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Original Article

Genetic association of cardiovascular disease related biomarkers with the overall survival of hepatocellular carcinoma: a Mendelian randomization analysis

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Abstract: Observational studies have reported associations between circulating biomarkers related to cardiovascular disease and the survival of patients with hepatocellular carcinoma. However, the relationship between these biomarkers and survival remains controversial. We conducted a two-sample Mendelian randomization analysis to investigate possible causal associations between cardiovascular disease biomarkers and hepatocellular carcinoma survival. Genetic risk scores, calculated using individual-level data from 866 cases of hepatitis B virus-related hepatocellular carcinoma in Guangxi, were utilized as proxies for four cardiovascular disease biomarkers: C-reactive protein, Apolipoprotein A-1, Cystatin C, and Lipoprotein(a). Associations between the genetic scores and survival were analyzed using Cox regression. The inverse-variance weighted method was used to estimate the summary statistics for the biomarkers and survival. Considering the multiple comparisons, the statistical significance was set at $P < 0.0125$. We observed a significant risk signal between genetically increased Cystatin C levels and poorer survival in hepatocellular carcinoma (HR for genetic scores = 1.29, 95% CI = 1.02-1.64; HR for inverse-variance weighted = 2.60, 95% CI = 1.45-4.65). Furthermore, we found a causal relationship of genetically determined Cystatin C and Lipoprotein(a) level with the survival of hepatocellular carcinoma patients with embolus. Our findings indicated the causal effects of increased levels of Cystatin C and Lipoprotein(a) on poorer survival in hepatocellular carcinoma.

Keywords: Cardiovascular disease biomarkers, hepatocellular carcinoma, Mendelian randomization, survival

Introduction

Primary liver cancer (PLC) is the sixth most commonly diagnosed cancer and the third leading cause of cancer death Worldwide [1]. According to the World Health Organization, more than 1 million people will die of PLC, a serious threat to human health, by 2030 [1]. Hepatocellular carcinoma (HCC) is the most common pathological type of PLC, accounting for 85% to 90% of all cases [2]. HCC has an insidious onset with no obvious early symptoms and is highly malignant, progressing rapidly. Although most patients can be treated with surgical resection, the rates of recurrence and mortality remain quite high. Therefore, the indicators that effectively reflect the prognosis of HCC patients undergoing liver resection may have significant guiding significance for further precise treatment of HCC patients. They also hold important value in reducing the mortality rate and prolonging the survival of patients with HCC.

Cardiovascular disease (CVD) may be a risk factor for cancer incidence or mortality, indepen-

dent of shared risk factors such as obesity, smoking, and diabetes [3, 4]. However, the exact mechanisms underlying the association of CVD and cancer remain unclear. Recent investigations have proposed that certain molecules associated with CVD, such as C-reactive protein (CRP), Apolipoprotein A-1 (APOA1), Cystatin C (CysC), and Lipoprotein(a) [LP(a)], may play a role in the development of HCC through common pathways [5-8]. These pathways include such as inflammation, oxidative stress, adiposity, and fibrosis. For example, it has been reported that CRP may participate in tumor metastasis by regulating HIF-1α activity through the MEK/ERK and PI3K/AKT signaling pathways [9]. Although multiple observational studies have suggested a close relationship between CVD markers and the disease process of HCC, there is no consistent conclusion regarding the direction of the associations between these CVD markers and the prognosis of HCC [6, 7, 10-15].

Mendelian randomization (MR) is an effective method for testing the causality between an exposure and an outcome. In this approach, genetic variants are primarily used as instrumental variables (IVs) to explore the causal relationship between exposure and outcome phenotypes [16]. The rationale for conducting MR analysis lies in the fact that it can overcome the limitations of observational studies, which are unable to establish causal inference [17]. By fully utilizing genetic data, MR defines biomarker levels based on genetic predispositions. MR can be used to evaluate the causal direction from the marker levels to the risk of disease outcome, while avoiding the influence of environmental effects. This provides a theoretical basis for the further selection of biomarkers with a clear causal direction. Therefore, we conducted two sample Mendelian randomization (MR) analyses in this study to identify CVD markers (exposure) that are causally associated with HCC survival (outcome).

Materials and methods

Genetic variant data on CVD biomarkers

We selected instrumental variables for analysis of the causal effects based on a suggestive significant association ($P < 5\times10^{-6}$) with cardiovascular disease-related circulating biomarkers, including CRP, APOA1, CysC, and LP(a). Single nucleotide polymorphism (SNP) associated with CRP level was selected from a GWAS of 10,112 individuals in the Biobank Japan Project [18]. The SNPs associated with APOA1, CysC, and LP(a) levels were selected from a GWAS of the UK biobank East Asian population [APOA1, n = 2325; CysC, n = 2573; LP(a), n = 2279] (https://pan.ukbb.broadinstitute.org/). The independent SNPs of CVD biomarkers were selected based on a linkage disequilibrium (LD) $R^2 < 0.001$ and kb = 10000. The F-value is calculated and used to evaluate whether there is a weak instrumental variable bias. The F-value is calculated using the formula F-value = $(\beta_{exposure})^2/(SE_{exposure})^2$.

GWAS data for hepatocellular carcinoma

The GWAS data for hepatocellular carcinoma (HCC) survival was collected from 866 HBV-HCC patients who were recruited from Guangxi Medical University Cancer Hospital between July 2007 and December 2017. The patients were selected based on predefined inclusion and exclusion criteria [19]. All 866 patients were followed up every three months for the first two years after discharge following hepatectomy, and every six months thereafter. The follow-up included information on subsequent treatment and death, with overall survival as the outcome event. The last follow-up was conducted in March 2020. Moreover, approval from the ethics committee and informed consent were obtained from the Guangxi Medical University Cancer Hospital. All 866 patients had 5 mL of peripheral blood extracted for DNA extraction. The process of DNA extraction, genotyping, quality control, and imputation can be found in an article we previously published [19].

Genetic risk score for CVD biomarker level

In order to predict the individual genetic levels of CRP, APOA1, CysC, and LP(a), we used the genotype data to construct the CVD biomarker genetic risk score (GRS) based on their associated SNPs. GRSs were calculated using the following formula:

$$\text{CVD\_biomarker-GRS}_i = \sum_{j=1}^{n} BjXij$$

Xij represents the number of alleles related to increased CVD biomarker levels for the *j*th SNP of the *i*th subject (where Xij can be 0, 1, or 2). *Bj*

Table 1. Characteristics and clinical features of patients

Covariances		Death/Total	HR	95% CI	*P*-value
Age (year)	< 47[1]	233/434	1	-	0.036
	≥ 47	186/432	0.81	(0.66, 0.99)	
Sex	Female	42/106	1	-	0.176
	male	377/760	1.26	(0.90, 1.76)	
Smoking	No	268/545	1	-	0.475
	Yes	151/321	0.91	(0.71, 1.17)	
Drinking	No	292/614	1	-	0.541
	Yes	127/252	1.08	(0.84, 1.41)	
BCLC	0/A	146/427	1	-	< 0.001
	B/C	273/439	1.98	(1.56, 2.52)	
Embolus	No	260/636	1	-	< 0.001
	Yes	159/230	1.74	(1.38, 2.21)	
AFP (ng/ml)	≤ 400	232/522	1	-	0.015
	> 400	187/344	1.29	(1.05, 1.57)	
Cirrhosis	No	184/390	1	-	0.702
	Yes	235/476	1.04	(0.85, 1.26)	

HR, hazard ratio; BCLC, Barcelona Clinic Liver Cancer stage; AFP, alpha fetoprotein. [1]The median age of this population.

refers to the beta estimates for the jth SNP obtained from the exposure GWAS in the BBJ project or the UKB East Asia population. The GRS could be used as an instrumental variable to represent the weighted number of alleles associated with increased CVD biomarker levels. It can then be used to predict the CVD biomarker level of each individual included in the Mendelian randomization analysis. The genetic levels of CRP, APOA1, CysC, and LP(a) were represented by 9, 9, 6, and 5 SNPs, respectively (see Table S1).

Mendelian randomization analysis

Two-sample MR analysis was used to examine the causal relationships between CVD biomarker levels and overall survival in HCC. The causal effects were estimated using the GRS method with our SNP collections. Namely, individual-level data was aggregated into a univariate score (GRS) for survival analysis. Conducting survival analysis on GRS and HCC patients' survival to infer the causal relationship between CVD biomarkers and HCC survival. We also utilized the inverse-variance weighted (IVW), MR Egger, and MR-PRESSO methods for conducting sensitivity analysis on the summary statistics data. Additionally, we employed Egger regression to assess the presence of pleiotropic effects in the CVD biomarker SNPs.

Statistical analysis

Cox proportional hazards regression models were used to analyze the association between GRS and overall survival of HCC. The models were adjusted for age, sex, smoking status, drinking status, alpha fetoprotein (AFP) levels, Barcelona Clinic Liver Cancer (BCLC) stage, embolus status, and cirrhosis status. The optimal cutoff values for GRS levels were determined using the "surv_cutpoint" function from the "survminer" R package. These cutoff values were then used to classify patients with HCC into two groups: high level and low level. The results of the MR analysis were considered significant at a *P* value of less than 0.0125 (Bonferroni correction: $P = 0.05/4$). A *p*-value between 0.0125 and 0.05 was considered suggestive evidence for a potential causal association [17]. The MR analyses utilized the "TwoSampleMR" and "MRPRESSO" packages. The image was created using the "TwoSampleMR" package and GraphPad Prism 8.

Results

Basic characteristics

The basic characteristics of 866 HBV-HCC patients were presented in **Table 1**. We observed significant associations of age, BCLC stage, embolus status, and AFP level with the overall survival (OS) of the 866 HBV-HCC patients. The group of individuals under the age of 47, with BCLC B or C stage, embolus, and AFP levels greater than 400 ng/ml had significantly poorer survival compared to the other group with corresponding characteristics. Therefore, in the subsequent Mendelian randomization analysis, we conducted a stratified analysis among the aforementioned factors.

GRS of CVD biomarker levels and HCC survival in the Southern Chinese population

We calculated the GRS of CRP, APOA1, CysC, and LP(a) using the hazard ratios (HRs) of each relevant SNP in additive models (see Tables S2, S3, S4 and S5). The optimal cutoff values for

Table 2. Mendelian randomization estimates for the causal effect of CVD biomarkers level on the overall survival of hepatocellular carcinoma in the Southern Chinese population

CVD biomarkers			Death/Total	HR[1]	95% CI	*P*-value
CRP	GRS	< 0.869	240/526	1	1	/
		≥ 0.869	179/340	1.09	0.90-1.32	0.394
	IVW		419/866	1.29	0.66-2.52	0.458
	MR Egger		419/866	0.24	0.01-2.16	0.250
	MR-PRESSO		419/866	1.29	0.79-1.79	0.354
ApoA1	GRS	< 1.783	344/724	1	1	/
		≥ 1.783	75/142	1.21	0.94-1.55	0.146
	IVW		419/866	1.03	0.74-1.44	0.858
	MR Egger		419/866	0.69	0.11-4.36	0.697
	MR-PRESSO		419/866	1.05	0.81-1.29	0.701
CysC	GRS	< 1.311	85/212	1	1	/
		≥ 1.311	334/654	1.29	1.02-1.64	0.035*
	IVW		419/866	2.60	1.45-4.65	0.001*
	MR Egger		419/866	2.53	0.85-7.53	0.169
	MR-PRESSO		419/866	2.60	2.29-2.91	0.002*
LP(a)	GRS	< 0.275	186/411	1	1	/
		≥ 0.275	233/455	1.21	1.00-1.47	0.056
	IVW		419/866	1.23	0.86-1.76	0.259
	MR Egger		419/866	1.09	0.58-2.03	0.810
	MR-PRESSO		419/866	1.23	0.87-1.59	0.322

CVD, cardiovascular disease; HR, hazard ratio; CRP, C-reactive protein; ApoA1, Apolipoprotein A-1; CysC, Cystatin C; LP(a), Lipoprotein(a); GRS, Genetic risk score; IVW, inverse-variance weighted; MR, mendelian randomization. [1]adjusted by age, sex, smoking status, drinking status, BCLC stage, embolus status, cirrhosis status, and AFP level in multivariate Cox regression analyses of GRS. *$P < 0.05$.

the GRS of CRP, APOA1, CysC, and LP(a) calculated using the Yoden Index are 0.869, 1.783, 1.311, and 0.275, respectively. According to the optimal cutoff, we divided the GRS into a binary variable (e.g., GRS < 0.869 or GRS ≥ 0.869, **Table 2**). The results of Egger regression analysis suggested that the instrumental variable of CRP rs12502614 exhibited directional pleiotropy (Egger-intercept = 0.305, $P = 0.025$). We evaluated the association between genetic CRP levels and HCC survival after removing rs12502614.

We found evidence of causal associations between CysC and overall survival in patients with HCC (**Table 2**). Genetically elevated CysC was associated with an increased risk of HCC mortality after adjusting for age, sex, smoking, drinking, BCLC, embolus, and AFP (GRS ≥ 1.311 vs. GRS < 1.311, HR = 1.29, 95% CI = 1.02-1.64, $P = 0.035$). The analysis revealed a consistent relationship between genetic CysC levels and HCC mortality, as demonstrated by inverse-variance weighted regression (HR = 2.59, 95% CI = 1.49-4.51, $P = 0.001$) and MR-PRESSO (HR = 2.60, 95% CI = 2.29-2.91, $P = 0.002$) (**Table 2**). Although no significant result was found using the MR Egger regression approach, the scatter plot indicated that the results obtained from both methods (IVW and MR Egger) were consistent in their directions (**Figure 1**). We also observed trends indicating a higher risk of mortality in HCC patients with genetically elevated levels of CRP, APOA1, and LP(a). However, these associations did not reach statistical significance.

Modification effect on the association between genetic CVD biomarker levels and HCC survival

In order to investigate the modification effect of clinical features on the relationship between genetic CVD biomarker levels and overall survival in HCC, we conducted a stratified analysis. However, no interaction was found between these clinical features and the GRS (see Table S6). We found a causal effect of genetic CysC levels and suggestive evidence of potential

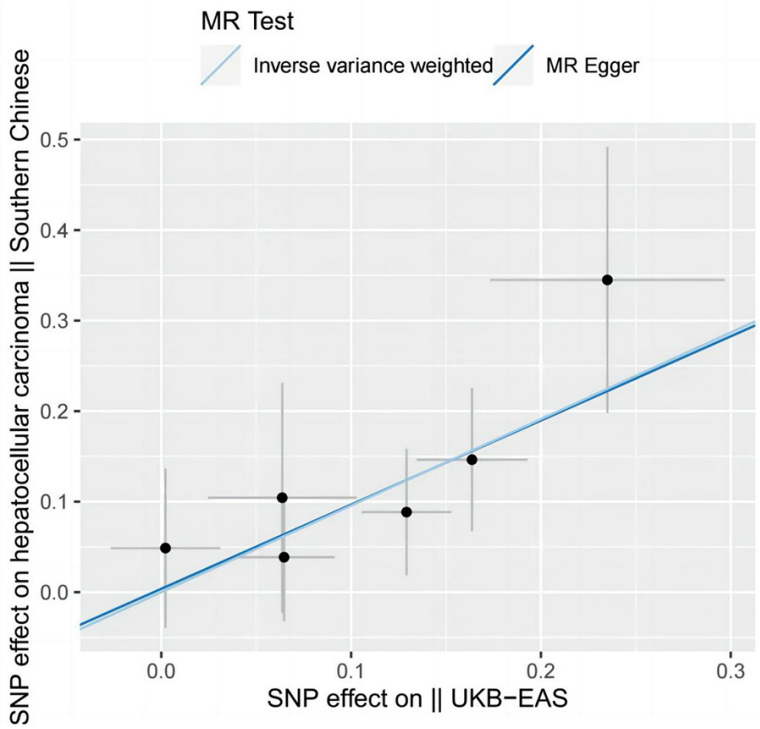

Figure 1. Scatter plot of the effect of each SNP on Cystatin C level and hepatocellular carcinoma survival. Scatter plots show the per-allele association with HCC survival plotted against the per-allele association with CysC levels, with vertical and horizontal black lines showing the 95% CI for each SNP. The scatter plot is overlaid with the Mendelian randomization estimate (slope of solid line with dashed lines showing 95% CI) of the effect of CysC on HCC survival.

causal effects of genetic LP(a) levels on overall survival in certain groups with HCC. The HCC patients with a genetically higher CysC level (GRS ≥ 1.311) had an increased risk of mortality compared to those with a lower CysC level (GRS < 1.311) in the age group < 47 years old [HR = 1.54 (95% CI = 1.10-2.15); *P* = 0.011], and in the group without embolus [HR = 1.38 (95% CI = 1.01-1.89); *P* = 0.042] (**Figure 2A** and **2E**). The HCC patients with a genetically higher LP(a) level (GRS ≥ 0.275) had an increased risk of mortality compared to those with a lower LP(a) level (GRS < 0.275) in the group without embolus (HR = 1.34, 95% CI = 1.05-1.72, *P* = 0.020) (**Figure 2E**). Although no significant associations were found in other subgroups, we observed consistent trends across all subgroups, with the same trends as discovered in the overall population (**Figure 2B-D**, **2F-H**).

Discussion

In this study, we conducted a two-sample Mendelian randomization analysis to examine the causal effects of CVD biomarkers on HCC survival. We utilized both individual data and summary data for our analysis. We constructed a weighted GRS as an instrumental variable, which is composed of genetic variants associated with CVD biomarkers that were identified in genome-wide association studies. Our findings mainly suggest a causal association of increased CysC levels and a potential causal association of LP(a) levels with reduced survival in HCC patients, particularly in those who are younger than 47 years or do not have embolus. The results from the summarized data (IVW & MR-PRESSO) agree with the trends observed in the two-stage regression analysis using individual-level genotype data (GRS). These results provide consistent evidence that an elevated CysC level is causally associated with survival in HCC patients.

CysC is a cysteine protease inhibitor encoded by the *CST3* gene. It is produced and secreted into the blood by various cells. CysC regulates bone resorption, neutrophil chemotaxis, tissue inflammation, and resistance to bacterial and viral infections. As a CVD biomarker, CysC is involved in the pathogenesis of acute coronary syndrome through inflammation [20]. However, the role of CysC as a biomarker in liver diseases and its pathogenic mechanism is still not fully elucidated. It has been reported that CysC is associated with the progression of chronic liver disease and cirrhosis. Previous studies have reported that serum CysC is closely related to the histological stage and the progression of chronic liver disease. It can be considered as a potential marker of liver fibrosis [21]. In addition, the serum CysC level was also reported as an independent predictor of mortality in patients with cirrhosis [22, 23]. CysC is differentially expressed in a variety of tumors and plays a role in promoting or suppressing tumors [24-28]. However, the role of CysC in the process of HCC has not been fully understood.

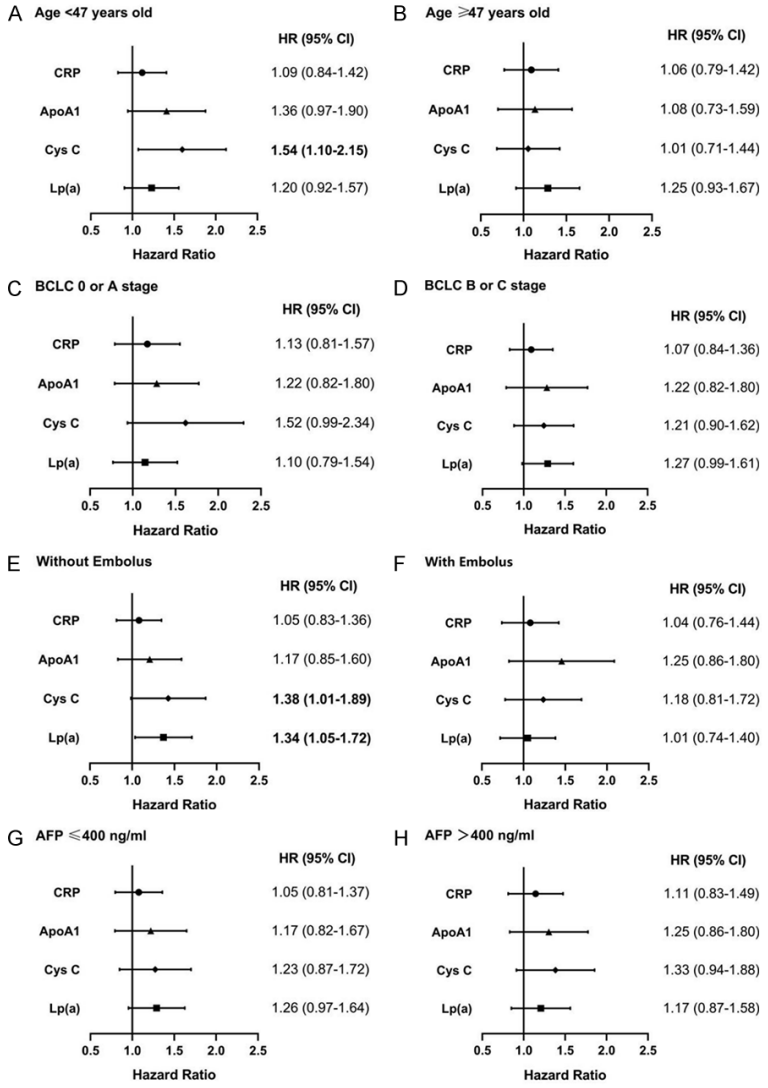

Figure 2. Associations of stratified analyses of each genetic risk score of cardiovascular disease biomarkers on hepatocellular carcinoma overall survival by age (A: < 47 years old; B: ≥ 47 years old), BCLC stage (C: 0 or A stage; D: B or C stage), embolus status (E: Without embolus; F: With embolus), and AFP (G: ≤ 400 ng/ml; H: > 400 ng/ml). CRP, C-reactive protein; ApoA1, Apolipoprotein A-1; CysC, Cystatin C; LP(a), Lipoprotein(a).

Recently, Ran [7] reported that the expression of CST3 in HCC tissues was significantly lower than that in adjacent tissues. Additionally, the serum CysC level of HCC patients were lower than that of the non-HCC population. In addition, Ran found that overexpression of CST3 inhibited the proliferation, migration, and invasion of HCC cells. However, He et al. reported opposite results in patients with primary liver cancer but without distant metastasis [13]. By using immunohistochemistry, they discovered that the level of CysC in HCC tissue was higher compared to that of the adjacent non-tumor tissue, distant normal tissue, and cirrhosis tissue. They also reported that the serum CysC levels of primary hepatic carcinoma patients were higher than those of the normal control. Another study on CysC provided a possible explanation for the phenomenon mentioned above in terms of biological mechanisms. They reported that CysC could be secreted from Huh7 cells (a hepatoma cell line) into the extracellular environment, promoting HCC cell proliferation by activating the TLR4 downstream signaling pathway [29].

In stratified analysis, we found that genetically elevated CysC levels were causally associated with poorer survival in HCC patients without embolus, which is consistent with the conclusion reached by He et al. One possible explanation is that CysC is an endogenous inhibitor of cysteine protease, which may diminish the impact of cysteine cathepsin (Cat) during tumor invasion and metastasis [13]. During the processes of tumor invasion and metastasis, Cat B and other cathepsins mediate the degradation of the extracellular matrix [30-32]. CysC is the primary inhibitor of Cat B, which can prevent Cat B from damaging interstitial tissues and basement membranes, thereby limiting tumor invasion and metastasis [28, 33]. Therefore, elevated CysC levels were associated with an increased risk of mortality in HCC patients without embolus, rather than in HCC patients with embolus. This reflects a modification effect of embolus status on the causal association between CysC and HCC survival. Moreover, we found that the causal association between CysC and HCC survival also existed in the population whose onset age was younger than 47 years old (median age of 866 HCC patients). This suggests that onset age could

also modify the association between CysC and HCC survival. Therefore, elevated CysC levels were associated with an increased risk of mortality in HCC patients without embolus, rather than in HCC patients with embolus. This reflects a modification effect of embolus status on the causal association between CysC and HCC survival. Moreover, we found that the causal association between CysC and HCC survival also existed in the population whose onset age was younger than 47 years old (median age of 866 HCC patients). This suggests that onset age could also modify the association between CysC and HCC survival [8]. Gao et al. reported that Lp(a) levels in HCC patients were significantly lower than those in healthy individuals. They also found that HCC patients with low Lp(a) levels had higher recurrence rates and shorter survival times compared to those with high Lp(a) levels [12]. Tang et al. reported that the level of Lp(a) in HCC patients was significantly higher than that in patients with liver cirrhosis, and the level of Lp(a) increased with the progression of HCC stage [11]. The result of our study is consistent with Tang et al.'s, providing evidence for the causal association between increased Lp(a) levels and worse HCC survival. Lp(a) can promote the proliferation of malignant tumor cells by activating platelets to release the platelet-derived growth factor. At the same time, however, Lp(a) can inhibit the activation of plasminogen, leading to the formation of a fibrin network and thrombus in a local area. This can promote the adhesion of malignant tumor cells to specific parts of blood vessels [34, 35], rather than allowing them to circulate freely in the bloodstream.

We applied Mendelian randomization to mitigate the bias of reverse causation when compared to traditional measures of CVD biomarkers after HCC occurrence. However, several limitations of this study should be discussed. First of all, the instrumental variables for CVD biomarkers were selected from the GWAS of the East Asian population. Therefore, there may be heterogeneity between the Guangxi population and the East Asian population. We conducted a heterogeneity test using summary data, which indicated no heterogeneity between the exposure GWAS and outcome GWAS populations. Additionally, all the *P*-values for the Q test were greater than 0.05. Secondly, the study has limited instrumental variables, which may result in a low explanatory power for the corresponding CVD biomarkers. This may impede our ability to uncover the causal associations between CRP or APOA1 and HCC survival in the current study. Finally, due to the limited sample size in this study, our findings need to be validated based on larger patient populations in the future.

In summary, our study clarifies the causal effect of CysC and suggests a potential causal effect of Lp(a) levels on HCC survival in the population of Guangxi. This finding offers new insights into the search for prognostic markers for hepatocellular carcinoma and provides clues for identifying common pathogenic mechanisms shared between cardiovascular diseases and cancer. CysC and Lp(a) may be used as biomarkers to indicate the survival of HCC patients, although this conclusion requires further validation in a larger population.

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Disclosure of conflict of interest

None.

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References

[1] Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A and Bray F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36

cancers in 185 countries. CA Cancer J Clin 2021; 71: 209-249.
[2] Zucman-Rossi J, Villanueva A, Nault JC and Llovet JM. Genetic landscape and biomarkers of hepatocellular carcinoma. Gastroenterology 2015; 149: 1226-1239, e4.
[3] de Boer RA, Meijers WC, van der Meer P and van Veldhuisen DJ. Cancer and heart disease: associations and relations. Eur J Heart Fail 2019; 21: 1515-1525.
[4] Aboumsallem JP, Moslehi J and de Boer RA. Reverse cardio-oncology: cancer development in patients with cardiovascular disease. J Am Heart Assoc 2020; 9: e013754.
[5] Lau ES, Paniagua SM, Liu E, Jovani M, Li SX, Takvorian K, Suthahar N, Cheng S, Splansky GL, Januzzi JL Jr, Wang TJ, Vasan RS, Kreger B, Larson MG, Levy D, de Boer RA and Ho JE. Cardiovascular risk factors are associated with future cancer. JACC CardioOncol 2021; 3: 48-58.
[6] Gao G, Dong F and Yan W. Clinical value of serum apolipoprotein A-1 (Apo A-1) detection in HBV-related liver cancer. J Med Res 2017; 46: 109-112.
[7] Ran R. The Mechanism of Cystatin C in the development of Hepatocellular Carcinoma. 2019. [Master's degree in Laboratory Medicine, Wuhan University]. https://d.wanfangdata.com.cn/thesis/ChhUaGVzaXNOZXdTMjAyMzA3MjgxMDM1MzQSCUQwMjYxOTEyNxoIeHBpZDgzNGs%3D.
[8] Lippi G, Targher G, Franchini M and Guidi GC. Biochemical correlates of lipoprotein(a) in a general adult population. Possible implications for cardiovascular risk assessment. J Thromb Thrombolysis 2009; 27: 44-47.
[9] Shen S, Gong J, Yang Y, Qin S, Huang L, She S, Yang M, Ren H and Hu H. Molecular mechanism of C-reaction protein in promoting migration and invasion of hepatocellular carcinoma cells in vitro. Int J Oncol 2017; 50: 1289-1298.
[10] Zamanian-Daryoush M and DiDonato JA. Apolipoprotein A-I and cancer. Front Pharmacol 2015; 6: 265.
[11] Tang Y, Xu H, Liu X, Liu L and Li Y. Clinical significance of PF4, VEGF, HIF-1a and Lpα in sera of patients with hepatocellular carcinoma. J Clin Transfus Lab Med 2017; 19: 478-481.
[12] Gao XH, Zhang SS, Chen H, Wang K, Xie W and Wang FB. Lipoprotein (a): a promising prognostic biomarker in patients with hepatocellular carcinoma after curative resection. Onco Targets Ther 2018; 11: 5917-5924.
[13] He H, Lü RX, Xu J, Liang LB, Zhang MF, An ZM and Huang HJ. Preliminary study on the expression of cystatin C in primary liver cancer. Sichuan Da Xue Xue Bao Yi Xue Ban 2013; 44: 920-923.
[14] Zhao X, Luo J, Li B, Liu S and Li D. The association between preoperative serum C-reactive protein and hepatocellular carcinoma recurrence in patients with chronic hepatitis B virus (HBV) infection-a retrospective study. PLoS One 2015; 10: e0116909.
[15] Peng C, Ye Y, Wang Z, Guan L, Bao S, Li B and Li W. Circulating microRNAs for the diagnosis of hepatocellular carcinoma. Dig Liver Dis 2019; 51: 621-631.
[16] Smith GD and Ebrahim S. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? Int J Epidemiol 2003; 32: 1-22.
[17] Larsson SC, Traylor M, Malik R, Dichgans M, Burgess S and Markus HS; CoSTREAM Consortium, on behalf of the International Genomics of Alzheimer's Project. Modifiable pathways in Alzheimer's disease: Mendelian randomisation analysis. BMJ 2017; 359: j5375.
[18] Kanai M, Akiyama M, Takahashi A, Matoba N, Momozawa Y, Ikeda M, Iwata N, Ikegawa S, Hirata M, Matsuda K, Kubo M, Okada Y and Kamatani Y. Genetic analysis of quantitative traits in the Japanese population links cell types to complex human diseases. Nat Genet 2018; 50: 390-400.
[19] Huang Q, Liu Y, Qiu M, Lin Q, Wei X, Zhou Z, Liang X, Li R, Chen W, Zhou X and Yu H. Potentially functional variants of MAP3K14 in the NF-κB signaling pathway genes predict survival of HBV-related hepatocellular carcinoma patients. Front Oncol 2022; 12: 990160.
[20] Vizovišek M, Fonović M and Turk B. Cysteine cathepsins in extracellular matrix remodeling: extracellular matrix degradation and beyond. Matrix Biol 2019; 75-76: 141-159.
[21] Ladero JM, Cárdenas MC, Ortega L, González-Pino A, Cuenca F, Morales C and Lee-Brunner A. Serum cystatin C: a non-invasive marker of liver fibrosis or of current liver fibrogenesis in chronic hepatitis C? Ann Hepatol 2012; 11: 648-651.
[22] Seo YS, Park SY, Kim MY, Kim SG, Park JY, Yim HJ, Jang BK, Park SH, Kim JH, Suk KT, Kim JD, Kim TY, Cho EY, Lee JS, Jung SW, Jang JY, An H, Tak WY, Baik SK, Hwang JS, Kim YS, Sohn JH and Um SH. Serum cystatin C level: an excellent predictor of mortality in patients with cirrhotic ascites. J Gastroenterol Hepatol 2018; 33: 910-917.
[23] Allen AM, Kim WR, Larson JJ, Colby C, Therneau TM and Rule AD. Serum cystatin C as an indicator of renal function and mortality in liver transplant recipients. Transplantation 2015; 99: 1431-1435.
[24] Tumminello FM, Flandina C, Crescimanno M and Leto G. Circulating cathepsin K and cys-

tatin C in patients with cancer related bone disease: clinical and therapeutic implications. Biomed Pharmacother 2008; 62: 130-135.
[25] Leto G, Incorvaia L, Flandina C, Ancona C, Fulfaro F, Crescimanno M, Sepporta MV and Badalamenti G. Clinical impact of cystatin C/cathepsin L and follistatin/activin a systems in breast cancer progression: a preliminary report. Cancer Invest 2016; 34: 415-423.
[26] Decock J, Obermajer N, Vozelj S, Hendrickx W, Paridaens R and Kos J. Cathepsin B, cathepsin H, cathepsin X and cystatin C in sera of patients with early-stage and inflammatory breast cancer. Int J Biol Markers 2008; 23: 161-168.
[27] Nakabayashi H, Hara M and Shimuzu K. Clinicopathologic significance of cystatin C expression in gliomas. Hum Pathol 2005; 36: 1008-1015.
[28] Yan Y, Zhou K, Wang L, Zhou Y, Chen X and Fan Q. Expression of cystatin C and its effect on EC9706 cells in esophageal carcinoma. Int J Clin Exp Pathol 2015; 8: 10102-11.
[29] Chen W. Hepatocellular carcinoma cells-secreted Cystatin-C as TLR4 agonist. 2017. [Master's degree in Biochemistry, National Yang-Ming University]. https://hdl.handle.net/11296/y8357n.
[30] Bakst RL, Xiong H, Chen CH, Deborde S, Lyubchik A, Zhou Y, He S, McNamara W, Lee SY, Olson OC, Leiner IM, Marcadis AR, Keith JW, Al-Ahmadie HA, Katabi N, Gil Z, Vakiani E, Joyce JA, Pamer E and Wong RJ. Inflammatory monocytes promote perineural invasion via CCL2-mediated recruitment and cathepsin B expression. Cancer Res 2017; 77: 6400-6414.
[31] Yang KM, Bae E, Ahn SG, Pang K, Park Y, Park J, Lee J, Ooshima A, Park B, Kim J, Jung Y, Takahashi S, Jeong J, Park SH and Kim SJ. Co-chaperone BAG2 determines the pro-oncogenic role of cathepsin B in triple-negative breast cancer cells. Cell Rep 2017; 21: 2952-2964.
[32] Gondi CS and Rao JS. Cathepsin B as a cancer target. Expert Opin Ther Target 2013; 17: 281-291.
[33] Gashenko EA, Lebedeva VA, Brak IV, Tsykalenko EA, Vinokurova GV and Korolenko TA. Evaluation of serum procathepsin B, cystatin B and cystatin C as possible biomarkers of ovarian cancer. Int J Circumpolar Health 2013; 72: 21215.
[34] Hancock MA, Boffa MB, Marcovina SM, Nesheim ME and Koschinsky ML. Inhibition of plasminogen activation by lipoprotein(a): critical domains in apolipoprotein(a) and mechanism of inhibition on fibrin and degraded fibrin surfaces. J Biol Chem 2003; 278: 23260-23269.
[35] Kim JS, Yu HK, Ahn JH, Lee HJ, Hong SW, Jung KH, Chang SI, Hong YK, Joe YA, Byun SM, Lee SK, Chung SI and Yoon Y. Human apolipoprotein(a) kringle V inhibits angiogenesis in vitro and in vivo by interfering with the activation of focal adhesion kinases. Biochem Biophys Res Commun 2004; 313: 534-540.

Table S1. Detail information of instrumental variables for cardiovascular disease biomarkers in exposure dataset

IVs of CVD biomarkers	Effect Allele	Other Allele	EAF	β	SE	*Pval*	*F*-value
CRP							
rs12502614	A	G	0.308	0.07	0.015	1.82×10^{-06}	21.778
rs17135743	G	A	0.063	-0.137	0.029	2.78×10^{-06}	22.317
rs17531365	T	C	0.027	0.208	0.043	1.55×10^{-06}	23.399
rs2097677	A	G	0.193	0.104	0.017	1.58×10^{-09}	37.426
rs2487015	G	T	0.762	-0.081	0.017	1.22×10^{-06}	22.702
rs3093059	G	A	0.162	0.16	0.019	5.74×10^{-18}	70.914
rs4420638	G	A	0.097	-0.135	0.026	2.91×10^{-07}	26.96
rs6678039	C	T	0.136	-0.094	0.02	3.38×10^{-06}	22.09
rs7310409	G	A	0.528	0.08	0.014	3.71×10^{-09}	32.653
APOA1							
rs10006678	A	T	0.713	0.149	0.032	4.02×10^{-06}	21.262
rs11954453	G	T	0.904	-0.218	0.046	2.49×10^{-06}	22.185
rs1883025	T	C	0.743	-0.206	0.031	2.13×10^{-11}	44.864
rs2358816	T	A	0.431	0.129	0.028	3.76×10^{-06}	21.388
rs254891	G	A	0.265	-0.18	0.039	4.48×10^{-06}	21.043
rs56228609	T	C	0.846	0.22	0.037	3.58×10^{-09}	34.842
rs57001330	A	G	0.734	-0.15	0.03	7.34×10^{-07}	24.539
rs61469338	A	C	0.632	0.128	0.028	4.65×10^{-06}	20.991
rs80123226	T	A	0.815	0.175	0.034	2.85×10^{-07}	26.357
CysC							
rs2380554	G	C	0.671	0.122	0.027	4.19×10^{-06}	21.152
rs32494	C	T	0.475	0.12	0.024	3.23×10^{-07}	26.118
rs369111629	A	G	0.962	-0.289	0.062	2.89×10^{-06}	21.893
rs6048956	T	C	0.894	-0.229	0.039	4.18×10^{-09}	34.528
rs61838231	G	T	0.793	-0.132	0.029	4.31×10^{-06}	21.109
rs7579970	C	T	0.212	-0.144	0.029	7.66×10^{-07}	24.436
LP(a)							
rs10837882	A	T	0.752	0.143	0.030	2.06×10^{-06}	22.551
rs10983564	C	A	0.600	0.129	0.027	1.43×10^{-06}	23.224
rs28450782	T	A	0.654	0.136	0.027	7.64×10^{-07}	24.457
rs56393506	T	C	0.889	0.664	0.042	7.35×10^{-57}	252.473
rs9538391	G	T	0.965	0.323	0.070	3.59×10^{-06}	21.464

CRP, C-reactive protein; ApoA1, Apolipoprotein A-1; CysC, Cystatin C; LP(a), Lipoprotein(a); EAF, effect allele frequency; SE, standard error.

Table S2. Associations of CRP IVs with hepatocellular carcinoma overall survival in the Southern Chinese population

IVs of CRP	Chr	Position	Gene	Alleles	HR[a]	95% CI	*P*-value[b]
rs12502614	4	48992748	CWH43	GG/AG/AA	1.28	1.10-1.50	0.002
rs17135743	16	2036631	GFER	AA/AG/GG	0.96	0.65-1.42	0.854
rs17531365	5	32571313	SUB1	CC/TC/TT	1.01	0.61-1.68	0.962
rs2097677	7	22732839	28 kb 5' of AC002480.2	GG/AG/AA	1.08	0.85-1.36	0.536
rs2487015	9	96521240	60 kb 5' of MIR4291	GG/TG/TT	1.14	0.97-1.34	0.113
rs3093059	1	159685136	CRP	AA/GA/GG	0.96	0.80-1.15	0.672
rs4420638	19	45422946	APOC1	GG/GA/AA	1.05	0.83-1.33	0.694
rs6678039	1	61597165	NFIA	CC/CT/TT	0.84	0.59-1.21	0.347
rs7310409	12	121424861	HNF1A	AA/AG/GG	0.96	0.83-1.11	0.564

CRP, C-reactive protein; IV, instrumental variable; HR, hazard ratio. [a]additive model. [b]$\alpha = 0.05/9 = 0.006$ (corrected by Bonferroni approach).

Table S3. Associations of ApoA1 IVs with the overall survival of hepatocellular carcinoma in the Southern Chinese population

IVs of ApoA1	Chr	Position	Gene	Alleles	HR[a]	95% CI	*P*-value[b]
rs10006678	4	167589611	65 kb 3' of SPOCK3	TT/AT/AA	0.91	0.78-1.06	0.229
rs11954453	5	84062963	276 kb 3' of CTD-2269F5.1	GG/GT/TT	0.98	0.75-1.29	0.903
rs1883025	9	107664301	ABCA1	TT/TC/CC	1.05	0.91-1.23	0.494
rs2358816	1	114419368	RP5-107303.5	AA/AT/TT	0.97	0.85-1.11	0.671
rs254891	5	171074931	123 kb 5' of AC011410.1	GG/AG/AA	1.00	0.85-1.19	0.963
rs56228609	16	56987765	3.4 kb 5' of AC012181.1	CC/CT/TT	1.05	0.88-1.26	0.564
rs57001330	12	5316804	26 kb 3' of RP11-319E16.1	AA/AG/GG	0.91	0.78-1.06	0.231
rs61469338	2	16197169	AC010145.4	CC/AC/AA	1.06	0.92-1.23	0.410
rs80123226	15	58676032	ALDH1A2	AA/AT/TT	0.99	0.84-1.17	0.909

ApoA1, Apolipoprotein A-1; IV, instrumental variable; HR, hazard ratio. [a]additive model. [b]$\alpha = 0.05/9 = 0.006$ (corrected by Bonferroni approach).

Table S4. Associations of CysC IVs with the overall survival of hepatocellular carcinoma in the Southern Chinese population

IVs of CysC	Chr	Position	Gene	Alleles	HR[a]	95% CI	*P*-value[b]
rs2380554	8	70490340	SULF1	CC/CG/GG	1.07	0.93-1.23	0.361
rs32494	5	55643774	27 kb 5' of RP11-155L15.1	TT/TC/CC	0.88	0.77-1.01	0.064
rs369111629	6	66016925	EYS	GG/AG/AA	0.79	0.59-1.05	0.109
rs6048956	20	23609301	CST3	TT/TC/CC	0.94	0.73-1.20	0.615
rs61838231	1	223503394	SUSD4	GG/GT/TT	1.00	0.84-1.19	0.980
rs7579970	2	233093136	DIS3L2	CC/CT/TT	0.85	0.73-0.99	0.038

CysC, Cystatin C; IV, instrumental variable; HR, hazard ratio. [a]additive model. [b]$\alpha = 0.05/6 = 0.008$ (corrected by Bonferroni approach).

Table S5. Associations of LP(a) IVs with the overall survical of hepatocellular carcinoma in the southern Chinese population

IVs of LP(a)	Chr	Position	Gene	Alleles	HR[a]	95% CI	*P*-value[b]
rs10837882	11	5372863	OR51B6	TT/AT/AA	1.21	1.04-1.40	0.015
rs10983564	9	119946647	ASTN2	AA/CA/CC	1.00	0.88-1.15	0.955
rs28450782	8	135251602	RP11-513H8.1	AA/TA/TT	0.98	0.85-1.12	0.737
rs56393506	6	161089307	1.9 kb 5' of LPA	CC/TC/TT	1.08	0.84-1.38	0.546
rs9538391	13	59921711	132 kb 5' of RNU7-88P	TT/GT/GG	1.22	0.76-1.97	0.408

LP(a), Lipoprotein(a); IV, instrumental variable; HR, hazard ratio. [a]additive model. [b]$\alpha = 0.05/5 = 0.01$ (corrected by Bonferroni approach).

Table S6. *P* for interaction between GRS of CVD biomarkers and covariances in the Southern Chinese population

Covariances		CRP	ApoA1	CysC	LP(a)
Age (year)	< 47	0.904	0.445	0.117	0.826
	≥ 47				
Sex	Female	0.339	0.343	0.789	0.442
	male				
Smoking	No	0.904	0.986	0.616	0.763
	Yes				
Drinking	No	0.279	0.538	0.183	0.299
	Yes				
BCLC	0/A	0.856	0.980	0.340	0.463
	B/C				
Embolus	No	0.890	0.670	0.467	0.122
	Yes				
AFP (ng/ml)	< 400	0.768	0.679	0.839	0.698
	≥ 400				
Cirrhosis	No	0.877	0.653	0.171	0.459
	Yes				

GRS, Genetic risk score; CVD, cardiovascular disease; CRP, C-reactive protein; ApoA1, Apolipoprotein A-1; CysC, Cystatin C; LP(a), Lipoprotein(a); BCLC, Barcelona Clinic Liver Cancer stage; AFP, alpha fetoprotein.