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Cathepsins and their role in gynecological cancers

Evidence from two-sample Mendelian randomization analysis

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Abstract

Prior studies have reported connections between cathepsins (CTS) and gynecological cancers; however, the exact causal links are yet to be fully understood. Leveraging publicly accessible genome-wide association study summary datasets, we performed a two-sample bidirectional Mendelian randomization (MR) and multivariate MR (MVMR) analysis, with the inverse variance weighted (IVW) method as the primary approach. MR analysis demonstrated inverse associations between CTSB and cervical cancer (IVW: odds ratio [OR] = 0.9995, 95% confidence interval [CI] = 0.9991–0.9999, $P = .0418$), CTSE and ovarian cancer (IVW: OR = 0.9197, 95% CI = 0.8505–0.9944, $P = .0358$), CTSZ and ovarian cancer (IVW: OR = 0.9449, 95% CI = 0.8938–0.9990, $P = .0459$), CTSE and high grade serous ovarian cancer (IVW: OR = 0.8939, 95% CI = 0.8248–0.9689, $P = .0063$), and CTSZ and high grade serous ovarian cancer (IVW: OR = 0.9269, 95% CI = 0.8667–0.9913, $P = .0268$). A positive correlation was identified between CTSH and clear cell ovarian cancer (IVW: OR = 1.1496, 95% CI = 1.0368–1.2745, $P = .0081$). Nevertheless, subsequent adjustment for the false discovery rate revealed that none of the *P*-values retained statistical significance ($PFDR > 0.05$). MVMR analysis results elucidated that CTSZ was inversely associated with cervical cancer (IVW: OR = 0.9988, 95% CI = 0.9981–0.9996, $P = .0022$). Moreover, a positive association was noted between CTSF and cervical cancer (IVW: OR = 1.0007, 95% CI = 1.0000–1.0014, $P = .0364$), and similarly, between CTSS and cervical cancer (IVW: OR = 1.0005, 95% CI = 1.0000–1.0011, $P = .0490$). CTSO exhibited a positive association with non-endometrioid endometrial cancer (IVW: OR = 1.4405, 95% CI = 1.1864–1.7490, $P < .001$), and CTSH was positively associated with clear cell ovarian cancer (IVW: OR = 1.1167, 95% CI = 1.0131–1.2310, $P = .0263$). The MVMR analysis findings reveal that CTSZ emerges as a protective element against cervical cancer, whereas CTSF and CTSS represent risk factors for this disease. CTSO stands out as a risk factor for non-endometrioid endometrial cancer, and CTSH acts as a risk factor for clear cell ovarian cancer. This study elucidates causative connections between CTS and gynecological cancers, providing innovative insights for diagnostic and therapeutic optimization.

Abbreviations: 95% CI = 95% confidence interval, CTS = cathepsins, FDR = false discovery rate, GWAS = genome-wide association study, IVs = instrumental variables, IVW = inverse variance weighted, MR = Mendelian randomization, MVMR = multivariate Mendelian randomization, OR = odds ratio, SNPs = single nucleotide polymorphisms, UVMR = univariable Mendelian randomization.

Keywords: cathepsins, causal inference, genome-wide association study, gynecological cancers, Mendelian randomization

1. Introduction

Gynecological cancers represent significant health risks for women. As per the most recent global cancer statistics report published by the International Agency for Research on Cancer, approximately 20 million new cancer diagnoses and 9.7 million cancer-related fatalities were recorded in 2022. Among these, cervical, endometrial, and ovarian cancers persist as the top 3 prevalent malignant neoplasms of the female reproductive system, comprising 7.0% of new diagnoses and 6.7% of mortality instances,[1] underscoring a profound menace to women's well-being. The occurrence and progression of cancer are influenced by a multitude of contributing factors. One such factor of significance is cathepsins (CTS), which display a range of enzymatic functionalities, being released by both tumor cells and tumor-related cells, ultimately becoming activated within lysosomes.[2] CTS contribute to the degradation of intracellular proteins and extracellular matrix components,[3] govern

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The datasets generated during and/or analyzed during the current study are publicly available.

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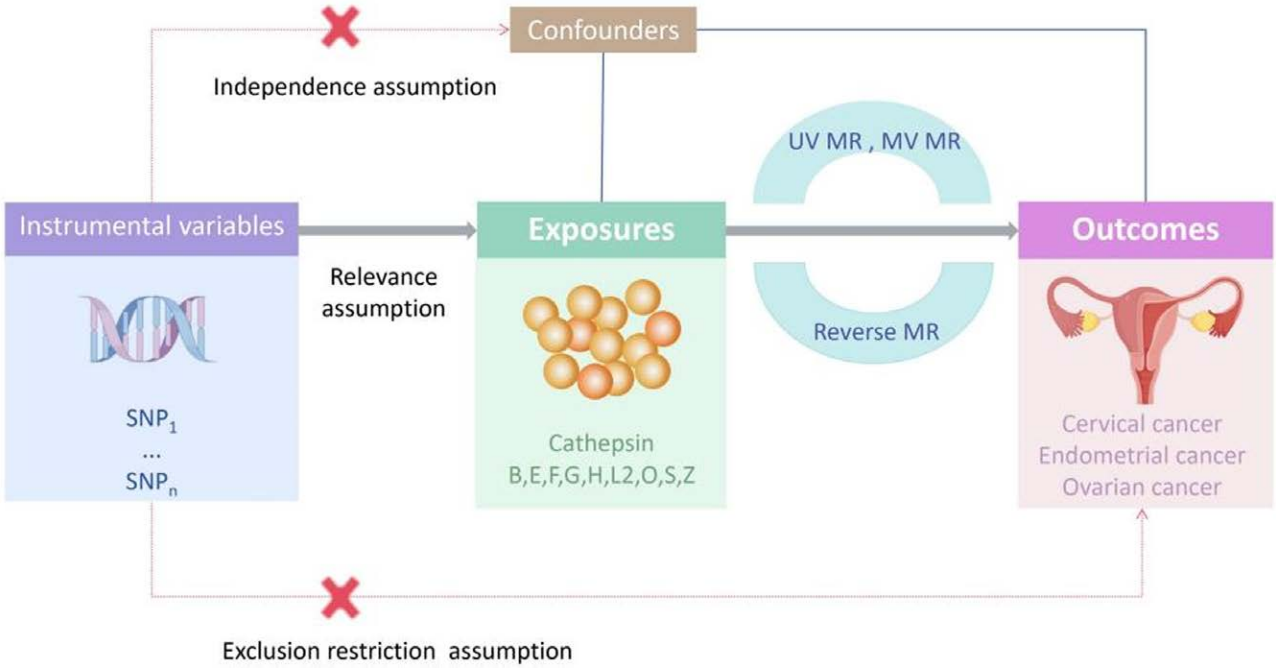

Figure 1. The core assumptions of MR and the flowchart illustrating the study procedure. UVMR = univariable Mendelian randomization.

lysosomal–mitochondrial homeostasis, modulate both intra- and extracellular signaling pathways, engage in processes of apoptosis and autophagy, release or trigger the activation of growth factors, and sway immune evasion mechanisms,[4–6] collectively shaping and influencing the dynamics of the tumor microenvironment.[7,8] Due to their multifaceted influence, CTS are deemed as valuable targets for the diagnosis and therapy of cancer.

Previous observational research has underscored the involvement of CTSB,[9–12] CTSL,[13–15] CTSS,[11,16] and CTSK[17,18] in the invasion and metastasis of cervical and ovarian cancers, highlighting the intimate connection between levels of CTS and the progression and prognosis of these diseases. However, the role of CTS in endometrial cancer remains inadequately defined. Furthermore, it is noteworthy that the CTS share analogous catalytic domains yet display distinct substrate specificities,[19] potentially enabling them to exert synergistic actions or manifest divergent, or even opposing, biological effects amidst different pathological contexts.[20,21] Presently, a dearth of extensive clinical investigations and direct experimental substantiation impedes a precise delineation of the distinct functions of various CTS in diverse subtypes of gynecological malignancies. Hence, conducting thorough and rigorous additional comprehensive investigations and verification of the causal links between CTS and cervical, ovarian, and endometrial cancers, including their respective subtypes, is a pressing need.

Mendelian randomization (MR) analysis, by employing genetic variations as instrumental variables (IVs), offers valuable insights into the causal relationships among diverse phenotypes.[22] In comparison to observational studies, it substantially mitigates the influence of confounding factors and effectively mitigates issues related to reverse causal bias.[23] Furthermore, multivariate MR (MVMR) analysis allows for the simultaneous evaluation of the independent impacts of various exposures on a singular outcome, adeptly navigating the challenges of pleiotropy and multicollinearity, thereby reducing the risk of bias in research conclusions.[24] As such, this study employs univariable two-sample bidirectional MR analysis to explore potential causal links between CTS and gynecological cancers. Moreover, MVMR analysis is conducted to unveil the diverse biological effects of CTS in gynecological cancers, shedding light on the distinct roles of each CTS subtype, thus offering novel perspectives for future diagnostic and therapeutic strategies.

2. Materials and methods

2.1. Study design

Two-sample bidirectional MR and MVMR analysis were leveraged to examine the causal relationships between the concentrations of 9 CTS (CTSB, CTSE, CTSF, CTSG, CTSH, CTSL2, CTSO, CTSS, and CTSZ) and the occurrence of 3 common gynecological cancers: cervical cancer, endometrial cancer, and ovarian cancer. Given the variability of tumors, different histological subtypes exhibit not only distinct molecular profiles but also possess specific origins, pathogenic mechanisms, clinical manifestations, and prognoses. As a result, our study encompassed 5 histological subtypes of ovarian cancer and 2 of endometrial cancer. MR analysis was employed to screen for single nucleotide polymorphisms (SNPs) as IVs in exposures, relying on 3 fundamental assumptions[25]: (i) the ultimate inclusion of IVs is strongly correlated with exposure; (ii) IVs are free from known confounding factors; and (iii) IVs cannot directly affect the outcome, but only through exposure factors. The flow chart of the research design is depicted in Figure 1. This study adhered to the STROBE-MR guidelines.[26]

2.2. Data source

The genome-wide association study (GWAS) data utilized in this research were sourced from the IEU OpenGWAS database, curated by the MRC Integrative Epidemiology Unit at the University of Bristol, United Kingdom. Specifically, the statistics for CTS B, E, F, G, H, L2, O, S, and Z were derived from the INTERVAL study, encompassing a cohort of 3301 individuals of European descent.[27] All donors supplied informed consent, and the study received approval from the National Research Ethics Committee (11/EE/0538). Further information can be found in Table 1.

The GWAS data pertaining to cervical cancer have been sourced from the Medical Research Council Integrated

Epidemiology Unit in the UK. Furthermore, the GWAS statistics concerning endometrial cancer, spanning both its endometrioid and non-endometrioid histological subtypes, have emanated from an extensive meta GWAS that incorporating findings from the studies carried out by the UK Biobank, the Endometrial Cancer Epidemiology Consortium and the Endometrial Cancer Association Consortium.[28] Additionally, the GWAS data relevant to ovarian cancer and its 5 histological subtypes, encompassing high grade serous ovarian cancer, low grade serous ovarian cancer, invasive mucinous ovarian cancer, clear cell ovarian cancer, and endometrioid ovarian cancer, have been derived from the Ovarian Cancer Association Consortium.[29] Each participant provided informed written consent, with all research protocols undergoing meticulous scrutiny and approval by the pertinent institutional ethics review boards. Therefore, this MR study stands exempt from the requirement to seek additional ethical approval or permission. Detailed specifics regarding the origin of GWAS data pertaining to gynecological cancers can be perused in Table 2.

2.3. Selection of IVs

To guarantee the comprehensiveness and precision of the results, we selected IVs in alignment with the prerequisites that must be met in the context of MR analyses: (1) a significance threshold of $P < 5 \times 10^{-6}$[30,31] was set to identify SNPs closely associated with exposure. However, when the outcome was cervical cancer, the restricted number of instruments meeting the criteria prompted us to adjust the significance threshold for exposure factors to $P < 5.0 \times 10^{-5}$ to bolster statistical power and attain more comprehensive research findings; (2) set $r^2 < 0.001$, window size = 10,000 kb to mitigate linkage disequilibrium; (3) SNPs with missing alleles and palindromic SNPs were removed; (4) PhenoScannerV2 search was applied to screen for SNPs linked to the target phenotype and to eliminate potential confounding factors; (5) retain SNPs with F-statistics exceeding 10 to prevent bias from weak instruments. The formula for calculation is as follows[32]: $F = [(N - K - 1)/K] \times [R^2/(1 - R^2)]$, $R^2 = 2 \times (1 - MAF) \times MAF \times \beta^2/SD^2$, $SD = SE \times \sqrt{N}$. Where N represents the overall sample size of the exposure data, K denotes the count of IVs, MAF stands for minor allele frequency, which can be interchangeable with the effect allele frequency when calculating R^2, β represents the value of the allele effect, while SE indicates the standard error of β.

2.4. Statistical analysis

The methods that methodologies utilized to examine the causal effects between exposure and outcome encompassed a variety of approaches, including the inverse variance weighted (IVW) method, MR-Egger, weighted median, weighted mode, and simple mode. Among them, the IVW method, by amalgamating the effect sizes of multiple SNPs and using the inverse of the standard errors as weights, ensures that the contribution of each SNP to the final estimation is commensurate with its statistical precision. This robust technique facilitates reliable causal inference, positioning it as the primary research method for MR analysis.[33] Concurrently, the remaining 4 methods functioned as supplementary strategies within the analysis.[34] The results of MR analysis were expressed as odds ratio (OR) and 95% confidence interval (95% CI), with statistical significance indicated by $P < .05$. Due to the multiple comparisons undertaken in this study, a false discovery rate (FDR) correction approach was implemented to adjust for multiple testing to bolster the credibility of the results.[35] $P_{FDR} < .05$ was statistically significant. Findings exhibiting $P < .05$ but $P_{FDR} > .05$ may furnish suggestive evidence of causality.

To mitigate the potential influence of biases and confounding factors and to enhance the credibility and interpretability of the causal effect garnered through MR analysis, we conducted a suite of sensitivity analyses.[36] The MR-PRESSO

Table 1

Summary of CTS data.

Cathepsins	Abbreviation	Sample size	Population	GWAS ID	Number of SNPs
Cathepsin B	CTSB	3301	European	prot-a-718	10,534,735
Cathepsin E	CTSE	3301	European	prot-a-720	10,534,735
Cathepsin F	CTSF	3301	European	prot-a-722	10,534,735
Cathepsin G	CTSG	3301	European	prot-a-723	10,534,735
Cathepsin H	CTSH	3301	European	prot-a-725	10,534,735
Cathepsin O	CTSO	3301	European	prot-a-726	10,534,735
Cathepsin S	CTSS	3301	European	prot-a-727	10,534,735
Cathepsin L2	CTSL	3301	European	prot-a-728	10,534,735
Cathepsin Z	CTSZ	3301	European	prot-a-729	10,534,735

CTS = cathepsins, GWAS = genome-wide association study, SNPs = single nucleotide polymorphisms.

Table 2

Summary of data on cervical cancer, endometrial cancer, and ovarian cancer and different subtypes.

Gynecological cancers	Ncase	Ncontrol	Population	GWAS ID
Cervical cancer	1889	461,044	European	ukb-b-8777
Endometrial cancer	12,906	108,979	European	ebi-a-GCST006464
Endometrial cancer (endometrioid histology)	8758	46,126	European	ebi-a-GCST006465
Endometrial cancer (Non-endometrioid histology)	1230	35,447	European	ebi-a-GCST006466
Ovarian cancer	25,509	40,941	European	ieu-a-1120
High grade serous ovarian cancer	13,037	40,941	European	ieu-a-1121
Low grade serous ovarian cancer	1012	40,941	European	ieu-a-1122
Invasive mucinous ovarian cancer	1417	40,941	European	ieu-a-1123
Clear cell ovarian cancer	1366	40,941	European	ieu-a-1124
Endometrioid ovarian cancer	2810	40,941	European	ieu-a-1125

GWAS = genome-wide association study.

technique was utilized to detect and address outliers, incorporating a comparison of causal estimates before and after the removal of outliers, with subsequent corrections made for horizontal pleiotropy. Cochran Q test was employed to assess heterogeneity among the selected SNPs. A *P*-value >.05 signified the lack of heterogeneity, leading to the selection of a fixed-effect IVW model. Otherwise, a random-effect IVW model was applied.[37] The MR-Egger intercept served as a tool to examine horizontal pleiotropy, with a *P*-value above .05 suggesting the lack of horizontal pleiotropy. Leave-one-out analysis was performed by systematically excluding individual SNPs to determine if any single SNP exerted substantial influence on the IVW estimates, thereby evaluating the stability of the data. Furthermore, to fortify the robustness of causal deductions, a MVMR analysis was executed building upon the framework of UVMR analysis, employing LASSO regression to mitigate multicollinearity, and comprehensively evaluated the distinct causal effects of various CTS on the outcome. All data analyses were carried out using the software packages "TwoSample MR" (version 0.5.8), "MendelianRandomisation," "MR-PRESSO" and "ggplot2" of the R (version 4.2.2).

3. Results

3.1. Effect of diverse CTS on gynecological cancers

In assessing the causal ramifications of differing levels of various CTS on common gynecological malignancies, we initially conducted UVMR analysis utilizing 9 unique types of CTS (CTSB, CTSE, CTSF, CTSG, CTSH, CTSL2, CTSO, CTSS, CTSZ) as exposures and investigated their causal associations with cervical cancer, endometrial cancer, ovarian cancer, and their respective histological subtypes as outcomes. The analysis demonstrated a negative causal relationship between CTSB and cervical cancer (IVW: OR = 0.9995, 95% CI = 0.9991–0.9999, *P* = .0418) (Fig. 2). Furthermore, elevated level of CTSE (IVW: OR = 0.9197, 95% CI = 0.8505–0.9944, *P* = .0358), CTSZ (IVW: OR = 0.9449, 95% CI = 0.8938–0.9990, *P* = .0459) reduced the risk of developing ovarian cancer (Fig. 3); higher levels of CTSE (IVW: OR = 0.8939, 95% CI = 0.8248–0.9689, *P* = .0063) and CTSZ (IVW: OR = 0.9269, 95% CI = 0.8667–0.9913, *P* = .0268) were linked to a decreased risk of developing high grade serous ovarian cancer (Fig. 4). Notably, CTSH exhibited a positive causal connection with the risk of clear cell ovarian cancer (IVW: OR = 1.1496, 95% CI = 1.0368–1.2745, *P* = .0081) (Fig. 5) (see Figures S1–S6, Supplemental Digital Content, http://links.lww.com/MD/O464, which illustrate that the scatter plots depict the results of the causal relationship between CTS and gynecological cancers). Nonetheless, none of these relationships remained statistically significant after adjusting for FDR correction (P_{FDR} > .05). While the uncorrected *P*-values had indicated significance (*P* < .05), the results post FDR correction demonstrated that the associations were not considered sufficiently to be robust. Yet, the outcomes of *P* < .05 but P_{FDR} > .05 could offer suggestive evidence of causality. Additionally, the IVW approach did not identify any causal links between other CTS and cervical cancer, endometrial cancer, and ovarian cancer, as well as among their respective subtypes. No potential outliers were detected using the MR-PRESSO method. Moreover, no conspicuous outliers were seen in the findings of the leave-one-out analytical strategy (see Figures S7–S12, Supplemental Digital Content, http://links.lww.com/MD/O464, which illustrate the findings of the leave-one-out analysis). The Cochran Q test, employed to evaluate the uniformity of the impact across various IVs, yielded findings that did not indicate any pronounced heterogeneity (see Figures S13–S18, Supplemental Digital Content, http://links.lww.com/MD/O464, which illustrate that the funnel plots additionally present the results of heterogeneity); in addition to this, the MR-Egger regression outcomes provided no indication of horizontal pleiotropy, affirming the suitability of the IVW method as the principal benchmark for analyzing causal relationships (see Table S1, Supplemental Digital Content, http://links.lww.com/MD/O463, which illustrates the sensitivity analyses of the two-sample bidirectional MR analysis).

3.2. Effect of gynecological cancers on various CTS

A reverse two-sample MR analysis was carried out in which cervical cancer, endometrial cancer, and ovarian cancer, along with their different histological subtypes, were considered as exposure variables. In this investigation, the focus was on exploring the likelihood of reverse causal effects wherein these common gynecological cancers may influence the levels of 9 CTS, treated as outcomes. The findings revealed a dearth of reverse causal evidence concerning CTSB and cervical cancer, as well as for CTSE and CTSZ in relation to ovarian cancer and high grade serous ovarian cancer. Similarly, no such evidence was observed for CTSH in the context of clear cell ovarian cancer. Nonetheless, the reverse MR analysis yielded evidence indicating that non-endometrioid endometrial cancer leads to a reduction in CTSB levels (IVW: OR = 0.9592, 95%

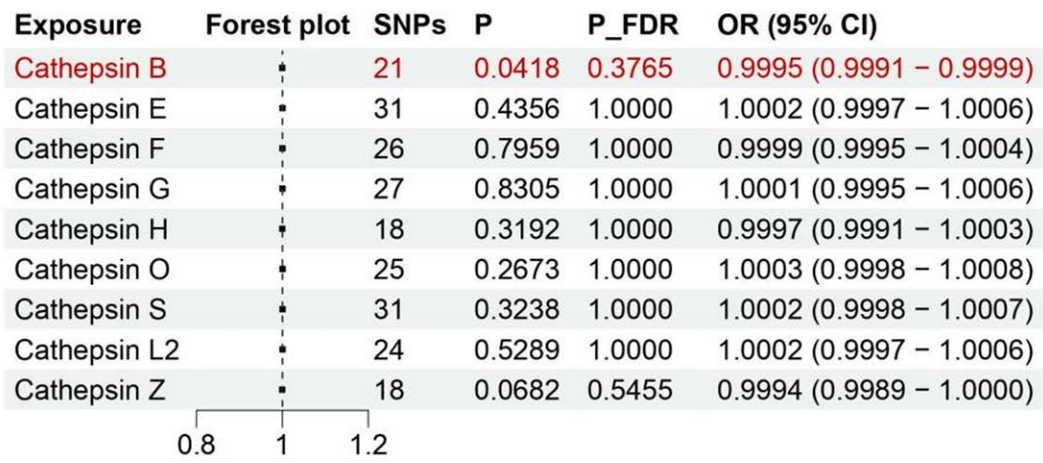

Figure 2. The effect of CTS on cervical cancer (IVW method). Marked red is having negative causality. 95% CI = 95% confidence interval; CTS = cathepsins, FDR = false discovery rate, IVW = inverse variance weighted, OR = odds ratio.

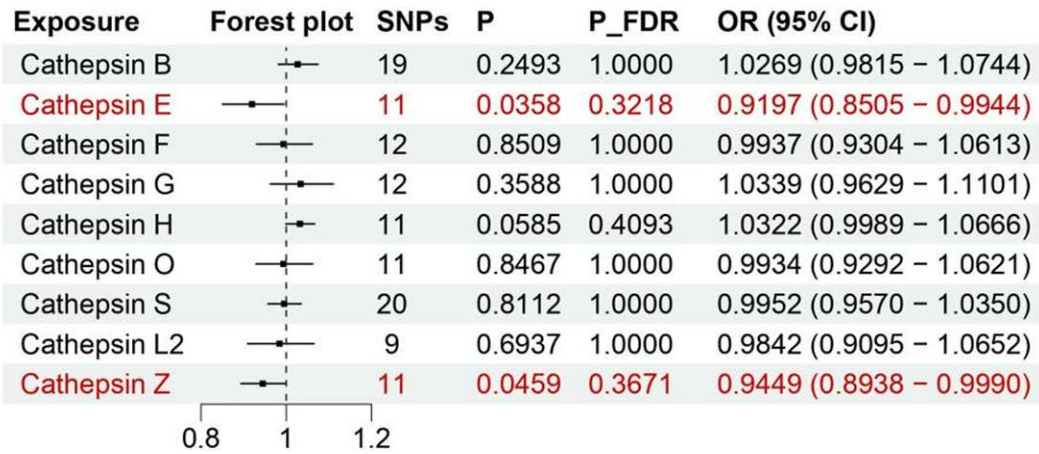

Figure 3. The effect of CTS on ovarian cancer (IVW method). Marked red is having negative causality. CTS = cathepsins, IVW = inverse variance weighted.

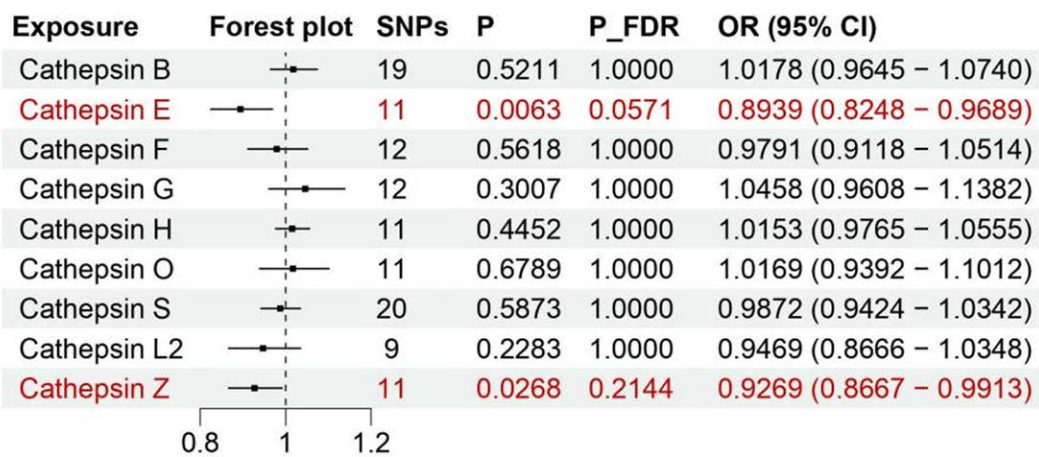

Figure 4. The effect of CTS on high grade serous ovarian cancer (IVW method). Marked red is having negative causality. CTS = cathepsins, IVW = inverse variance weighted.

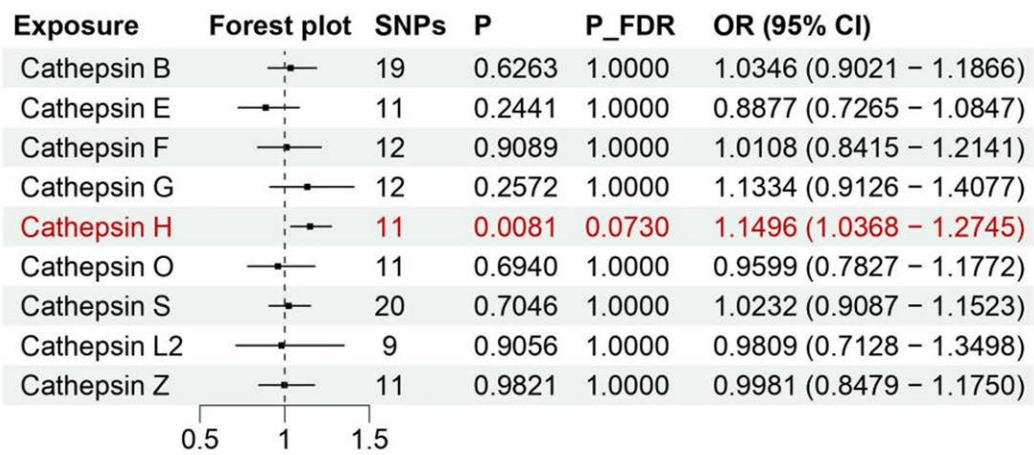

Figure 5. The effect of CTS on clear cell ovarian cancer (IVW method). Marked red is having positive causality. CTS = cathepsins, IVW = inverse variance weighted.

CI = 0.9237–0.9960, $P = .0303$) (Fig. 6), while both endometrial cancer (IVW: OR = 0.9022, 95% CI = 0.8192–0.9936, $P = .0366$) and endometrioid endometrial cancer (IVW: OR = 0.9019, 95% CI = 0.8310–0.9789, $P = .0135$) are associated with decreased levels of CTSO (Fig. 7). Furthermore, it was observed that endometrioid ovarian cancer leads to a reduction in CTSS levels (IVW: OR = 0.8785, 95% CI = 0.7983–0.9668, $P = .0080$) (Fig. 8), while endometrial cancer results in a decrease in CTSZ levels (IVW: OR = 0.9191, 95% CI = 0.8465–0.9979, $P = .0444$) (Fig. 9) (see Figures S19–S23, Supplemental Digital Content, http://links.lww.com/MD/O464, which illustrate that the scatter plots present the outcomes of the causal relationship

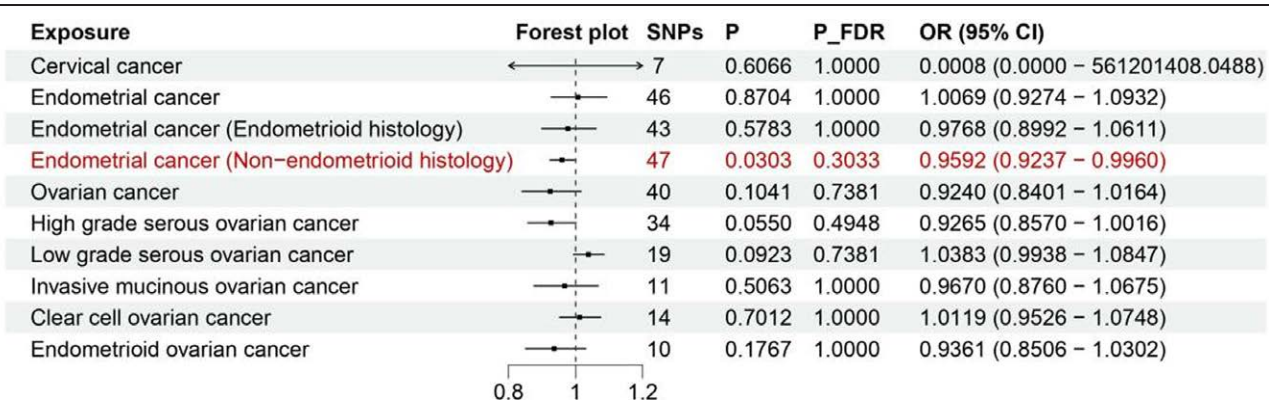

Figure 6. The effect of gynecological cancers on CTSB (IVW method). Marked red is having negative causality. CTS = cathepsins, IVW = inverse variance weighted.

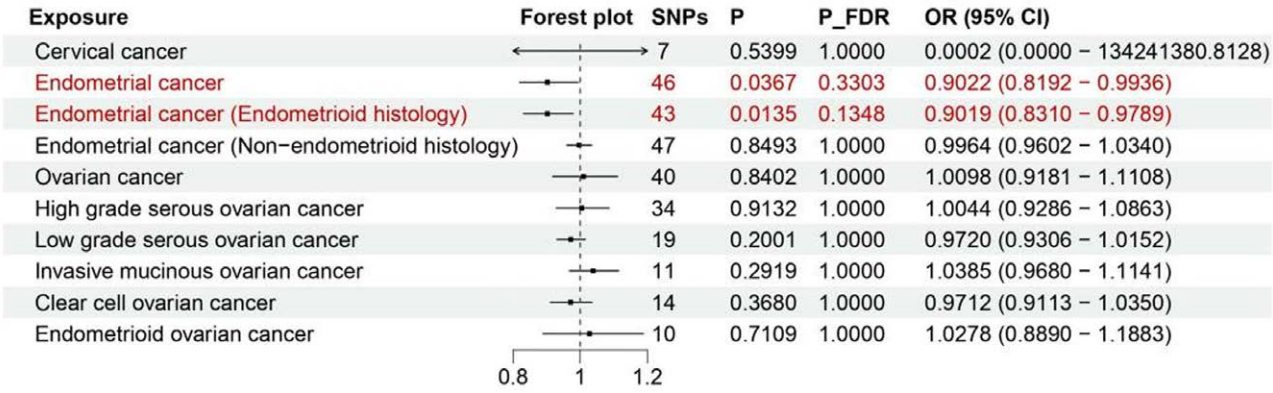

Figure 7. The effect of gynecological cancers on CTSO (IVW method). Marked red is having negative causality. CTS = cathepsins, IVW = inverse variance weighted.

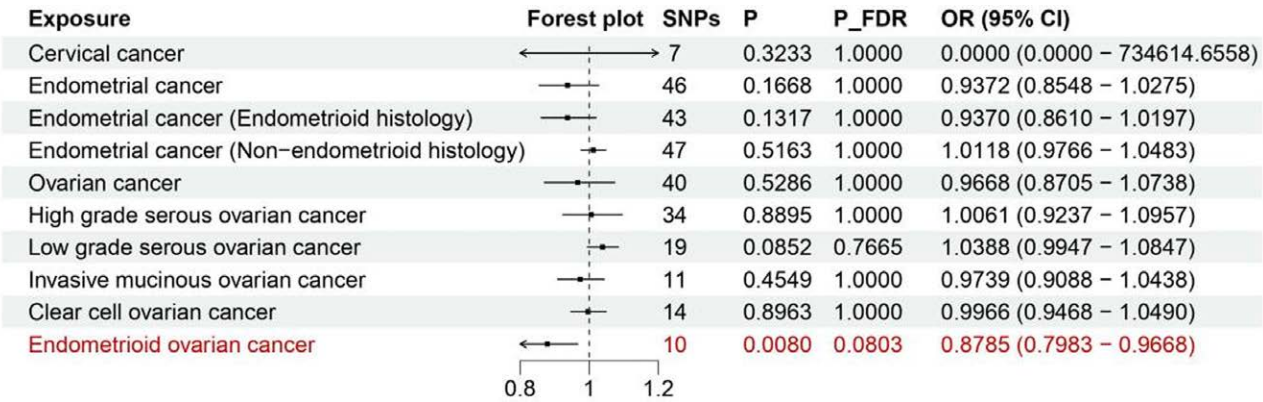

Figure 8. The effect of gynecological cancers on CTSS (IVW method). Marked red is having negative causality. CTS = cathepsins, IVW = inverse variance weighted.

between gynecological cancers and CTS). Besides, the IVW method showed a positive causal relationship between high grade serous ovarian cancer and CTSZ (IVW: OR = 1.0872, 95% CI = 1.0056–1.1754, $P = .0357$) (Fig. 9), in stark contrast, the MR-Egger method presented a conflicting perspective, suggesting a negative causal relationship (MR-Egger: OR = 0.9393, 95% CI = 0.8465–0.9979, $P = .4875$). The divergent causal effects disclosed by the IVW method and the MR-Egger method, coupled with the insignificant result from the MR-Egger analysis, might be ascribed to limited statistical power and the likelihood of outliers, thus compromising the robustness of the results. Following the application of the FDR correction, all P_{FDR} exceeded 0.05, signifying no statistical significance. Moreover, the IVW method did not identify any changes in the levels of other CTS induced by cervical cancer, endometrial cancer, and ovarian cancer, as well as their respective subtypes. In the sensitivity analysis of the reverse MR analysis (see Table S1, Supplemental Digital Content, http://links.lww.com/MD/O463, which illustrates the sensitivity analyses of the two-sample bidirectional MR analysis), the MR-PRESSO technique did not identify any possible anomalies. Cochran Q test results pointed towards heterogeneity in the case of endometrial cancer as the exposure and CTSO as the outcome, leading to the application of the random-effect IVW model (see

Figures S24–S28, Supplemental Digital Content, http://links.lww.com/MD/O464, which illustrate that the funnel plots also present the findings related to heterogeneity). The leave-one-out analysis uncovered no evident outliers (see Figures S29–S33, Supplemental Digital Content, http://links.lww.com/MD/O464, which illustrate the findings of the leave-one-out analysis), and the MR-Egger regression displayed no notable horizontal pleiotropy, underscoring the solidity of the results.

3.3. Results of MVMR analyses

While the amino acid sequences within the CTS family exhibit homology, variations in function may exist. Employing MVMR analysis allows for the mitigation of collinearity issues among diverse CTS, aiming to verify whether the estimates from UVMR analysis are biased. The MVMR analysis revealed that, upon adjusting for other types of CTS and removing collinearity, solely elevated CTSH levels remained closely associated with a heightened risk of clear cell ovarian cancer (IVW: OR = 1.1365, 95% CI = 1.0314–1.2522, $P = .0495$). Moreover, heightened concentrations of CTSF (IVW: OR = 1.0007, 95% CI = 1.0000–1.0014, $P = .0364$) and CTSS (IVW: OR = 1.0005, 95% CI = 1.0000–1.0011, $P = .0490$) were found to be associated with an elevated vulnerability to cervical cancer. Conversely, elevated levels of CTSZ were correlated with a diminished risk of cervical cancer (IVW: OR = 0.9988, 95% CI = 0.9981–0.9996, $P = .0022$). Additionally, heightened CTSO levels demonstrated a positive correlation with an increased propensity for non-endometrioid endometrial cancer (IVW: OR = 1.4405, 95% CI = 1.1864–1.7490, $P < .001$). In contrast, the causal connections between CTSB and cervical cancer, as well as CTSE and CTSZ with ovarian cancer and high grade serous ovarian cancer, failed to persist statistical significance. The forest plot illustrating these results is depicted in Figure 10. In the sensitivity analysis results, heterogeneity was identified in the outcomes of endometrial cancer, endometrioid endometrial cancer, and endometrioid ovarian cancer. Horizontal pleiotropy was also detected in the outcome of endometrioid endometrial cancer. Notably, sensitivity analyses of other MVMR studies yielded no substantial heterogeneity or signs of horizontal pleiotropy (see Table S2, Supplemental Digital Content, http://links.lww.com/MD/O463, which illustrates the sensitivity analyses of the MVMR analysis).

4. Discussion

CTS, a group of cysteine proteases ubiquitously present intra- and extracellularly, exhibit profound protein and biomolecule degradation capabilities. Their activation and secretion are thought to play a crucial role in the progression and metastasis of gynecological malignancies, potentially via extracellular matrix degradation, modification of the tumor microenvironment, initiation of apoptosis, and promotion of immune evasion.[38] This research leveraged genetic methodologies to explore the causal interplay between 9 CTS and 3 prevalent gynecological malignancies, including various subtypes, from a genetic standpoint. Our findings underscored that CTSF and CTSS demonstrated protective attributes against cervical cancer, in contrast to CTSZ, which emerged as a risk factor for this particular gynecological cancer. Additionally, CTSH emerged as a hazardous factor for clear cell ovarian cancer, whereas CTSO was recognized as a risk element for non-endometrioid endometrial cancer. Furthermore, the 6 associations identified through UVMR analysis, which exhibited a significance level of $P < .05$ prior to FDR correction but $P_{FDR} > .05$ post-correction, hint that CTSB acts as a suggestive protective factor in cervical cancer, while both CTSE and CTSZ may be suggestive protective factors for ovarian cancer and high grade serous ovarian cancer. Nevertheless, these discoveries warrant additional validation through further research. Sensitivity analyses unveiled no evidence of heterogeneity or horizontal pleiotropy. The findings of this study underscore the substantial involvement of CTS in the inception and advancement of gynecological malignancies.

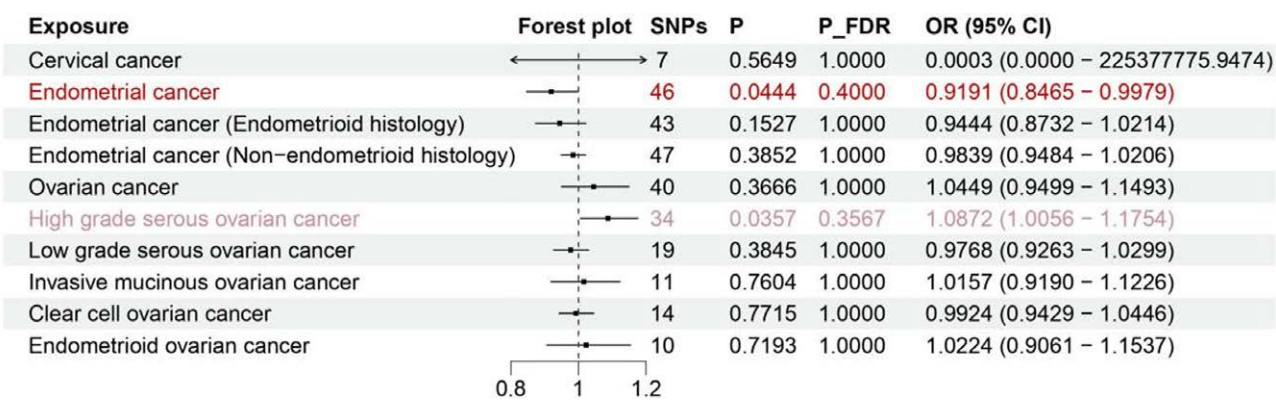

Figure 9. The effect of gynecological cancers on CTSZ (IVW method). Marked red is having negative causality. The pink marking denotes that the causal impact identified by the IVW method contradicts that of the MR-Egger method. CTS = cathepsins, IVW = inverse variance weighted.

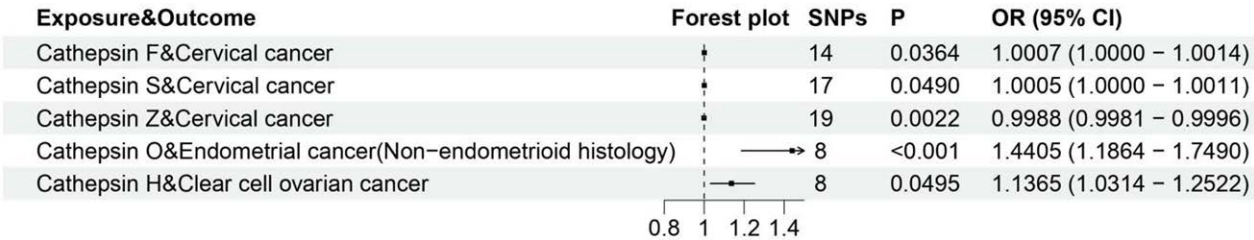

Figure 10. Forest plot of the causal relationship between CTS and gynecological cancers based on the IVW method of MVMR analysis. CTS = cathepsins, IVW = inverse variance weighted, MVMR = multivariate Mendelian randomization.

Cervical cancer stands as the predominant gynecological malignancy affecting women on a global scale.[39] As reported in literature, CTS may act as potential biological markers in clinical research on cervical cancer,[40] contributing significantly to its onset and advancement.[6,41] The MVMR results unveiled that elevated levels of CTSF and CTSS heightened the likelihood of developing cervical cancer, while heightened levels of CTSZ lowered the risk of cervical cancer. In previous investigations, elevated levels of CTSS have been shown to facilitate perineural invasion in cervical cancer through the degradation of the extracellular matrix,[42,43] and diminished activity can impede invasion and migration of cervical cancer cells by dampening their motility and metastatic ability.[44] While prior research has not definitively established a direct correlation between CTSF and CTSZ with cervical cancer, these 2 CTS have been implicated in the pathogenesis of various other cancer types.[45] Given the structural and functional parallels observed among members of the CTS family, alongside the intricate nature of the proteolytic network, it is conceivable that CTSF and CTSZ may be involved in the initiation and metastasis of cervical cancer. In addition, the UVMR analysis revealed that diminished levels of CTSB were associated with a heightened risk of cervical cancer. However, this statistical significance did not persist in both FDR corrected and MVMR analyses after accounting for other types of CTS. Hence, a definitive causal link between CTSB and the risk of cervical cancer remains elusive. Yet earlier investigations have shown a marked increase in CTSB expression in invasive cervical cancer tissues as opposed to normal tissues, with a positive correlation in the depth of infiltration.[41] CTSB possesses the capability to trigger the activation of enzymes such as urokinase-type plasminogen activator and matrix metalloproteinases, which in turn leads to the breakdown of the extracellular matrix and basement membrane. Impairment of its function or hinderance of its transport can result in diminished lysosomal activity and disrupted mitochondrial phagocytosis, ultimately impeding the proliferation of cervical cancer cells.[46,47] This inconsistency may be attributed to a multitude of factors, including the presence of numerous confounding variables in observational studies, inadequate matching in experimental models, and the relatively limited number of cases in the GWAS data for CTS and cervical cancer utilized in the MR analysis.

The onset of ovarian cancer is insidious, and at present, there is a deficiency in efficacious early screening modalities.[48] The 5-year survival rate for patients is <30%,[49] and the risk of recurrence ranges from approximately 65% to 80%,[50] thereby posing a major threat to the overall health of women worldwide. Antecedent studies have elucidated the pivotal involvement of CTS in fostering the proliferation, invasion, and migration of ovarian cancer cells,[13,15] suggesting their promise as biomarkers or therapeutic targets for the management of ovarian cancer. The outcomes of the MVMR analysis unveiled a positive correlation between CTSH and clear cell ovarian cancer. Furthermore, UVMR analysis revealed that CTSE and CTSZ might have been suggestive protective factors against ovarian cancer and high grade serous ovarian cancer ($P < .05$ 、 $P_{FDR} > 0.05$), with no substantiated links observed between other CTS types and the risk of ovarian cancer and its diverse subtypes. CTSH exhibits distinctive aminopeptidase functionality and is widely expressed across several organ systems.[51] Its expression in theca cells is implicated in crucial physiological processes including follicle development, growth and rupture of follicles, whereas damage and subsequent repair of ovarian epithelial cells due to repetitive ovulation may contribute to the pathogenesis of ovarian cancer.[52] CTS enhance tumor cell invasion and growth by degrading the extracellular matrix, altering the physical properties of the tumor microenvironment, modulating immune responses and employing other mechanisms. These combined processes establish a conducive environment, ultimately facilitating tumor invasion and metastasis. Recent observational studies and experiments have shown that the expression levels of CTSB and CTSL are higher in ovarian cancer,[53–55] but there remains a notable paucity of direct evidence associating CTSE and CTSZ expression with the risk of developing ovarian cancer and high grade serous ovarian cancer. According to the findings from MR analysis, it can be inferred that the correlations between CTSB and CTSL expression levels and ovarian cancer are unlikely to be directly driven by genetic factors, but rather influenced indirectly through other pathways, such as environmental factors or lifestyle choices. Besides, CTSE and CTSZ may suggestively exert an influence on both the initiation and progression of ovarian cancer. Furthermore, reverse MR analysis has unveiled a correlation where endometrioid ovarian cancer is associated with a decline in CTSS levels. CTSS is also an emerging ovarian cancer marker,[16] which regulates tumor cell proliferation, migration, inflammation and apoptosis by specifically cleaving and activating the G-protein-coupled receptor PAR2 located in the cell membrane, triggering downstream signaling and affecting ovarian cancer-related genes and signaling pathways, such as Wnt/β-catenin and MAPK.[56] Additionally, CTSS can also modulate antigen processing and CD4 + T and CD8 + T cell activity, impacting immune responses and promoting tumor growth and metastasis.[57] Consequently, the levels of CTS could potentially serve as valuable serum biomarkers, aiding in the diagnosis of ovarian cancer and the prediction of patients' prognoses.

Endometrial cancer ranks among the top 3 most prevalent gynecological malignancies, with the occurrence influenced by factors such as aging, obesity, diabetes, endogenous and exogenous estrogen, genetic predisposition, and mutations in cancer-related genes.[58] Moreover, CTS are expressed in the normal endometrium and are crucial factors for the functioning of the endometrium.[59] MVMR analysis revealed a positive causal association between CTSO and non-endometrioid endometrial cancer. Furthermore, reverse MR analysis provided suggestive evidence that endometrial cancer and endometrioid endometrial cancer might have led to decreased levels of CTSO. As a newly emerging member of the CTS family, the role of CTSO in tumorigenesis remains a subject of ongoing investigation. While existing research has yet to definitively reveal its direct link with endometrial cancer, considering the similarities among members of the CTS family and the complexity of the proteolytic network,[60] it is plausible to speculate that CTSO may be involved in processes like cell cycle regulation, apoptosis, and cell signaling, indirectly affecting the occurrence and development of non-endometrioid endometrial cancer. Reverse MR analysis yielded suggestive indications that endometrial cancer could potentially lead to reduced levels of CTSZ, and that non-endometrioid endometrial cancer might cause a decrease in the levels of CTSB. Earlier research has uncovered a notable correlation between CTSB positivity and the FIGO staging of endometrial cancer, with a clear negative association evident in terms of survival rates.[9] Heightened expression of CTSB can predict more aggressive tumor behavior and is regarded as an unfavorable independent tumor marker for individuals with endometrial cancer undergoing long-term follow-up. However, there is presently insufficient epidemiological research to substantiate the notion that endometrial cancer may cause decreased levels of CTSZ. Further in-depth research is needed to delve into the biological mechanisms underpinning the relationships between various CTS and endometrial cancer, including its subtypes.

The main benefits of this study are as follows: Firstly, this study is the first to utilize public databases to conduct two-sample bidirectional MR analysis and MVMR analysis to explore the causal links between CTS and gynecological cancers as well as their different subtypes from a genetic viewpoint. This methodology circumvents issues of reverse causation and potential confounding variables, while also averting the waste of human, material, and financial resources. Secondly, the GWAS

data used in this study exclusively pertained to individuals of European descent, mitigating the potential inaccuracies that could arise from cross-population subgroup comparisons. Thirdly, the selection of IVs in our MR analysis adhered to strict criteria, and sensitivity analyses undertaken to evaluate the reliability of the findings. Nonetheless, there are limitations to this study. The utilization of GWAS data derived solely from European populations may restrict the generalizability of the results to other demographic groups. The sample size in the GWAS dataset is relatively small, and a more lenient threshold was applied during selecting IVs. Despite the implementation of multiple strategies for assessment and correction, confounding factors may still have been inadvertently introduced, resulting in a certain degree of bias within the outcomes. Therefore, we look forward to larger-scale GWAS datasets in the future to validate our discoveries and enhance the accuracy and dependability of MR analysis.

In summary, our research findings suggest a causal relationship between CTS and 3 common gynecological cancers. To be more precise, CTSF and CTSS are delineated as protective elements against cervical cancer, whereas CTSZ is acknowledged as a risk factor for this particular malignancy. Additionally, CTSH is linked to an elevated risk factor of clear cell ovarian cancer, and CTSO is associated with an increased risk of non-endometrioid endometrial cancer. CTS could potentially act as one of the pivotal biomarkers for gynecological cancers, offering novel strategies for optimizing diagnostic modalities and therapeutic interventions.

Author contributions

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