Original Article

Association of Genetic Markers of Hypercholesterolemia with Gastric Cancer: A Study of Mendelian Randomization Methods

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Abstract

Background: Research has indicated links between pure hypercholesterolemia (PH) and multiple cancers; however, its direct effect on gastric cancer remains uncertain. **Methods:** To investigate the potential direct effect of PH on gastric cancer, this study employed a two-sample MR approach, utilizing data from genome-wide association studies. Instrumental variables were identified based on single nucleotide polymorphisms associated with PH at a genome-wide significance level ($p < 5 \times 10^{-8}$) and with linkage disequilibrium ($r^2 < 0.001$). Causal inference was drawn using methods including IVW, MR-Egger, and the WM approach, with a significance threshold set at $p < 0.05$. Heterogeneity and horizontal pleiotropy were evaluated through Cochran's Q-test and the MR-Egger intercept, respectively. Robustness checks were conducted via omission diagnostics. **Results:** The MR analysis indicated a causal link between PH and a reduced risk of gastric cancer [IVW: OR: 0.84, p = 0.019; MR-Egger: OR: 0.813, p = 0.268; WM: OR: 0.838, p = 0.013]. The absence of significant heterogeneity and horizontal pleiotropy affirmed the reliability of these findings. **Conclusion:** The findings suggest a significant inverse relationship between PH and the risk of gastric cancer, indicating that elevated serum cholesterol levels may decrease the risk or slow the progression of gastric cancer, whereas lower levels may expedite cancer progression and are associated with a worse prognosis.

Keywords: Mendelian Randomization, Gastric Cancer, Simple Hypercholesterolemia.

Introductory

Gastric carcinoma, identified as a malignancy of epithelial origin within the stomach, was reported in 2020 data from China to occupy the third position in both incidence and mortality rates among diverse malignancies.[1] The body of research dedicated to the detection, prevention, and therapeutic approaches for gastric carcinoma underscores the significant influence of lifestyle choices, including the prevalence of Helicobacter pylori infection, genetic predispositions, alongside tobacco and alcohol usage, on the genesis and dissemination of this disease.[2] Moreover, recent academic discourse has introduced dyslipidemia as a contributing factor to the onset, spread, and outcome of gastric cancer. Lipids, which include both triglycerides and cholesterol, serve critical roles in energy storage and provision, in addition to being fundamental components of cellular membranes. Disrupted lipid metabolism is thus implicated in influencing cellular proliferation and vital regulatory mechanisms through alterations in the synthesis of cell membranes.[3] Hypercholesterolemia, affecting an estimated 35 million individuals globally, presents a challenge in detection and diagnosis, with fewer than 10% identified promptly. Additionally, the majority of those under treatment do not achieve the desired targets for low-density lipoprotein (LDL) cholesterol.[4,5] As a recognized class 1 genomic disorder, the significant implications of hypercholesterolemia for public health have been underscored by research emphasizing its

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phenotype frequency.[6,7] Dyslipidemia's role in altering gene expression and, consequently, the function of encoded proteins, alongside its impact on key cytokines and signaling pathways integral to cellular metabolism, has been documented. This alteration can facilitate tumorigenesis.[8,9] Furthermore, the rapid proliferation of cancer cells can intensify lipid metabolism, wherein metabolites or active substances produced by cancerous tissues may indirectly or directly influence membrane receptor mechanisms, thus altering the functional characteristics of cholesterol and LDL.[10] Several epidemiological investigations have demonstrated a significant inverse relationship between total blood cholesterol levels and the risk of various cancers, including hepatocellular, oesophageal, lung, and gastric carcinomas. These findings highlight cholesterol's pivotal role in oncogenesis, indicating its diverse effects across tumor types. While numerous studies have linked PH with gastric cancer, the exact causal connection and the mechanisms underlying this association are yet to be fully elucidated. This gap in knowledge is attributed to limitations and biases inherent in prior research.[11]

MR serves as an epidemiological technique that employs genetic variations as proxies, or IVs, to elucidate causal connections between specific exposures and health outcomes. This method draws a parallel to the concept of randomization used in clinical trials, where the natural random allocation of genetic variants at conception for a phenotype simulates the random allocation to treatment groups, providing a basis for causal inference. According to the foundational principles of genetics established by Gregor Mendel, these genetic variations, distributed independently during the process of meiosis, are expected to remain uninfluenced by external environmental factors.[12,13] The constancy of genetic variations from the moment of conception inherently guards against the potential for reverse causality, enhancing the credibility of MR studies.[14] This investigation conducted comprehensive bidirectional MR analyses, utilizing publicly accessible data from GWAS, to rigorously explore the potential causal linkage between PH and the incidence of gastric cancer.

Mrthodologies

Study Design

In this research, bidirectional two-sample MR analysis was applied to determine the causal connections between specific exposures and outcomes. For genetic variants to qualify as effective IVs in this context, they are required to satisfy three essential conditions: First, there must be a robust association between the SNPs and the exposure of interest. Second, these SNPs should exhibit no correlation with potential confounding factors that could distort the analysis. Third, the pathway through which SNPs influence the outcome must proceed solely through the exposure in question, ensuring there is no direct impact on the outcome independent of the exposure, as depicted in Figure 1 and Figure 2.[15]

Relevance Assumptions: SNPs strongly correlated with exposure

Independence Assumptions: SNPs are not associated with confounding factors

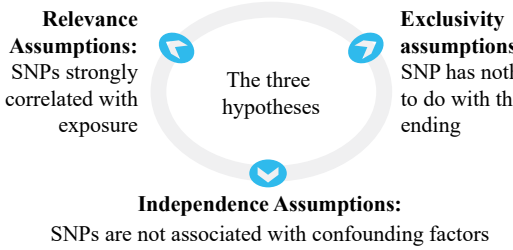


Figure 1: Three Big Assumptions

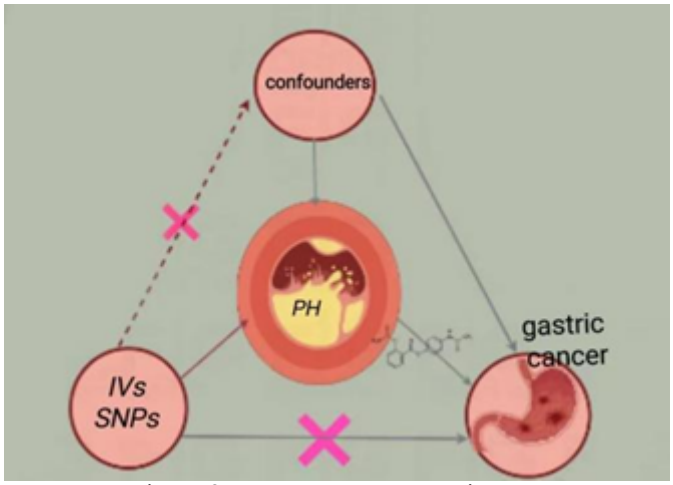
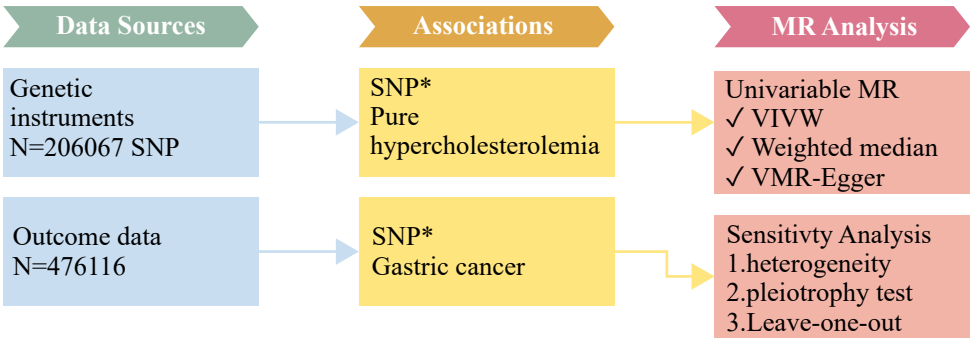


Figure 2: Exposure-outcome Diagram

To explore the possibility of a reverse association, our study also delved into reverse causality by treating PH as the outcome and gastric cancer as the variable of exposure in the MR analyses.

Process



Data Sources

The dataset for the Mendelian randomization analyses was sourced from the FinnGen biobank, facilitated by the IEU OpenGWAS project. Table 1 presents the dataset details for PH, gastric cancer, among others, featuring IEU GWAS IDs and the number of participants.

Table 1: Data Sources and Details.

GWAS Data	GWAS IEU ID	Source	Sample Size	Population
PH	finn-b-E4_HYPERCHOL	FinnGen biobank	206067	European
GASTRIC CANCER	ebi-a-GCST90018849	FinnGen biobank	476116	European

Selection of IVs

In our research, the initial phase involved isolating SNPs that demonstrated a significant correlation with the exposure, reaching a level of genome-wide significance ($p < 5 \times 10^{-8}$), thereby qualifying them as IVs. To address the issue of estimate distortion due to the tight linkage disequilibrium among IVs, we meticulously selected SNPs that were not only beyond a 10,000 kb window size but also exhibited a linkage disequilibrium coefficient lower than ($r^2 < 0.001$). Moreover, to enhance the accuracy of our causal analysis, we established a criterion for the MAF of SNPs, setting a minimum threshold of 0.05 to ensure sufficient statistical power. The f-statistic was used as a test for a strong connection between the IVs and the exposure, removing those IVs with weak associations, that is, f-statistic values below 10.0. In order to improve the precision of our estimates, we proceeded to systematically exclude SNPs with palindromic sequences to ensure that the effect attributed to an SNP on both the exposure and the outcome was the same across the same allele. SNPs associated with PH but not found in the exposure dataset were also eliminated. Consistently with the third hypothesis, we conducted a full PheWAS to eliminate SNPs linked to the exposure, in order to refine our causal search.

Statistical Analyses

To clarify the causative relationship between PH and gastric cancer in this study, we used three different statistical methods, among which the use of the IVW approach was the most productive. By combining the Wald ratios produced by each SNP with the outcome and estimating the overall causal effect assuming net bias of zero across all IVs, the IVW method provides us with the cornerstone analysis method.[16] The presence of heterogeneity in the estimates generated from individual SNPs through both IVW and MR-Egger methods was assessed using Cochran's Q test. Furthermore, to affirm the robustness and accuracy of the MR outcomes, we conducted a comprehensive sensitivity analysis aimed at evaluating the influence of specific SNPs on the overall results. Outcomes were deemed statistically significant with a p-value less than 0.05.

The statistical analyses for this investigation were conducted utilizing the "Two sample MR" package within the R software environment (version 4.3.2). The use of publicly available data negated the need for further ethical approvals.

In the End

Selection of Instrumental Variables

Initially, sixteen SNPs were identified for their potent correlation with PH, demonstrating significance at the genome-wide level ($p < 5 \times 10^{-8}$) and exhibiting linkage disequilibrium ($r^2 < 0.001$). Following the criteria of setting the MAF greater than 0.05 and excluding IVs with weak association (F-statistic > 10), five SNPs, namely rs11591147, rs149603090, rs77645768, rs182695896, and rs117733303, were removed from consideration. Additionally, two SNPs with palindromic sequences, rs115478735 and rs964184, were also excluded. Consequently, nine effective SNPs for PH were retained for further analysis. These selected SNPs will serve as instrumental variables to investigate the causal relationship between PH and gastric cancer. (Table 2 indicates the details)

Table 2: Chosen Snps and Relationships.

Chr	SNP	Id	Beta	Se	P
1	rs646776	finn-b-E4-HYPERCHOL	0.1473	0.02	1.65E-13
2	rs499883	finn-b-E4-HYPERCHOL	0.1105	0.0164	1.75E-11
3	rs72875462	finn-b-E4-HYPERCHOL	-0.1706	0.0297	9.21E-09
4	rs1367117	finn-b-E4-HYPERCHOL	0.1229	0.0181	1.09E-11
5	rs12916	finn-b-E4-HYPERCHOL	0.0922	0.0164	1.71E-08
6	rs2954017	finn-b-E4-HYPERCHOL	-0.0995	0.0163	1.12E-09
7	rs9644859	finn-b-E4-HYPERCHOL	0.1089	0.0165	3.78E-11
8	rs55791371	finn-b-E4-HYPERCHOL	-0.2586	0.0274	3.58E-21
9	rs7412	finn-b-E4-HYPERCHOL	-0.4115	0.0377	8.42E-28

Causal Effect of PH on Gastric Cancer

Utilizing the R statistical environment (version 4.3.2) and RStudio for analysis, this investigation uncovered a reverse causation between PH and the incidence of gastric cancer. Detailed results from the IVW method indicated an OR of 0.843 with a 95% CI from 0.731 to 0.972, achieving a p-value of 0.019. The MR-Egger regression offered an OR of 0.813, with a 95% CI spanning from 0.581 to 1.139, alongside a p-value of 0.268. Concurrently, the WM approach yielded an OR of 0.838, with its 95% CI between 0.729 to 0.964, and a p-value of 0.013, as demonstrated in Figure 3. The investigation found no significant pleiotropy (MR-Egger intercept = -0.005; standard error = 0.023; p = 0.824). The absence of heterogeneity in these findings (IVW: Q-pval = 0.070) was evident in Figure 4. Leave-one-out analysis further validated the consistency and dependability of these results, as shown in Figure 5.

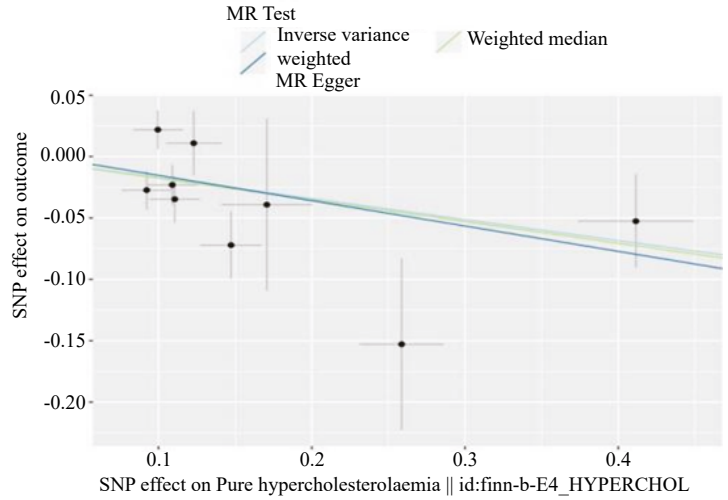


Figure 3: MR Scatter Plot for Relationship of PH with Gastric Cancer.

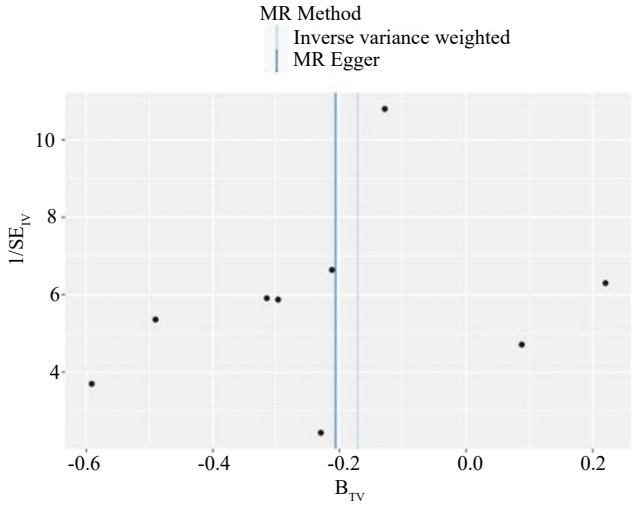


Figure 4: MR Funnel Plot for Relationship of PH with Gastric Cancer.

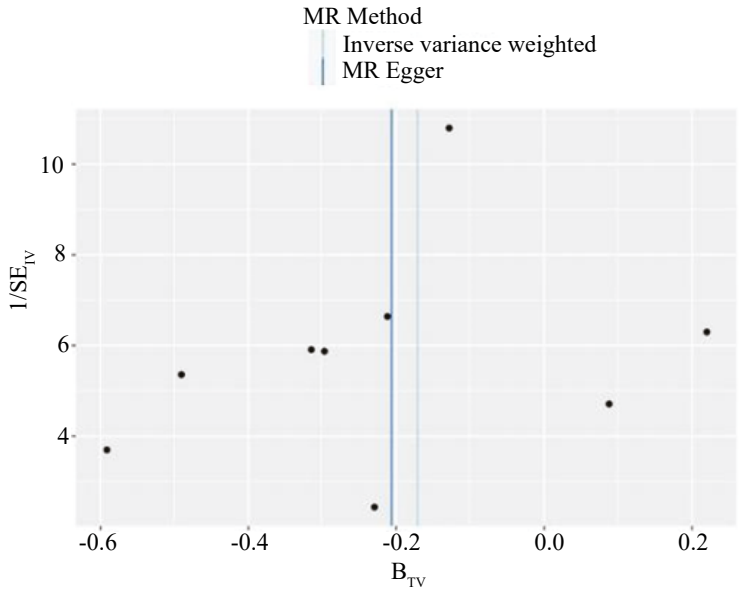


Figure 5: MR Leave-one-out Plot for Relationship of PH with Gastric Cancer.

Inverse MR Analysis

Investigations were expanded to explore the possibility of reverse causation through conducting MR analysis with gastric cancer as the presumed causative factor and PH as the resultant condition. This analytical approach did not reveal any evidence of a reverse causal link between gastric cancer and PH. The IVW method presented an OR of 1.031, with the 95% CI spanning from 0.941 to 1.128, leading to a p-value of 0.514, which does not signify a substantial association. Correspondingly, the MR-Egger regression analysis produced an OR of 0.943, with a 95% CI ranging between 0.731 and 1.212, and a p-value of 0.667. The WM technique also indicated a similar non-significant correlation, with an OR of 1.006 and a 95% CI of 0.905 to 1.119, alongside a p-value of 0.667, which are showed in Figure 6. Additionally, comprehensive sensitivity analyses of various studies found no evidence of either pleiotropy or heterogeneity, as depicted in Figure 7. The robustness and reliability of these outcomes were further confirmed by omission analyses, as depicted in Figure 8.

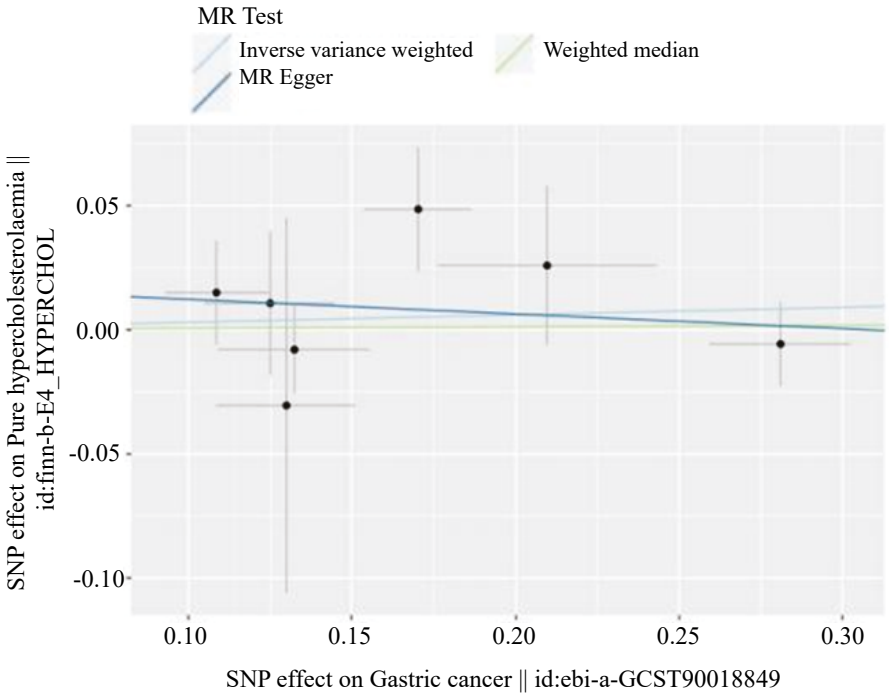


Figure 6: MR Scatter Plot for Relationship of Gastric Cancer with PH.

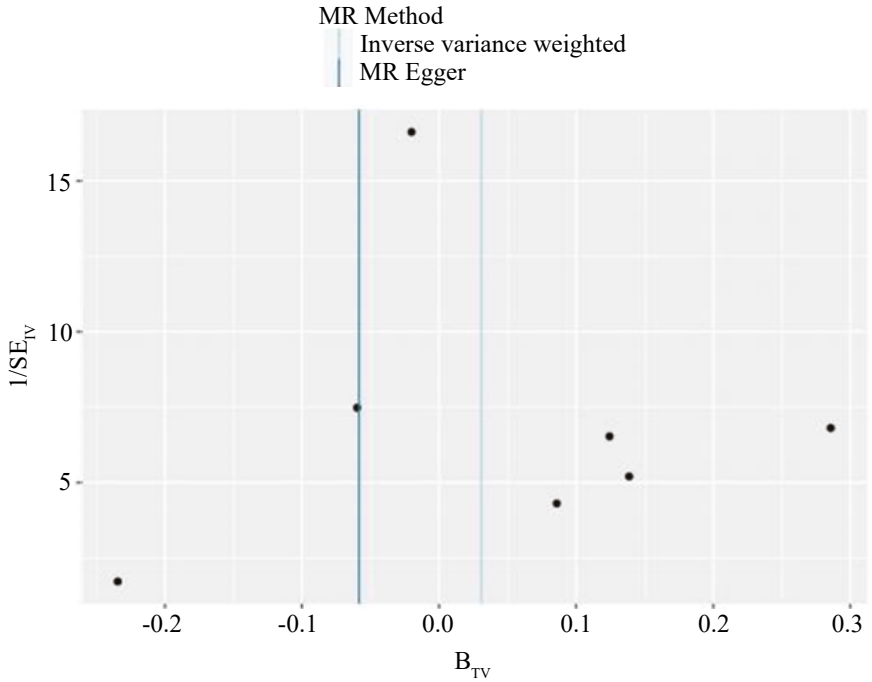


Figure 7: MR Funnel Plot for Relationship of Gastric Cancer with PH.

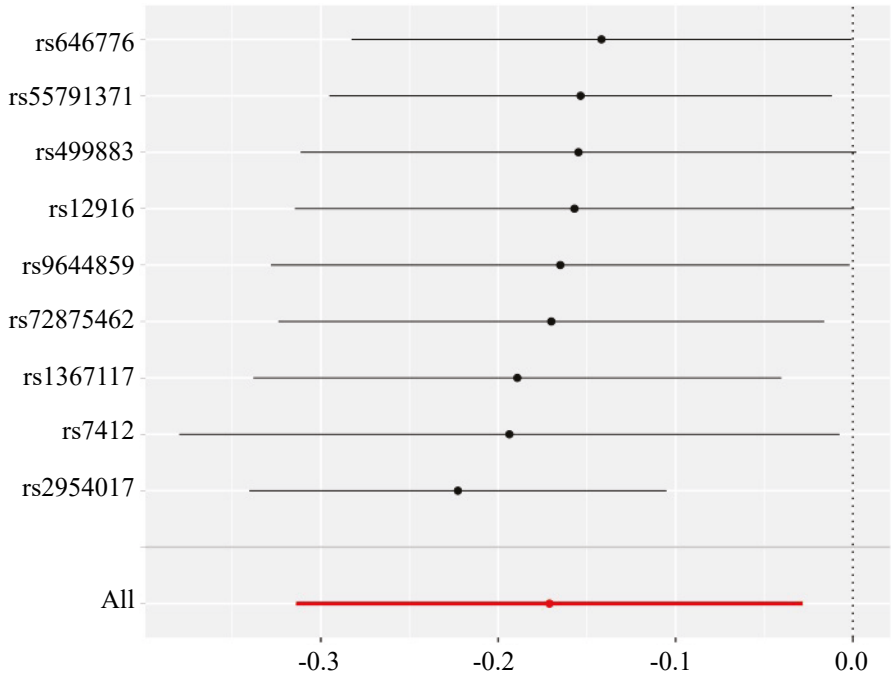


Figure 8: MR Leave-one-out Plot for Relationship of Gastric Cancer with PH.

Discussion

While studies regarding the impact of altered blood cholesterol levels on cancer risk present inconsistent findings, emerging clinical data increasingly supports the notion that abnormal cholesterol metabolism contributes to a poorer prognosis in cancer patients, particularly those with lymphatic metastases and renal cell carcinoma (RCC). It has been observed that lower blood cholesterol levels are markedly linked with aggressive tumor behavior and diminished postoperative recovery. A case-control study focusing on lipid metabolism and gastric cancer risk[17] discovered that

higher total serum cholesterol (TC) levels were more common in early-stage gastric cancer among females. As the cancer advanced, a gradual decline in lipid levels was noted. Moreover, a comprehensive multi-centre cohort study encompassing 3,046 RCC patients demonstrated a strong correlation between lower preoperative serum cholesterol and more aggressive tumor features, as well as inferior cancer-specific survival outcomes.[18] Another prospective study exploring the correlation between serum cholesterol levels and prognosis in malignant tumor patients found that lower serum cholesterol was indicative of a poorer prognosis, interpreted as a sign of heightened cancer activity. In a separate observational clinical study,[19] low serum TC was associated with increased cancer incidence and mortality. When adjusting for preoperative and surgical factors, low TC correlated with a higher rate of complications (serious complication rate: 15.2% for low TC, compared to 4.7% for normal TC and 5.5% for high TC; $p = 0.004$). Multivariate analyses further revealed that lower TC was linked to poorer OS and RFS in the weighted population [OS: HR = 0.92; 95% CI = 0.867-0.980; $p = 0.009$ and RFS: HR = 0.93; 95% CI = 0.873-0.988; $p = 0.02$]. Evaluation of TC before surgery emerges as a valuable indicator for predicting survival and the likelihood of complications post-surgery in patients diagnosed with stage I-III GC. It holds potential in pinpointing patients at higher risk, thereby facilitating the implementation of appropriate treatment strategies, including nutritional interventions and systematic follow-up.[20] In an analytic research of 40 patients with gastric cancer, significant elevation of serum TC, LDL, and HDL levels in the postoperative period compared to preoperative levels was recorded ($P<0.05$). This brought the conclusion that TC, LDL, and HDL levels in gastric cancer patients are related to the differentiation level of gastric cancer tissues. It was found that the lesser the differentiation of the malignant tissues, the lesser were the serum TC, LDL and HDL levels.

In summary, the results obtained from our MR analysis consistently point to a causal association between high cholesterol levels and reduced odds of gastric cancer incidence or recurrence. This suggests that a slight rise in the amount of cholesterol in the diet may reduce the prevalence of gastric cancer. In addition, the elevation of serum cholesterol levels after surgery suggests less recurrence, and thus better prognosis. Such observations may potentially reveal new information on the pathophysiological basis of the relationship between PH and CA. Strengths: Our study has several strengths. Most importantly, the use of MR in our study helps to address and attenuate biases arising from unmeasured confounders. This approach, using genetic differences as a key instrumental variable, greatly strengthens the validity of our causal inferences, making them more robust than those that can be made from traditional observational studies. In addition, the intentional inclusion of participants only from European populations further strengthens the validity of our results by minimizing the risk of bias due to population stratification.

Disadvantages

There are some limitations in our research that require acknowledgment. The key limitation is that the data that is used is solely based on European data. However, this specificity limits the generalizability of our results universally given the differences in the prevalence and features of gastric cancer among various ethnic populations. Additionally, hyperlipidemia is a broad term that includes many conditions, such as hypercholesterolemia, hypertriglyceridemia, and mixed hyperlipidemia. The subject of our study is cholesterol in the form of HDL-C and LDL-C. But the GWAS database does not have complete datasets for these subtypes and therefore there are no further detailed analyses of these hyperlipidemia subtypes. Moreover, the lack of information about some gastric cancer subtypes in the GWAS database restricted our potential to investigate these polymorphisms.

Reach A Verdict

This MR analysis underscores a significant inverse relationship between PH and cancer activities (CA), suggesting a pivotal role for PH in the etiology of gastric cancer. The insights garnered from this study could pave the way for groundbreaking strategies in the prevention and management of gastric cancer, potentially altering the current understanding of its pathogenesis and offering a new dimension to therapeutic interventions.

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