Lipids in Health and Disease

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Association between remnant cholesterol and the risk of 4 site-specific cancers: evidence from a cross-sectional and Mendelian randomization study

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Abstract

Background Recent studies have implicated remnant cholesterol (RC) in the etiology, progression, and prognosis of cancer. However, very few of them concentrated on the study of the precise relationship between serum RC levels and cancer risk, leaving this subject unexplored. Consequently, this study aims to investigate the association between serum RC levels and 4 site-specific cancers, employing a dual approach that combines observational and mendelian randomization (MR) analysis.

Methods Based on data from the National Health and Nutrition Examination Survey (NHANES) from 1999 to 2020, this study collected data from18,067 participants. To rule out confounders, this study utilized weighted multivariable logistic regression and assessed non-linear associations using restricted cubic spline (RCS) regression, followed by two-piecewise linear regression. Sensitivity analysis conducted in this study included subgroup analysis, multiple imputation, outlier removal, and propensity score matching. To strengthen causal inference, this study employed univariable and multivariable MR analysis. The robustness and reliability of the findings were estimated by the application of replication and meta-analysis.

Results The results of multivariable logistic regression analysis demonstrated a significant association between serum RC levels and breast cancer, showing that individuals in the higher logRC category had a higher risk of breast cancer compared to those in the lower category (Q3 vs. Q1: OR = 1.71, 95% CI: 1.01–2.88, *P* = 0.044). Weighted RCS revealed an inverted L-shape association between RC and the risk of breast cancer (*P*-nonlinear = 0.0386, *P*-overall = 0.010). Primary MR analysis provided evidence for an increased risk of breast (IVW: OR = 1.08, 95% CI: 1.03–1.12, *P* = 0.000951) and colorectal cancer (IVW: OR = 1.12, 95% CI: 1.00-1.24, *P* = 0.0476) associated with RC. However, the results of replication and meta-analysis did not support a significant causal association of RC with the risk of breast cancer (OR = 1.04, 95% CI: 0.95–1.13), lung cancer (OR = 0.95, 95% CI: 0.88–1.03), colorectal cancer (OR = 1.05, 95% CI: 0.92–1.19), and prostate cancer (OR = 1.01, 95% CI: 0.95–1.08).

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Conclusion Although a non-linear relationship was observed in the cross-sectional study between remnant cholesterol levels and breast cancer risk, MR analyses failed to provide any causal evidence.

Keywords Remnant cholesterol, Cancer, NHANES, Mendelian randomization

Introduction

Cancer has been a significant global health concern, ranking as one of the predominant contributors to mortality on a worldwide scale, especially among individuals under the age of 70 [1]. In 2020, the World Health Organization (WHO) reported a staggering global cancer burden of approximately 19.3 million newly diagnosed cases and 10 million cancer-related deaths [2]. As cancer poses substantial challenges to global healthcare systems, breast, lung, colorectal, and prostate cancers have become the most common types of malignancies [3], the consequential effect has also have an impact on economic growth and social well-being. These phenomena underscore the critical need for early detection, prevention, and effective treatment strategies.

Serum lipids serve as potential biomarkers for a variety of diseases such as cancer [4], cardiovascular and cerebrovascular disorders [5, 6], metabolic illnesses [7], and infectious diseases [8]. Cholesterol substantially influences the progression, proliferation and prognosis of cancer by facilitating cellular replication, motility and invasion. Consequently, as a more efficacious predictor of disease, cholesterol has garnered increasing attention [9]. Studies have revealed that heightened levels of serum cholesterol are combined with higher risk of colorectal [10], breast [11], and prostate cancers [12]. For instance, a 10 mg/dL increment in cholesterol is associated with a 9% increased likelihood of prostate cancer returning [12]. However, a conflicting evidence suggested that lower cholesterol levels could potentially increase the risk of certain types of cancer [13]. Another study revealed an inverse relationship between blood total cholesterol levels and the incidence and mortality of cancer, particularly in males [14]. A large prospective study found that low serum cholesterol increased the risk of gastric cancer [15]. This suggests the inclusiveness of the association between cholesterol and cancer, which requests further investigation on this relationship [15]. Previous lipid-related studies primarily concentrated on high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C), which, nevertheless, are not the sole cause of cancer risk. Apart from these lipid profiles, other forms of lipid abnormalities also contribute to an increased cancer risk [16]. Recent findings highlighted the role of very low-density lipoproteins (VLDL). By providing a continuous energy source to cancer cells through lipid uptake, VLDL could promote the progression of breast and liver cancer [17].Therefore, reinforcing the understanding and anticipation of susceptibility to cancer requires the investigation of novel lipid parameters beyond the traditional ones.

Remnant cholesterol (RC), a novel biomarker representing cholesterol esters in triglyceride-rich lipoproteins, encompassing remnants such as VLDL, IDLs, and chylomicron remnants [18]. Numerous studies have implicated RC in cardiovascular diseases, hypertension, diabetes, and ischemic stroke [13, 19]. Much attention has also been paid to the derived ratios, including RC/HDL, RC/LDL, and RC/TC, which have the potential to affect the disease risk assessment [20]. A study revealed that low levels of remnant cholesterol may be linked to elevated cancer incidence and poorer prognosis in cancer patients [13]. Furthermore, another investigation highlighted that increased levels of RC may serve as a prognostic indicator for individuals with breast cancer [21]. The unexplored relationship between RC and cancer susceptibility indicated that cancer patients are still facing the challenge in managing the residual risk.

In summary, all these findings suggest that lipids are strongly associated with the development of several cancers, and that RC as a potential novel marker for predicting cancer development has rarely been investigated in large-scale studies. Thus, further research is essential to specify relationship between RC and cancers. Such work may contribute to a deeper understanding of this novel lipid biomarker and its implication for cancers, potentially paving the way for the tailored development of interventions targeting cancer risk factors. Therefore, this study used National Health and Nutrition Examination Survey (NHANES) 1999–2020 database and MR analysis to jointly investigate the association of RC with four common cancers.

Methods

Sample source for cross-sectional study

An overview of the study design is presented in Fig. 1. The study utilized data obtained from NHANES (1999–2020) for the purpose of analysis. NHANES is a comprehensive and ongoing study that plays a vital role in understanding the overall well-being of the U.S. population and informing strategies to improve public health. It is designed to be nationally representative and has received endorsement from the NCHS. All data from this survey are available with free access through the website. The exclusion criteria for this study include: (1) Age < 20 years. (2) Pregnant women. (3) Missing data on remnant cholesterol, cancer and covariables. (4) Missing data on weight or weight of 0.

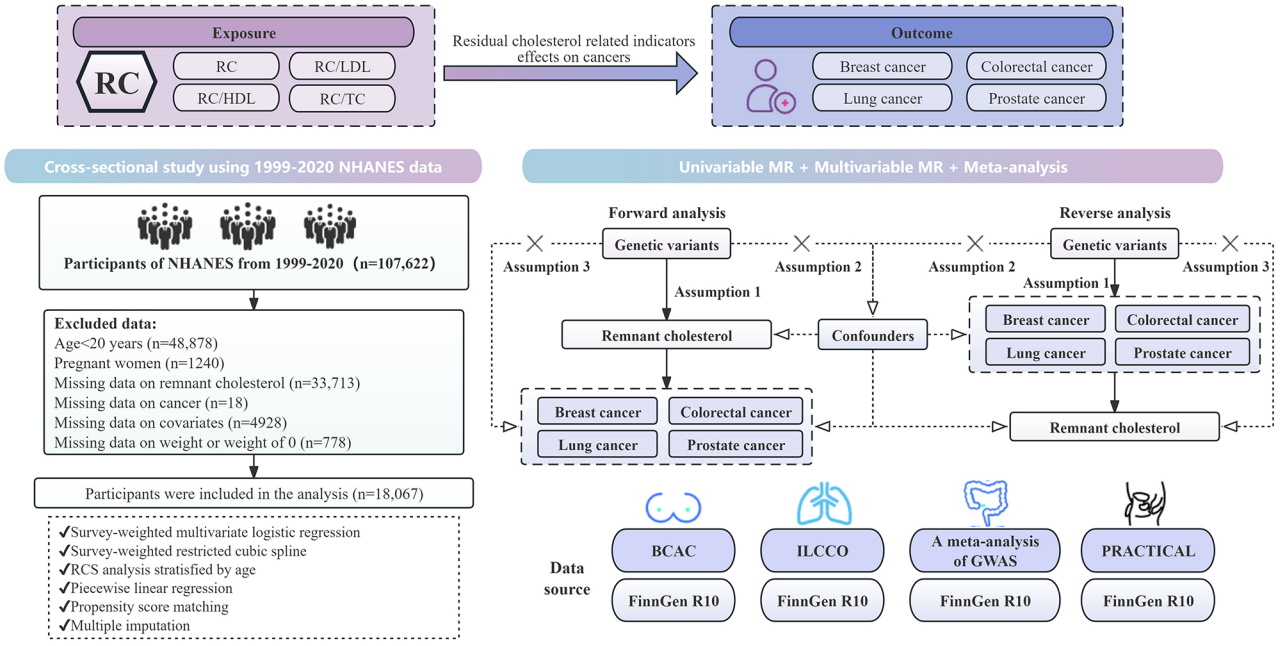

Fig. 1 Outline of the study design

Exposure variables, outcome variables, and covariables in cross-sectional study

This study designated RC, RC/HDL ratio, RC/LDL ratio, and RC/TC ratio as exposure variables for analysis. RC is calculated using the formula RC=TC - LDL - HDL. The four prevalent malignancies- breast, lung, colorectal, and prostate cancer- comprised the principal outcome variables. Cancer diagnosis was primarily based on two self-report questions: "Ever told you had cancer or malignancy?" and "What kind of cancer?". Sociodemographic covariables in this study included age, sex, and race. Socioeconomic factors were also accounted for, specifically incorporating the poverty income ratio (PIR) and education level. Additionally, lifestyle behaviors (drinking status and smoking status), statin use, possible comorbidities (hypertension and diabetes), and BMI were also considered in this study. The information about statin use was extracted from the NHAENS drug data, which is displayed in the Table S1.

Statistical analysis of cross-sectional study

This study employed sampling weights. These weights were calculated by the following criteria: a four-year fasting subsample weight was used for the 1999–2002 period (WTSAF4YR×4/21.2), and a two-year fasting subsample weight for 2003–2016 (WTSAF2YR×2/21.2). For the 2017–2020 data influenced by the pandemic, a special weight (WTSAFPRP×3.2/21.2) was employed.

Due to the skewed distribution of RC and its derived metrics, this study replaced the original variables with the natural logarithmic transformations of the values (Fig. S1). To investigate the independent influence of RC on cancer risk, this study utilized multivariable weighted logistic regression and progressively controlled for three models in the analysis. Considering the condition that the relationship between RC and cancer risk might be nonlinear, this study employed RCS curves and stratified RCS by age to delve deeper into the association between RC and cancer risk. We used a cubic spline model with 3 knots (10th, 50th and 90th percentiles). The inflection point in a non-linear pattern was estimated by maximum likelihood. Then, the two-piecewise linear regression was employed to examine the link between RC and cancer risk before and after the inflection point. The study utilized a propensity score matching (PSM) strategy, employing 1:1 nearest neighbor matching and 0.05 caliper matching to establish a comparable control group and mitigate the impact of confounding factors. Multiple imputation was employed to address missing data, with comparison conducted across 5 complete datasets (Fig. S3). The optimal imputed dataset was selected based on Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) values. Due to the distinct gender distribution of prostate and breast cancers, we employed weighted logistic regression and age-stratified analyses to more effectively investigate the impact of RC on the risk of these specific types of cancer. Additionally, this study conducted sensitivity analysis using methods such as subgroup analysis, outlier removal, and unweighted logistic regression to test the stability of the findings.

All statistical analyses of this study used gtsummary (1.7.2) in R 4.3.3, survey (4.2-1), rms (6.7.1),

VIM (6.2.2), MatchIt (4.5.5), medium (3.16.0), ggplot2 (3.4.4) packages. All *P* values were two-tailed, $P<0.05$ was considered nominally significant, and $P<0.0125$ (Bonferroni correction $P=0.05/4$) was considered a significant association.

Data source for mendelian randomization study

This research employed genome-wide association study (GWAS) data to explore the putative causal relationship between RC and four distinct types of site-specific cancers. The summary data in GWAS for RC from UK Biobank included 115,082 subjects. The RC calculations are consistent with the previous study (RC=TC - LDL - HDL). The levels of TC, HDL-C, and LDL-C were assessed by reference to the high-throughput magnetic resonance metabolomics data, which is accessible through Nightingale Health's biomarker quantification version 2020. Following the steps above, these measurements were rigorously account for variables such as age, gender, fasting condition, and genetic profile data to ensure statistical validity and enhance the analysis' precision [22]. GWAS data for breast cancer, lung cancer, colorectal cancer, and prostate cancer were sourced from the BCAC (122,977 cases and 228,951 controls), ILCCO (11,348 cases and 15,861 controls), a meta-analysis study [23] (19,948 cases and 12,124 controls) and PRACTICAL (79,148 cases and 61,106 controls), respectively. Cancer data for replication and meta-analysis were provided by the most recent FinnGen R10 database. Detailed information of MR data sources is shown in the Table S12.

Selection of genetic instruments

This study selected SNPs that demonstrated a strong association with RC, ensuring both statistical independence ($R^2<0.001$ within a window of 10000 kb) and an F-statistic value exceeding 10 (Table S14). The R^2 and F statistic were calculated from the previous studies [24, 25]. LDlink website was used to remove the influence of confounders related to BMI, diabetes, smoking, and alcohol drinking (https://ldlink.nih.gov/?tab=home). Then two SNPs (rs112875651 and rs58542926) associated with BMI and type 2 diabetes mellitus (T2DM) were removed respectively (Table S13). Furthermore, this study removed inconsistent alleles and ambiguous palindromic SNPs (rs1293261) that could not be corrected, which harmonized the SNPs for exposure and outcome. Harmonization data used in MR analysis are shown in Table S16-S23. SNPs associated with outcome ($P<5E-08$) were also excluded to ensure that the SNP could only influence outcome through exposure. Finally, radial MR was employed to detect and exclude outliers from this analysis (Table S15 and Fig. S5).

Statistical analysis of mendelian randomization

The inverse variance weighting method was used to evaluate the causal association of RC with four cancers. Other analysis methods include maximum likelihood, MR-Egger, weighted median, MR Pleiotropy RESidual Sum and Outlier (MR-PRESSO), and robust adjusted profile score (RAPS). In addition, this study used a rigorous series of sensitivity analyses to assess the conformity of the causal estimates to the MR methodological principles. Cochran's Q test is a reliable statistical method for assessing heterogeneity. In leave-one-out analysis, the stability of the results was tested by sequentially removing the SNPs. The MR-PRESSO global test was utilized to detect horizontal pleiotropy, while the MR-Egger intercept test serves as a valuable method for evaluating the presence of directional horizontal pleiotropy. To evaluate the statistical power of the study, the mRnd online platform was utilized for its computational capabilities (https://shiny.cnsgenomics.com/mRnd/). This study also performed a reverse MR analysis to rule out reverse causality. The variable Mendelian randomization analyses (MVMR) method was employed to investigate the specific influence of RC on cancer, during which process the confounders (TC, LDL, HDL, TG, smoking, alcohol consumption, BMI, and diabetes) were adjusted to minimize their impact on this analysis. To further substantiate the causal effect, this study conducted a replication analysis using the FinnGen R10 dataset and a meta-analysis to validate the robustness of the causal effect. The IVW random effect model was employed for individuals exhibiting heterogeneity exceeding 50%, whereas the IVW fixed effect model was utilized for those with heterogeneity not surpassing 50%. A robust causal estimate is characterized by the following criteria: (1) The IVW analysis resulted in a p-value of less than 0.05, and these findings were robust across the five alternative sensitivity analyses. (2) The robustness of sensitivity analysis methods, ensuring no heterogeneity, horizontal pleiotropy, and stable leave-one-out results. (3) MVMR-IVW analysis, adjusted for confounders, must yield a p-value below 0.05. (4) The replication and meta-analysis must yield a p-value below 0.05. All *P* values were two-tailed, $P<0.05$ was considered nominally significant, and $P<0.0125$ (Bonferroni correction $P=0.05/4$) was considered a significant association.

This study used TwoSampleMR (0.5.7), MendelianRandomization (0.9.0), RadialMR (1.1), mr.raps (0.4.1) package for Mendelian randomization analysis, metafor (4.4-0), meta (7.0–0) package for meta-analysis and visualization of MR.

Results

Association between RC and cancers in the NHANES database

The study involved a total of 18,067 participants, which were classified into a cancer group and a non-cancer group, as shown in Table 1. Cancer patients were found to be older, female, non-Hispanic Whites, with higher income, and with higher prevalence of smoking and alcohol consumption compared to non-cancer patients. This study produced notably different results between the two groups ($P<0.05$) in terms of RC, HDL-C and other derived indices (RC/LDL and RC/TC). In Table S2, the participants were further divided into quartiles based on logRC, revealing significant differences in age, gender, race, education level, PIR, BMI, smoking habits, alcohol consumption, comorbidities, statin use, breast cancer, and lung cancer ($P<0.05$). Nonetheless, no notable differences were observed in colorectal or prostate cancer ($P>0.05$).

The survey-weighted logistic regression model was subsequently implemented to investigate the associations between RC and the specific cancers (breast cancer and lung cancer). After fully adjusting for covariables (model 3), this study found the logRC for the Q3 group to be associated with a heightened risk of breast cancer compared to the Q1 group (Q3 vs. Q1: OR=1.71, 95% CI: 1.01–2.88, $P=0.044$). With Q3 in model 3 served as the reference, the study showed a 41% decrease in the logRC level among individuals in the Q4 group, indicating a lower risk of developing breast cancer (Q4 vs. Q3: OR=0.59, 95% CI: 0.37–0.94, $P=0.028$) (Table 2). Additionally, no linear association was detected (P for trend>0.05). Table S3 showed no significant relationship between log(RC/TC), log(RC/LDL), log(RC/HDL), and breast cancer ($P>0.05$). Furthermore, In Model 3, no statistically significant correlation was observed between RC or its derived indicators and lung cancer ($P>0.05$) (Table S4). The RCS analysis, when weighted, disclosed an inverse L-shaped association between RC and the risk of breast cancer (Fig. 2A, P-nonlinear=0.0386), while unweighted RCS showed a nearly nonlinear association between them (Fig. 2B, P-nonlinear=0.0804). Furthermore, both weighted and unweighted age-stratified RCS analyses were employed to further investigate the role of age stratification on the relationship between RC and breast cancer risk. The results indicated that participants aged 60 and above had a higher cancer risk compared to those under 60 years (Fig. 2C and D). Considering the non-linear relationship between RC and breast cancer risk, this study found the logRC cut-off point of 3.296 (RC=27.00 mg/dL), which had the most significant likelihood ratio test P-value in a two-piecewise linear regression model (P for likelihood ratio test=0.031) (Table 3). When logRC is less than 3.296, a 60% increase in breast cancer risk is observed with each having an additional unit of the logRC value (OR=1.60, 95% CI: 1.04–2.53, $P=0.038$). However, this effect saturated when logRC exceeded 3.296 (95% CI: 0.28–1.15, $P=0.132$).

The subgroup analysis disclosed a statistically significant association between increased RC levels and the risk of breast cancer, particularly in participants with the following features: those aged 60 years or older (Q3 vs. Q1: OR=1.89, 95% CI: 1.03–3.46), those with a PIR in the 1.3–3.5 range (Q3 vs. Q1: OR=2.77, 95% CI: 1.20–6.43), those with a history of alcohol consumption (Q4 vs. Q1: OR=4.77, 95% CI: 1.18–19.40), smokers (Q3 vs. Q1: OR=2.39, 95% CI: 1.08–5.29), those with hypertension (Q3 vs. Q1: OR=2.13, 95% CI: 1.05–4.58), and statin users (Q2 vs. Q1: OR=3.88, 95% CI: 1.43–10.60) (Fig.S4). No significant interactions were observed among these factors (all P for interaction>0.05). All sensitivity analyses confirmed a strong association between logRC and breast cancer. Specifically, the removing of potential outliers did not affect the statistically significant association between logRC and breast cancer (Table S5). Neither did the unweighted logistic regression models significantly alter the results (Table S6). A visual representation of the missing data distribution for covariables is provided in Fig.S2. Based on the AIC and BIC results of 5 imputation models in the Table S7, imputation 2 was identified as the most suitable choice. Furthermore, survey-weighted multivariate logistic analysis after multiple imputation and PSM yielded consistent findings (Table S8 and S9). The study discovered a nominal significant association between increased RC levels in women aged 60 years and above and the risk of breast cancer (Q3 vs. Q1: OR=1.88, 95% CI: 1.03–3.45, $P=0.041$) (Table S10), but did not find a significant association between RC levels and prostate cancer in the male population ($P>0.05$) (Table S11).

Association between RC and cancers in the MR analyses

In view of the limitations of cross-sectional designs in establishing causal relationships, this study strategically employed MR analysis to enhance its investigation. Based on the instrumental variable selection criteria, this study identified 38, 39, 39, and 40 SNPs (with phenotypic variation explained of 4.40%, 5.37%, 5.60%, and 5.63% respectively), respectively associated with breast cancer, lung cancer, colorectal cancer, and prostate cancer. These SNPs were included in the subsequent analyses. The SNPs used in the primary and replication MR analyses are given more details in the additional file 1 (Table S24-S25). The univariable MR analysis disclosed a significant positive association between RC levels and breast cancer incidence (IVW: OR=1.08, 95% CI: 1.03–1.12, $P=0.000951$). A weak but nominal significant association effect was also observed between RC and colorectal cancer (IVW: OR=1.12, 95% CI: 1.00-1.24, $P=0.0476$).

Table 1 Baseline population characteristics from 1999 to 2020

Characteristic	Overall (N = 18,067)	No cancer (N = 16,325)	Cancer (N = 1,742)	*P* value
Age category, %				**< 0.001**
< 60 years	11,732 (74.44%)	11,257 (78.39%)	475 (36.78%)	
≥ 60 years	6,335 (25.56%)	5,068 (21.61%)	1,267 (63.22%)	
Age, years	47.24 (16.78)	45.64 (16.19)	62.55 (14.44)	**< 0.001**
Sex, %				**< 0.001**
Female	9,051 (50.92%)	8,139 (50.15%)	912 (58.30%)	
Male	9,016 (49.08%)	8,186 (49.85%)	830 (41.70%)	
Race, %				**< 0.001**
Mexican American	2,957 (7.52%)	2,842 (8.08%)	115 (2.16%)	
Non-Hispanic White	8,460 (70.35%)	7,215 (68.63%)	1,245 (86.67%)	
Non-Hispanic Black	3,581 (10.58%)	3,359 (11.14%)	222 (5.32%)	
Other Race	3,069 (11.55%)	2,909 (12.15%)	160 (5.85%)	
Education level, %				0.376
Below high school	1,872 (5.11%)	1,714 (5.16%)	158 (4.69%)	
High school or equivalent	6,683 (34.67%)	6,055 (34.79%)	628 (33.50%)	
College or above	9,512 (60.22%)	8,556 (60.05%)	956 (61.82%)	
PIR, %				**< 0.001**
< 1.3	5,175 (19.78%)	4,811 (20.40%)	364 (13.93%)	
1.3–3.5	7,026 (36.55%)	6,308 (36.51%)	718 (36.92%)	
> 3.5	5,866 (43.67%)	5,206 (43.09%)	660 (49.15%)	
BMI category, %				0.833
Underweight	250 (1.51%)	227 (1.51%)	23 (1.53%)	
Normal	5,001 (29.41%)	4,530 (29.50%)	471 (28.62%)	
Overweight	6,133 (33.38%)	5,529 (33.43%)	604 (32.90%)	
Obese	6,683 (35.69%)	6,039 (35.56%)	644 (36.95%)	
BMI, kg/m^2	27.70 (24.10, 32.21)	27.70 (24.09, 32.20)	27.62 (24.22, 32.60)	0.456
Drinking status, %				**< 0.001**
Never	2,306 (10.17%)	2,097 (10.23%)	209 (9.60%)	
Former	3,450 (15.90%)	2,975 (15.05%)	475 (24.02%)	
Mild	6,251 (37.10%)	5,534 (36.50%)	717 (42.84%)	
Moderate	2,661 (17.15%)	2,477 (17.52%)	184 (13.61%)	
Heavy	3,399 (19.69%)	3,242 (20.71%)	157 (9.94%)	
Smoking status, %				**< 0.001**
Never smoker	9,683 (53.25%)	8,904 (54.04%)	779 (45.76%)	
Former smoker	4,721 (26.05%)	4,018 (24.75%)	703 (38.45%)	
Current smoker	3,663 (20.69%)	3,403 (21.21%)	260 (15.79%)	
Diabetes, %				**< 0.001**
No	14,723 (86.23%)	13,477 (87.28%)	1,246 (76.21%)	
Yes	3,344 (13.77%)	2,848 (12.72%)	496 (23.79%)	
Hypertension, %				**< 0.001**
No	9,826 (60.30%)	9,235 (62.45%)	591 (39.87%)	
Yes	8,241 (39.70%)	7,090 (37.55%)	1,151 (60.13%)	
Statin use, %				**< 0.001**
No	14,742 (83.59%)	13,599 (85.22%)	1,143 (68.06%)	
Yes	3,325 (16.41%)	2,726 (14.78%)	599 (31.94%)	
LDL-C, mg/dL	113.00 (90.00, 137.00)	113.00 (91.00, 137.00)	113.00 (88.00, 138.00)	0.505
HDL-C, mg/dL	51.00 (42.00, 62.00)	51.00 (42.00, 62.00)	53.00 (43.00, 65.00)	**< 0.001**
TC, mg/dL	191.00 (165.00, 218.00)	190.00 (165.00, 217.00)	194.00 (166.00, 223.00)	0.052
RC, mg/dL	21.00 (14.00, 30.00)	20.07 (14.00, 30.00)	22.00 (15.00, 31.00)	**< 0.001**
RC/TC	0.11 (0.08, 0.16)	0.11 (0.08, 0.15)	0.11 (0.08, 0.17)	**0.002**
RC/LDL	0.18 (0.13, 0.27)	0.18 (0.13, 0.26)	0.20 (0.13, 0.29)	**< 0.001**
RC/HDL	0.40 (0.24, 0.67)	0.39 (0.24, 0.66)	0.40 (0.24, 0.69)	0.278
Breast cancer				

Table 1 (continued)

Characteristic	Overall (N = 18,067)	No cancer (N = 16,325)	Cancer (N = 1,742)	*P* value
No	17,811 (98.54%)			
Yes	256 (1.46%)			
Lung cancer				
No	18,023 (99.78%)			
Yes	44 (0.22%)			
Colorectal cancer				
No	17,932 (99.43%)			
Yes	135 (0.57%)			
Prostate cancer				
No	17,789 (98.98%)			
Yes	278 (1.02%)			

PIR poverty income ratio; RC Remnant cholesterol, BMI body mass index, HDL-C High-density lipoprotein cholesterol; LDL-C Low-density lipoprotein cholesterol, TC Total cholesterol

Table 2 Association between remnant cholesterol and breast cancer

Characteristic	Model 1			Model 2			Model 3		
	OR	95% CI	*P* value	OR	95% CI	*P* value	OR	95% CI	*P* value
logRC category									
Q1	—	—		—	—		—	—	
Q2	1.61	0.95, 2.72	0.078	1.30	0.76, 2.22	0.343	1.34	0.78, 2.29	0.288
Q3	1.92	1.21, 3.06	**0.006**	1.57	0.95, 2.60	0.081	1.71	1.01, 2.88	**0.044**
Q4	1.23	0.75, 2.01	0.413	0.95	0.56, 1.62	0.855	1.01	0.59, 1.75	0.965
logRC category									
Q2	—	—		—	—		—	—	
Q1	0.62	0.37, 1.05	0.078	0.77	0.45, 1.32	0.343	0.75	0.44, 1.28	0.288
Q3	1.20	0.76, 1.89	0.436	1.21	0.76, 1.92	0.418	1.28	0.79, 2.07	0.317
Q4	0.76	0.47, 1.24	0.274	0.73	0.45, 1.21	0.223	0.76	0.45, 1.27	0.292
logRC category									
Q3	—	—		—	—		—	—	
Q1	0.52	0.33, 0.83	**0.006**	0.59	0.35, 0.99	**0.044**	0.59	0.35, 0.99	**0.044**
Q2	0.83	0.53, 1.32	0.436	0.78	0.48, 1.27	0.317	0.78	0.48, 1.27	0.317
Q4	0.64	0.41, 1.00	**0.048**	0.59	0.37, 0.94	**0.028**	0.59	0.37, 0.94	**0.028**
logRC category									
Q4	—	—		—	—		—	—	
Q1	0.81	0.50, 1.33	0.413	1.05	0.62, 1.79	0.855	0.99	0.57, 1.71	0.965
Q2	1.31	0.81, 2.12	0.274	1.36	0.83, 2.24	0.223	1.32	0.78, 2.23	0.292
Q3	1.57	1.00, 2.45	**0.048**	1.65	1.05, 2.59	**0.030**	1.69	1.06, 2.69	**0.028**
P trend			0.304			0.960			0.750

Model 1: unadjusted

Model 2: adjusted for age, sex, race, PIR and education level

Model 3: adjusted for age, sex, race, PIR, education level, BMI, smoking status, drinking status, diabetes, hypertension, and statins

Findings from Cochran's Q test, MR-PRESSO global test and MR-Egger intercept analysis did not indicate heterogeneity or horizontal pleiotropy. And the results of the leave-one-out tests remained stable after excluding each SNP (Fig. S6-S9). However, this discovery did not succeed in establishing a direct cause-and-effect association between RC levels and the incidence of lung or colorectal cancer ($P>0.05$). All statistical methods used in this study maintained robust statistical power. This study also employed scatter plots for visual representation of the analysis (Fig. S10). The funnel plot did not display any influential anomalies (Fig. S11). The reverse MR analysis did not uncover any indication of a causal relationship between RC and any form of cancer (Table S26). In the multivariable analysis, a nominal significant and independent positive causal relationship between RC and colorectal cancer was established, even after controlling for the confounding factor of T2DM (OR = 1.26, 95% CI: 1.05–1.51, $P=0.014$) (Table S27). No other findings indicated a distinct causal effect of RC on cancer incidence ($P>0.05$). Finally, the results from the replication and meta-analysis indicated no significant causal association

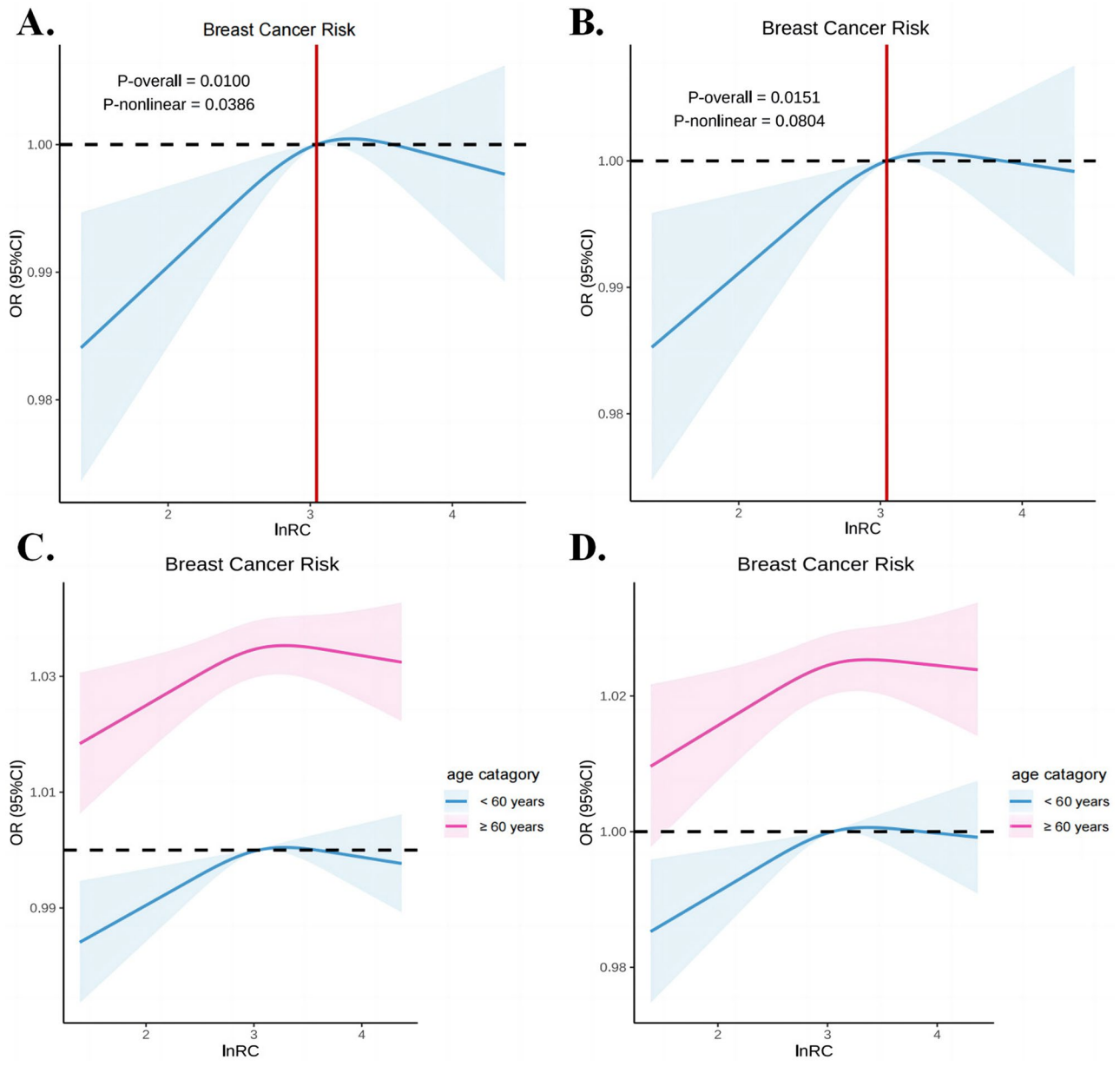

Fig. 2 RCS plot illustrating the association between RC and breast cancer. (**A**) Weighted RCS for Association of RC with breast cancer. (**B**) Unweighted RCS for Association of RC with breast cancer. (**C**) Weighted age RCS for Association of RC with breast cancer. (**D**) Unweighted age RCS for Association of RC with breast cancer

Table 3 Threshold analysis for the association between remnant cholesterol and individuals diagnosed with breast cancer

Outcome: breast cancer	Adjusted OR (95%CI)	*P* value
Model 1 Fitting model by standard linear regression	1.12 (0.85–1.48)	0.408
Model 2 Fitting model by two-piecewise linear regression		
Inflection point		3.296
logRC ≤ 3.296 (*N* = 12,506)	1.60 (1.04–2.53)	**0.038**
logRC > 3.296 (*N* = 5,561)	0.58 (0.28–1.15)	0.132
P for likelihood ratio test		**0.031**

between RC and breast cancer (OR = 1.04, 95% CI: 0.95–1.13), lung cancer (OR = 0.95, 95% CI: 0.88–1.03), colorectal cancer (OR = 1.05, 95% CI: 0.92–1.19), or prostate cancer (OR = 1.01, 95% CI: 0.95–1.08) (Table S25 and Fig. 3).

Discussion

This research, utilizing a comprehensive cross-sectional survey and MR analysis, is the initial effort to explore the association between RC and the susceptibility to 4 specific types of cancer. The observational study revealed an inverted L-shaped association between RC and breast

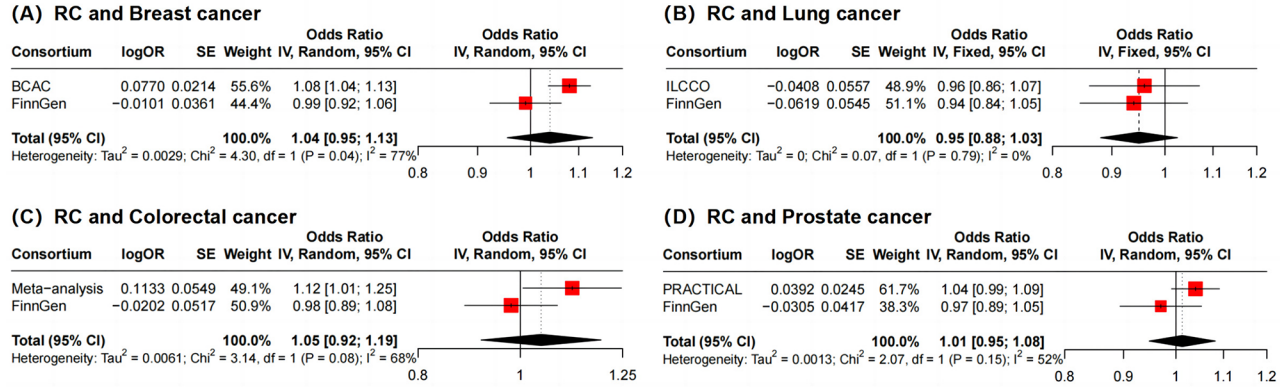

Fig. 3 Meta-analysis of RC and 4 site-specific cancers. (**A**) RC and breast cancer. (**B**) RC and lung cancer. (**C**) RC and colorectal cancer. (**D**) RC and prostate cancer

cancer, but the MR analysis failed to establish causality. The reliability and persuasiveness of the results of this study were further enhanced through a range of sensitivity analyses. Notably, none of the findings from observational research and MR analysis demonstrated any link between RC levels and the risk of the other three cancers.

The reproduction of cell membranes and the synthesis of hormones heavily depend on cholesterol. Thus, it is crucial to keep it within rational range, otherwise its excessive accumulation can lead to various bad conditions, including cardiovascular diseases, cancers, and metabolic disorders. In a fasting state, RC, primarily composed of VLDL and intermediate-density lipoprotein (IDL) [13], constitutes approximately one-third of total cholesterol, a proportion that has been increasingly recognized by numerous studies [19, 21, 26]. The link between RC and the mortality of cancer has been confirmed in several clinical investigations, which produced inconsistent findings. A study of a cohort revealed a negative association between levels of remnant cholesterol and the likelihood of all-cause or cancer-specific mortality, such as hepatocellular carcinoma and gastric cancer [13]. A study was undertaken involving 709 female breast cancer patients to investigate the association between RC and breast cancer mortality, revealing that higher RC levels were linked to poorer prognosis [21]. Despite the paucity of research on the association between RC and cancer incidence, the existing studies offered some insights. It has been reported that VLDL, a significant component of RC, is linked to hepatocellular and breast cancer. With its facilitation of angiogenesis, cell migration, and invasion, VLDL could be a potential cause for promoting proliferation and progression of breast cancer cells [17]. Furthermore, there is an increase in VLDL levels among individuals with lung cancer as compared to those without cancer [27]. An in vitro study discovered that, as a substance derived from the hydrolysis of VLDL and chylomicrons (CM), remnant lipoproteins can stimulate the proliferation of prostate cancer PC-3 cells [28]. A separate Mendelian randomization study revealed a consistent association between elevated levels of IDL and VLDL, and an augmented susceptibility to distal colon cancer [29]. These findings indicated a potential link between RC and cancer risk, although this indication was not substantiated by direct epidemiological evidence connecting circulating serum RC to cancer incidence.

The link between levels of cholesterol and breast cancer is still a topic of debate. Previous research has presented conflicting findings regarding the relationship between cholesterol levels and breast cancer risk. Some research has suggested a 30-33% higher risk of breast cancer with elevated cholesterol levels [30–33], while others report a negative relationship between cholesterol and breast cancer risk [34–36]. Additionally, two cohort studies suggested no significant association between the two [37, 38]. With the purpose of figuring out their actual association and potentially providing a more plausible explanation for it, this study creatively explored the dose-response association between RC and breast cancer. The results showed that, prior to a threshold, breast cancer risk incrementally rises with increasing RC levels. However, as RC levels approach this point, the risk appears to be reaching a plateau. A possible mechanism is that the growth of estrogen receptor-positive breast cancer cells could be induced by cholesterol, particularly through its derivative 27-hydroxycholesterol [39, 40]. Meanwhile, cholesterol is implicated in the modulation of inflammatory responses and the induction of oxidative stress, contributing to the progression of cancer. Furthermore, several studies have corroborated the subgroup analysis findings of this study, revealing that factors like aging over 60, smoking, alcohol consumption, and postmenopausal hypertension, particularly excessive drinking, early smoking, and hypertension in women, are associated with higher breast cancer risk [41, 42]. This may be related with unhealthy lifestyle habits and hormonal

fluctuations before and after menopause. Importantly, while epidemiological evidence does not directly link the use of statins to a reduction in breast cancer occurrence, it does suggest that statins, particularly atorvastatin, may have a protective effect against the recurrence of breast cancer [43]. This finding contrasts with the outcomes of this study, necessitating a cautious interpretation of the potential link between statin use and increased risk of breast cancer in the present investigation. The causes of the inconsistency among study results might be disparities in study populations, methodologies, outcome definitions, and unaccounted confounding factors. Future research should prioritize experimental studies and larger, longer-term randomized controlled trials (RCTs) to address these discrepancies and provide a more comprehensive and plausible understanding.

Contrary to observational findings, the MR results, supposedly offering more reliable evidence than observational ones, failed to uncover a causal relationship between RC and breast cancer. To mitigate potential biases from horizontal pleiotropy, this study employed multiple sensitivity analyses and used a bidirectional MR to rule out reverse causality, thereby providing stronger evidence, which was further validated through replication analyses by using data from different sources. Overall, no evidence has been uncovered to substantiate the association between RC and the susceptibility to breast cancer. Both the observational data and MR analysis failed to substantiate a connection between RC and the other three cancers.

Strengths and limitations

This study's strength lies in its large-scale, complementary cohort and MR analysis, which thoroughly examined the associations between RC and its derived indices with 4 site-specific cancers. Observational studies employed weighted random sampling to yield results that are more representative. Sensitivity analyses, including multiple imputation and propensity score matching, were employed to bolster the robustness of this study. Multivariable MR and meta-analysis were used to reinforce the reliability of the results. Nevertheless, several limitations are present in this study. First, despite adjusting for multiple confounders and the use of multiple sensitivity analyses, residual confounding and selection bias may still pose a challenge. For example, self-reported cancer diagnoses in patients introduced the possibility of reporting bias. Future studies may consider combining objective medical assessments with self-reported data to obtain a more comprehensive understanding. Second, the observational findings revealed a nonlinear relationship between RC and breast cancer, but only linear causation can be ruled out in MR studies not nonlinear causation. Third, the MR sample originated from European populations, while the observational data were from the US. Such difference of sample origin introduced heterogeneity.

Conclusion

In conclusion, RC, a new lipid marker associated with residual risk, is drawing attention from numerous studies, but further studies with deeper research are requested. The current issue is that, the standardized testing, normal range values, and targeted therapeutic measures for RC remain controversial. This study investigated the associations between serum RC levels and four site-specific cancers by using a dual-method approach. Cross-sectional findings identified a non-linear association between remnant cholesterol and breast cancer risk, whereas findings based on MR analysis did not support a causal link. To resolve this dispute, a further investigation of the relationship between RC and breast cancer prevalence may be necessary, based on larger population and participants of different ages. Therefore, this study suggests that, for populations at high risk of cancer, in addition to vigilance against conventional lipid markers, more attention should be paid to their residual cholesterol levels. For these populations, routine assessment of RC and immediate dynamic monitoring could be helpful for their further in-depth research and analysis.

Abbreviations

BMI	Body Mass Index
RC	Remnant Cholesterol
HDL-C	High-Density Lipoprotein Cholesterol
LDL-C	Low-Density Lipoprotein Cholesterol
TC	Total Cholesterol
NHANES	National Health and Nutrition Examination Survey
MR	Mendelian Randomization
PIR	Poverty Income Ratio
VLDL	Very-Low-Density Lipoprotein
IDL	Intermediate-Density Lipoprotein
OR	Odds Ratio
CI	Confidence Interval
RCS	Restricted Cubic Spline
PSM	Propensity Score Matching
MVMR	Multivariable Mendelian Randomization Analyses
RAPS	Robust Adjusted Profile Score
MR-PRESSO	MR Pleiotropy RESidual Sum and Outlier

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

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Not applicable.

Author contributions

M. L. and Q.L. participated in the design of the study, conducted analysis and wrote the article. M. S., M. F. and G. H. is responsible for the Writing review &

editing. The manuscript has been approved by all authors and is ready for publication.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The data used in this investigation were obtained from publicly available summary data, eliminating the need for additional ethical review.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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