), tea consumption ($n = 152,653$), milk consumption ($n = 152,965$), and yoghurt consumption ($n = 152,097$). The data for the exposures were obtained by a standardized questionnaire. For coffee, tea (green tea and other traditional tea), milk, and yoghurt, participants were required to identify the consumption frequency based on a four-point scale (1, almost every day; 2, 3–4 days per week; 3, 1–2 days per week; and 4, rarely). For alcohol drinking, participants were asked to provide the details of alcohol type, volume (milliliters), and frequency (per week). Two alcohol-related phenotypes were then included, which were ever (current or former) versus never drinker and alcohol consumption (per week). The latter was calculated by multiplying the percentage of alcohol by the volume and frequency. Age², sex, and status of diseases were included as the covariates in the GWAS of exposures. In this study, SNPs significantly related to the exposures were selected ($p < 5 \times 10^{-8}$) as IVs. SNPs with minor allele frequencies ≤ 0.05 , SNPs in high linkage disequilibrium (LD) ($LD r^2 > 0.01$ within 10,000 kilobases), and palindromic SNPs (SNPs with A/T or C/G) with effect allele frequencies between 0.4 and 0.7 were excluded.^[10,11,13] Associated phenotypes of all used SNPs were checked in Ensembl (*Homo sapiens* as phenotype) (http://grch37.ensembl.org/Homo_sapiens/Info/Index) (Table S1).^[14]

For the outcome measure, 1866 HCC cases in the BBJ^[12] (1384 men and 482 women, aged 68.0 ± 8.4

TABLE 1 Power calculation for each association between six dietary habits and hepatocellular carcinoma risk

Phenotypes	VE per IV	Cases proportion	Minimum detectable OR at 80% power	Power to detect an OR of 0.90 or 1.10
Ever/never drinkers	3.97%	0.94%	1.33	14%
Alcohol consumption	1.11%	0.94%	1.62	7%
Coffee consumption	0.30%	0.94%	2.19	6%
Tea consumption	0.01%	0.94%	6.79	5%
Milk consumption	0.01%	0.94%	6.79	5%
Yoghurt consumption	0.02%	0.94%	5.36	5%

Note: Based on two-sided $\alpha = 0.05$. The sample size for all phenotypes was $n = 197,611$ (1866 cases and 195,745 controls).

Abbreviations: OR, odds ratio; VE per IV, variation explained per instrumental variable.

years) and 195,745 controls (97,655 men and 98,090 women, aged 61.6 ± 13.9 years) were recruited. For the case group, clinical data related to the diagnosis, surgery, chemotherapy, radiotherapy, and tumor markers of HCC were collected through reviewing the medical records of cooperating institutions. HCC was diagnosed by physicians at each cooperating institution. For the control group, participants were recruited from four other Japanese population-based prospective cohorts and the BBJ (participants without HCC). Those with esophageal cancer, gastric cancer, colorectal cancer, biliary tract cancer, and pancreatic cancer were further excluded from the control group. GWAS were conducted to identify HCC-related SNPs with age, sex, and the top five principal components as covariates.

Statistical analysis

We extracted HCC data from the BBJ based on the IVs established for each dietary habit. No SNPs were absent in the outcome data set. We harmonized the GWAS data of exposures and outcomes to ensure the effect allele was related to higher levels of exposures.

MR analyses were performed using various MR methods. For consumption of tea, milk, and yoghurt, the Wald ratio (WR) was used because these IVs contain only a single SNP. For ever/never drinkers and coffee consumption (number of SNPs greater than two), inverse variance weighted (IVW) (fixed effects [FE] or random effects [RE]), MR Egger, weighted median, and weighted mode were applied. Scatter plots were further conducted to visualize the results. Leave-one-out plots were built to assess the association between each SNP in an IV and HCC risk. For alcohol consumption, only IVW was used due to the limited SNPs ($n = 2$). Cochran's Q test was used to assess SNPs heterogeneity. $p < 0.05$ was regarded as high heterogeneity, and the RE IVW was used. The odds ratios (ORs) and 95% confidence intervals (CIs) of HCC risk for one-unit change in exposures were computed.

For an MR study to be valid, an IV should satisfy the following three assumptions: (1) the relevance assumption that the IV is associated with the exposure; (2) the

independence assumption that the IV is not associated with confounders; and (3) the exclusion restriction assumption that the IV affects the outcome only through its effect on exposure.^[15] In this study, the first assumption was tested by calculating the proportion of explained variance (R^2) and the F statistics of IVs.^[15–17] $F > 10$ was considered to be a strong IV.^[17] The second and third assumptions are difficult to prove directly but can be partially met if horizontal pleiotropy (an IV influences the outcome through another exposure other than the one under investigation) is absent.^[15] Horizontal pleiotropy could be tested by whether the MR-Egger intercept is significantly different from 0.^[15] $p > 0.05$ for the MR-Egger intercept (p value of pleiotropy) provides no evidence that horizontal pleiotropy is present.^[15] The MR-Egger intercept test was only conducted for two exposures (ever/never drinkers and coffee consumption) because the SNPs used in the other four exposures were inadequate for the MR-Egger method (two or fewer SNPs).

For the power calculation, the minimum detectable ORs at 80% power and the power to detect an OR of 0.90 or 1.10 were assessed through an online tool with a type-1 error rate of 0.05 (<http://cnsngenomics.com/shiny/mRnd/>).^[18,19] The R^2 of IVs, sample size of outcome, and case proportion were needed for the power calculation. The minimum detectable ORs at 80% power were 1.33 for ever/never drinkers, 1.62 for alcohol consumption, 2.19 for coffee consumption, 6.79 for tea and milk consumption, and 5.36 for yoghurt consumption. The power to detect an OR of 0.90 or 1.10 were 14% for ever/never drinkers, 7% for alcohol consumption, 6% for coffee consumption, and 5% for consumption of tea, milk, and yoghurt (Table 1).

All analyses were conducted using the TwoSampleMR package (version 0.5.6) in R (version 4.1.0). A two-sided $p < 0.05$ was regarded as statistically significant.

RESULTS

Nine SNPs related to six dietary habits were extracted from the BBJ. Among them, five (rs1260326, rs1229984,

rs3043, rs8187929, rs671), two (rs1229984, rs671), and six (rs6681426, rs1260326, rs4410790, rs671, rs58806801, rs5760444) SNPs were selected as IVs for ever/never drinkers, alcohol consumption, and coffee consumption, respectively. One SNP (rs671) was used for consumption of tea, milk, and yoghurt. The R^2 of IVs ranged from 0.01% (tea and milk consumption) to 3.97% (ever/never drinkers). The F statistics of IVs were 6827 for ever/never drinkers, 657 for alcohol consumption, 451 for coffee consumption, 22 for tea and milk consumption, and 26 for yoghurt consumption (Table S2).

People who had ever consumed alcohol had a higher HCC risk compared to never drinkers (RE IVW: OR 1.11; 95% CI, 1.05–1.18; $p < 0.001$). The positive association was robust when MR Egger (OR, 1.13; 95% CI, 1.05–1.21; $p = 0.049$), weighted median (OR, 1.11; 95% CI, 1.07–1.16; $p < 0.001$), and weighted mode (OR, 1.12; 95% CI, 1.06, 1.18; $p = 0.015$) were used (Table 2; Figure S1). Similarly, alcohol consumption was positively associated with HCC risk (FE IVW: OR, 1.57; 95% CI, 1.32–1.86; $p < 0.001$) (Table 2). On the contrary, coffee consumption was inversely related to HCC risk based on RE IVW (OR, 0.69; 95% CI, 0.53–0.90; $p = 0.007$), MR Egger (OR, 0.57; 95% CI, 0.35–0.92; $p = 0.082$), weighted median (OR, 0.71; 95% CI, 0.57–0.88; $p = 0.002$), and weighted mode (OR, 0.62; 95% CI, 0.49–0.77; $p = 0.009$) (Table 2; Figure S2). High consumption of tea, milk, and yoghurt were also inversely associated with HCC risk, with WR ORs (95% CIs) of 0.11 (0.05–0.26), 0.18 (0.09–0.34), and 0.18 (0.09–0.34), respectively (all $p < 0.001$) (Table 2). Leave-one-out analyses indicated that rs671 was the most important SNP for the associations of ever/never

drinkers and coffee consumption with HCC risk (Figures S3 and S4). Furthermore, in an MR-Egger intercept test, no horizontal pleiotropy was found for SNPs used in ever/never drinkers or coffee consumption (p of pleiotropy = 0.575 and 0.391, respectively) (Table 2).

DISCUSSION

In the present study, we conducted MR analyses to explore the potential causal associations between six dietary habits and HCC risk in an East Asian population. Ever/never drinkers and alcohol consumption were positively associated with HCC risk. In contrast, the consumption of coffee, tea, milk, and yoghurt were inversely associated with HCC risk.

Consistent with previous observational studies, our study suggested that alcohol-related phenotypes (ever/never drinkers and alcohol consumption) were positively associated with HCC risk. A meta-analysis of 16 cohort studies showed that heavy drinkers (three or more drinks per day) had higher liver cancer risk than non-drinkers (relative risk [RR], 1.16; 95% CI, 1.01–1.34).^[4] Another meta-analysis of 11 case-control studies also indicated that alcohol consumption was positively associated with liver cancer risk (OR, 1.83; 95% CI, 1.39–2.40; higher versus lower intake).^[20] A similar positive relationship between alcohol and HCC risk in Chinese populations was reported in a meta-analysis of 18 case-control studies (3812 HCC cases and 10,927 controls) (OR, 1.56; 95% CI, 1.16–2.09; ever drinkers versus never drinkers).^[21] There are several mechanisms that could explain the positive association between alcohol drinking and HCC/liver cancer risk. First, acetaldehyde,

TABLE 2 MR estimates of associations between six dietary habits and hepatocellular carcinoma risk

Phenotypes	Number of SNPs	MR methods	OR (95% CI)	p value	p of heterogeneity	p of pleiotropy
Ever/never drinkers	5				0.088	0.575
		RE IVW	1.11 (1.05, 1.18)	<0.001		
		MR Egger	1.13 (1.05, 1.21)	0.049		
		Weighted median	1.11 (1.07, 1.16)	<0.001		
		Weighted mode	1.12 (1.06, 1.18)	0.015		
Alcohol consumption	2	FE IVW	1.57 (1.32, 1.86)	<0.001	0.750	NA
Coffee consumption	6				0.016	0.391
		RE IVW	0.69 (0.53, 0.90)	0.007		
		MR Egger	0.57 (0.35, 0.92)	0.082		
		Weighted median	0.71 (0.57, 0.88)	0.002		
		Weighted mode	0.62 (0.49, 0.77)	0.009		
Tea consumption	1	WR	0.11 (0.05, 0.26)	<0.001	NA	NA
Milk consumption	1	WR	0.18 (0.09, 0.34)	<0.001	NA	NA
Yoghurt consumption	1	WR	0.18 (0.09, 0.34)	<0.001	NA	NA

Abbreviations: CI, confidence interval; FE, fixed effects; IVW, inverse variance weighted; MR, Mendelian randomization; NA, not applicable; OR, odds ratio; RE, random effects; SNP, single-nucleotide polymorphism; WR, Wald ratio.

which is the first metabolic product of alcohol, could induce oxidative stress. It could generate DNA adducts, modify genes related to alcohol-metabolizing enzymes, and hence promote liver carcinogenesis.^[22] Second, long-term and heavy alcohol intake could lead to alcoholic cirrhosis, which may progress to HCC/liver cancer.^[20] Third, alcohol may act as a solvent, which could promote the penetration of other carcinogens into cells.^[23] In addition, alcohol drinking itself could impair hepatic detoxification and body immunity.^[20]

Coffee consumption was a protective factor for HCC in this MR study; this is in line with previous observational studies. A meta-analysis of 18 cohort studies and eight case-control studies showed that an extra two cups of coffee per day was associated with a 35% reduced HCC risk (RR, 0.65; 95% CI, 0.59–0.72).^[5] An umbrella review also revealed an inverse association between coffee consumption and liver cancer risk (RR, 0.48; 95% CI, 0.40–0.58).^[24] Similar results were reported in some other meta-analyses.^[25–27] Several biological mechanisms were reported for the protective association between coffee intake and HCC/liver cancer. First, some bioactive compounds in coffee (such as chlorogenic acids and polyphenols) may exert an anticarcinogenic effect due to their antioxidant and anti-inflammatory properties.^[28,29] Second, coffee compounds, like chlorogenic acid, could also inhibit the production of hyperplastic liver cell foci^[30] and pre-neoplastic liver lesions,^[31] hence suppressing growth of liver tumor.^[30] In addition, cafestol and kahweol in coffee may be able to improve metabolism and excretion of carcinogens by promoting the activity of phase 2 liver enzymes.^[32,33] Furthermore, caffeine in coffee could inhibit the activity and replication of hepatitis virus, thereby preventing the development of liver cancer.^[34] Moreover, coffee may be able to reduce insulin resistance and decrease the risk of type 2 diabetes, which are important risk factors for liver cancer.^[35,36]

Tea consumption was inversely associated with HCC risk in this study, which is in agreement with previous observational studies. A recent umbrella review and meta-analysis showed that higher intake of green tea (versus lower intake) was related to a reduced liver cancer risk (RR, 0.87; 95% CI, 0.78–0.98; $p = 0.026$).^[37] Another meta-analysis of nine prospective cohort studies (465,274 participants with 3694 liver cancer cases) also indicated a significant inverse association between green tea consumption and liver cancer risk in Asian populations (RR, 0.88; 95% CI, 0.81–0.97; the highest versus lowest intake).^[6] One potential mechanism for the inverse association between tea intake and HCC is related to tea polyphenols. It was reported that some polyphenols (such as epigallocatechin gallate [EGCG], epigallocatechin, epicatechin gallate, and epicatechin) could inhibit tumor cell growth by scavenging reactive oxygen species,^[38] promoting tumor cell apoptosis,^[39] as well as suppressing angiogenesis,^[40]

DNA methylation,^[41] and related enzymes.^[42] Several studies have also reported significantly lower tumor incidence and recurrence rates in animals treated with EGCG than in controls.^[43,44]

Inverse associations between consumption of dairy products (milk and yoghurt) and HCC risk were observed in this study, while previous epidemiological studies had mixed results. Compatible with our study findings, a meta-analysis of seven cohort studies and eight case-control studies found yogurt intake was inversely associated with liver cancer risk (RR, 0.50; 95% CI, 0.27–0.91; highest versus lowest intake).^[7] The dose–response analysis also showed that a 40 g/day increment of yogurt intake was associated with a 5.4% decreased risk of liver cancer ($p = 0.002$).^[7] Nevertheless, in the same study, consumption of milk (RR, 1.17; 95% CI, 0.87–1.57), total dairy products (RR, 1.08; 95% CI, 0.78–1.51), and cheese and curd (RR, 1.16; 95% CI, 0.83–1.52) were not related to liver cancer risk (highest versus lowest intake).^[7] Another meta-analysis of four related studies (472 HCC cases and 954,592 controls) also indicated an absence of association between fermented dairy products (yogurt or cheese) and HCC risk (RR, 0.75; 95% CI, 0.37–1.54; intake versus no intake).^[45] Several mechanisms might explain the possible protective associations between dairy products and HCC risk. First, dairy foods contain many beneficial components that could reduce cancer risk. For example, calcium was suggested to bind toxic secondary bile acids and free fatty acids, hence inhibiting their carcinogenic effect. Lactoferrin has an antitumor property due to its ability to reduce DNA damage, which could enhance immune function and suppress inflammation.^[46] Second, dairy products, especially yogurt, are associated with several cancer-related gut microbiota. For instance, *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* are two lactic acid bacteria that are involved in the production of yogurt from milk, and they have been shown to be effective in preventing carcinogenesis and stimulating the immune system.^[47] However, the protective associations of milk and yogurt intake with HCC risk in this MR study have not been significantly presented in previous observational studies.^[7,45] This discrepancy could be explained in part by the inherent limitations of observational studies, namely the presence of potential uncontrolled confounders that could mask the true association, while the MR design of this study might overcome this limitation.^[7]

Assumptions of relevance, independence, and exclusion restriction should be fulfilled for an MR study to be valid.^[15] In the present study, the relevance assumption was met because all IVs had an F statistic greater than 10. There was potential biological plausibility for the associations between the used genetic variants and the six dietary habits. In the case of alcohol drinking, the family member of aldehyde dehydrogenase (*ALDH*)

and alcohol dehydrogenase (*ADH*) are known alcohol-related genes in East Asian populations, especially for the *ALDH2* gene (rs671). The minor A allele of rs671 could slow acetaldehyde metabolism, thereby leading to the flush response and other adverse effects. As a result, people without the A allele may have a greater capacity for alcohol drinking than those with the A allele.^[15] For coffee, many factors are involved in its metabolism and biological functions. For example, cytochrome P450 1A2 (*CYP1A2*) is an important enzyme for caffeine metabolism, and aryl hydrocarbon receptor (*AHR*) is an upstream inducer for *CYP1A2* transcription.^[48] Cafestol may induce cell apoptosis through lowering the myeloid cell leukemia 1 level,^[49] and caffeine is an antagonist for adenosine A2a receptor.^[11] Therefore, the polymorphisms in the encoded genes of these factors may influence coffee consumption. For instance, people who carried the C allele of rs4410790 (*AHR* gene) had higher coffee consumption than those who did not carry it.^[48] Moreover, the associations between rs671 and consumption of tea, milk, and yoghurt may be mediated by alcohol drinking because rs671 has a stronger effect on alcohol-related phenotypes than other dietary habits and these dietary habits could be influenced by alcohol drinking.^[11]

For the independence and exclusion restriction assumptions, a common SNP (rs671) was used as IVs for each dietary habit, suggesting that the six dietary habits may have pleiotropic effects (i.e., the SNP could affect the outcome through multiple pathways^[15]). However, the pleiotropic effects can be classified into horizontal pleiotropy (the SNP affects the outcome through another phenotype other than the one under investigation) and vertical pleiotropy (the SNP first affects the phenotype of interest, which could then influence other phenotypes, ultimately affecting the outcome).^[15] The presence of horizontal pleiotropy indicates the violation of independence and exclusion restriction assumptions. The vertical pleiotropy, however, is not only acceptable in MR but is also essential to MR as it shows that an exposure affects a downstream outcome.^[15] In this study, the pleiotropic effects were more likely to be vertical pleiotropy instead of horizontal pleiotropy as the effects of rs671 on ever/never drinkers and alcohol consumption were the strongest among the six dietary habits and alcohol drinking itself could affect other dietary habits.^[11] Furthermore, in the sensitivity analyses, the p values of the MR-Egger intercept (p values of pleiotropy) were used to test horizontal pleiotropy for SNPs used in ever/never drinkers and coffee consumption. The results showed that all values of pleiotropy were $p > 0.05$, suggesting that horizontal pleiotropy was not presented.

Although this study evaluated the potential causal associations between dietary habits and HCC risk in an East Asian population, several limitations should be mentioned. First, the robustness of the results of

several exposures (consumption of alcohol, tea, milk, and yoghurt) could not be tested by using different MR methods because these exposures had a small number of SNPs. Second, owing to the low number of HCC cases in the outcome data set and the low explained variations in exposures for IVs, the statistical power for each association between the six dietary habits and HCC risk was low. Third, the summary-level data imposed restrictions on performing subgroup analyses based on important covariates (such as age, sex, and comorbid conditions). Fourth, the consumption of coffee, tea, milk, and yoghurt was obtained by a questionnaire that only included the consumption frequency but not the actual consumption amount. Therefore, the ORs did not reflect the alteration of HCC risk for changes in the actual intake of these dietary habits; instead, they only indicated the change in HCC risk for higher versus lower consumption frequency. Fifth, in a two-sample MR study, bias caused by the winner's curse will occur when data on the exposures and outcome are derived from the same population.^[10] Nevertheless, for a binary outcome involving cases and controls, unbiased results can be obtained even in a one-sample setting if the associations between SNPs and exposures are assessed only in the controls.^[50] In this study, all participants in the exposure database had absence of HCC.⁽¹¹⁾ Last, the results of this study should be interpreted with caution when generalizing to other ethnic populations due to the use of East Asian GWAS data.

In conclusion, the present MR study showed that, in this East Asian population, ever drinkers (versus never drinkers) and higher alcohol consumption are associated with higher HCC risk. On the contrary, higher consumption of coffee, tea, milk, and yoghurt are associated with lower HCC risk. These findings highlight the importance of maintaining healthy diet habits in HCC prevention. Further MR analyses with more and stronger SNPs and larger HCC cases are needed. External validation of our finding in other ethnic populations based on larger HCC data sets is also necessary.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Yunyang Deng conceived and designed the current study, conducted the data acquisition, analyzed the data, and drafted the manuscript. Junjie Huang proofread the data and manuscript. Martin C. S. Wong interpreted the data and critically revised the manuscript. All authors approved the submitted version. Yunyang Deng and Martin C. S. Wong had full access to all the

data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

DATA AVAILABILITY STATEMENT

All data used in this study are publicly available on the JENGER website (<http://jenger.riken.jp/en/>) and the National Bioscience Database Center Human Database (<https://humandbs.biosciencedbc.jp/en/>) without any access restriction. To assess the data, please contact the corresponding author.

ORCID

Yunyang Deng  <https://orcid.org/0000-0003-4848-2953>

Junjie Huang  <https://orcid.org/0000-0003-2382-4443>

Martin C. S. Wong  <https://orcid.org/0000-0001-7706-9370>

REFERENCES

- Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2021;71:209–49.
- Tian T, Xiao F, Li H, Ding D, Dong W, Hou G, et al. Association between serum iron status and primary liver cancer risk: a Mendelian randomization analysis. *Ann Transl Med.* 2021;9:1533.
- McGlynn KA, Petrick JL, El-Serag HB. Epidemiology of hepatocellular carcinoma. *Hepatology.* 2021;73(Suppl 1):4–13.
- Turati F, Galeone C, Rota M, Pelucchi C, Negri E, Bagnardi V, et al. Alcohol and liver cancer: a systematic review and meta-analysis of prospective studies. *Ann Oncol.* 2014;25:1526–35.
- Kennedy OJ, Roderick P, Buchanan R, Fallowfield JA, Hayes PC, Parkes J. Coffee, including caffeinated and decaffeinated coffee, and the risk of hepatocellular carcinoma: a systematic review and dose–response meta-analysis. *BMJ Open.* 2017;7:e013739.
- Huang Y-Q, Lu X, Min H, Wu Q-Q, Shi X-T, Bian K-Q, et al. Green tea and liver cancer risk: a meta-analysis of prospective cohort studies in Asian populations. *Nutrition.* 2016;32:3–8.
- Zhao Q, He Y, Wang K, Wang C, Wu H, Gao L, et al. Dairy consumption and liver cancer risk: a systematic review and dose-response meta-analysis of observational studies. *Nutr Cancer.* 2021;73:2821–31.
- Guo J-Z, Xiao Q, Gao S, Li X-Q, Wu Q-J, Gong T-T. Review of Mendelian randomization studies on ovarian cancer. *Front Oncol.* 2021;11:681396.
- Kho P-F, Amant F, Annibaldi D, Ashton K, Attia J, Auer PL, et al. Mendelian randomization analyses suggest a role for cholesterol in the development of endometrial cancer. *Int J Cancer.* 2021;148:307–19.
- Skrivankova VW, Richmond RC, Woolf BAR, Davies NM, Swanson SA, VanderWeele TJ, et al. Strengthening the reporting of observational studies in epidemiology using Mendelian randomisation (STROBE-MR): explanation and elaboration. *BMJ.* 2021;375:n2233.
- Matoba N, Akiyama M, Ishigaki K, Kanai M, Takahashi A, Momozawa Y, et al. GWAS of 165,084 Japanese individuals identified nine loci associated with dietary habits. *Nat Hum Behav.* 2020;4:308–16.
- Ishigaki K, Akiyama M, Kanai M, Takahashi A, Kawakami E, Sugishita H, et al. Large-scale genome-wide association study in a Japanese population identifies novel susceptibility loci across different diseases. *Nat Genet.* 2020;52:669–79.
- Hemani G, Zheng J, Elsworth B, Wade KH, Haberland V, Baird D, et al. The MR-Base platform supports systematic causal inference across the human phenome. *eLife.* 2018;7:e34408.
- Wang T, Xu L. Circulating vitamin E levels and risk of coronary artery disease and myocardial infarction: a Mendelian randomization study. *Nutrients.* 2019;11:2153.
- Davies NM, Holmes MV, Davey Smith G. Reading Mendelian randomisation studies: a guide, glossary, and checklist for clinicians. *BMJ.* 2018;362:k601.
- Mozaffarian D, Kabagambe EK, Johnson CO, Lemaitre RN, Manichaikul A, Sun QI, et al. Genetic loci associated with circulating phospholipid trans fatty acids: a meta-analysis of genome-wide association studies from the CHARGE Consortium. *Am J Clin Nutr.* 2015;101:398–406.
- Xu L, Hao YT. Effect of handgrip on coronary artery disease and myocardial infarction: a Mendelian randomization study. *Sci Rep.* 2017;7:954.
- Burgess S. Sample size and power calculations in Mendelian randomization with a single instrumental variable and a binary outcome. *Int J Epidemiol.* 2014;43:922–9.
- Brion MJ, Shakhbazov K, Visscher PM. Calculating statistical power in Mendelian randomization studies. *Int J Epidemiol.* 2013;42:1497–501.
- He F, Sha Y, Wang B. Relationship between alcohol consumption and the risks of liver cancer, esophageal cancer, and gastric cancer in China: meta-analysis based on case-control studies. *Medicine (Baltimore).* 2021;100:e26982.
- Hartling L, Li Y, Yang H, Cao J. Association between alcohol consumption and cancers in the Chinese population—a systematic review and meta-analysis. *PLoS One.* 2011;6:e18776.
- Seitz HK, Stickel F. Molecular mechanisms of alcohol-mediated carcinogenesis. *Nat Rev Cancer.* 2007;7:599–612.
- Papadimitriou N, Markozannes G, Kannelopoulou A, Critselis E, Alhardan S, Karafousia V, et al. An umbrella review of the evidence associating diet and cancer risk at 11 anatomical sites. *Nat Commun.* 2021;12:4579.
- Zhao L-G, Li Z-Y, Feng G-S, Ji X-W, Tan Y-T, Li H-L, et al. Coffee drinking and cancer risk: an umbrella review of meta-analyses of observational studies. *BMC Cancer.* 2020;20:101.
- Tanaka K, Hara M, Sakamoto T, Higaki Y, Mizuta T, Eguchi Y, et al. Inverse association between coffee drinking and the risk of hepatocellular carcinoma: a case-control study in Japan. *Cancer Sci.* 2007;98:214–8.
- Bravi F, Tavani A, Bosetti C, Boffetta P, La Vecchia C. Coffee and the risk of hepatocellular carcinoma and chronic liver disease: a systematic review and meta-analysis of prospective studies. *Eur J Cancer Prev.* 2017;26:368–77.
- Tanaka K, Tamakoshi A, Sugawara Y, Mizoue T, Inoue M, Sawada N, et al.; Research Group for the Development and Evaluation of Cancer Prevention Strategies in Japan. Coffee, green tea and liver cancer risk: an evaluation based on a systematic review of epidemiologic evidence among the Japanese population. *Jpn J Clin Oncol.* 2019;49:972–84.
- Scalbert A, Johnson IT, Saltmarsh M. Polyphenols: antioxidants and beyond. *Am J Clin Nutr.* 2005;81(Suppl):215S–217S.
- Salomone F, Galvano F, Li Volti G. Molecular bases underlying the hepatoprotective effects of coffee. *Nutrients.* 2017;9:85.
- Tanaka T, Nishikawa A, Shima H, Sugie S, Shinoda T, Yoshimi N, et al. Inhibitory effects of chlorogenic acid, reserpine, polyphenolic acid (E-5166), or coffee on hepatocarcinogenesis in rats and hamsters. *Basic Life Sci.* 1990;52:429–40.
- Ferk F, Huber WW, Grasl-Kraupp B, Speer K, Buchmann S, Bohacek R, et al. Protective effects of coffee against induction of DNA damage and pre-neoplastic foci by aflatoxin B₁. *Mol Nutr Food Res.* 2014;58:229–38.
- Cavin C, Holzhaeuser D, Scharf G, Constable A, Huber WW, Schilter B. Cafestol and kahweol, two coffee specific

- diterpenes with anticarcinogenic activity. *Food Chem Toxicol.* 2002;40:1155–63.
33. Huber WW, Scharf G, Rossmann W, Prustomsky S, Grasl-Kraupp B, Peter B, et al. The coffee components kahweol and cafestol induce gamma-glutamylcysteine synthetase, the rate limiting enzyme of chemoprotective glutathione synthesis, in several organs of the rat. *Arch Toxicol.* 2002;75:685–94.
 34. Batista MN, Carneiro BM, Braga AC, Rahal P. Caffeine inhibits hepatitis C virus replication in vitro. *Arch Virol.* 2015;160:399–407.
 35. Tunncliffe JM, Shearer J. Coffee, glucose homeostasis, and insulin resistance: physiological mechanisms and mediators. *Appl Physiol Nutr Metab.* 2008;33:1290–300.
 36. Tanaka K, Tsuji I, Tamakoshi A, Matsuo K, Wakai K, Nagata C, et al.; Research Group for the Development and Evaluation of Cancer Prevention Strategies in Japan. Diabetes mellitus and liver cancer risk: an evaluation based on a systematic review of epidemiologic evidence among the Japanese population. *Jpn J Clin Oncol.* 2014;44:986–99.
 37. Kim TL, Jeong GH, Yang JW, Lee KH, Kronbichler A, van der Vliet HJ, et al. Tea consumption and risk of cancer: an umbrella review and meta-analysis of observational studies. *Adv Nutr.* 2020;11:1437–52.
 38. Higdon JV, Frei B. Tea catechins and polyphenols: health effects, metabolism, and antioxidant functions. *Crit Rev Food Sci Nutr.* 2003;43:89–143.
 39. Yang GY, Liao J, Kim K, Yurkow EJ, Yang CS. Inhibition of growth and induction of apoptosis in human cancer cell lines by tea polyphenols. *Carcinogenesis.* 1998;19:611–6.
 40. Shimizu M, Deguchi A, Lim JT, Moriwaki H, Kopelovich L, Weinstein IB. (-)-Epigallocatechin gallate and polyphenon E inhibit growth and activation of the epidermal growth factor receptor and human epidermal growth factor receptor-2 signaling pathways in human colon cancer cells. *Clin Cancer Res.* 2005;11:2735–46.
 41. Fang MZ, Wang Y, Ai N, Hou Z, Sun Y, Hong L, et al. Tea polyphenol (-)-epigallocatechin-3-gallate inhibits DNA methyltransferase and reactivates methylation-silenced genes in cancer cell lines. *Cancer Res.* 2003;63:7563–70.
 42. Yang CS, Wang X, Lu G, Picinich SC. Cancer prevention by tea: animal studies, molecular mechanisms and human relevance. *Nat Rev Cancer.* 2009;9:429–39.
 43. Dreosti IE, Wargovich MJ, Yang CS. Inhibition of carcinogenesis by tea: the evidence from experimental studies. *Crit Rev Food Sci Nutr.* 1997;37:761–70.
 44. Lu YP, Lou YR, Lin Y, Shih WJ, Huang MT, Yang CS, et al. Inhibitory effects of orally administered green tea, black tea, and caffeine on skin carcinogenesis in mice previously treated with ultraviolet B light (high-risk mice): relationship to decreased tissue fat. *Cancer Res.* 2001;61:5002–9.
 45. Zhang K, Dai H, Liang W, Zhang L, Deng Z. Fermented dairy foods intake and risk of cancer. *Int J Cancer.* 2019;144:2099–108.
 46. Norat T, Riboli E. Dairy products and colorectal cancer. A review of possible mechanisms and epidemiological evidence. *Eur J Clin Nutr.* 2003;57:1–17.
 47. Pala V, Sieri S, Berrino F, Vineis P, Sacerdote C, Palli D, et al. Yogurt consumption and risk of colorectal cancer in the Italian European prospective investigation into cancer and nutrition cohort. *Int J Cancer.* 2011;129:2712–9.
 48. Josse AR, Da Costa LA, Campos H, El-Sohehy A. Associations between polymorphisms in the AHR and CYP1A1-CYP1A2 gene regions and habitual caffeine consumption. *Am J Clin Nutr.* 2012;96:665–71.
 49. Choi MJ, Park EJ, Oh JH, Min K-J, Yang ES, Kim YH, et al. Cafestol, a coffee-specific diterpene, induces apoptosis in renal carcinoma Caki cells through down-regulation of anti-apoptotic proteins and Akt phosphorylation. *Chem Biol Interact.* 2011;190:102–8.
 50. Burgess S, Davies NM, Thompson SG. Bias due to participant overlap in two-sample Mendelian randomization. *Genet Epidemiol.* 2016;40:597–608.

SUPPORTING INFORMATION

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

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