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Relationship between telomere length and the prognosis of breast cancer based on estrogen receptor status: A Mendelian randomization study

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Objective: To identify the relationship between telomere length and the prognosis of breast cancer with different status of estrogen receptor (ER).

Methods: We collected single nucleotide polymorphisms (SNPs) associated with telomere length and breast cancer prognosis from the MRCIEU GWAS database and the dataset of a large meta-analysis conducted by the Breast Cancer Association Consortium (BCAC), respectively. The relationship was identified using inverse-variance weighted (IVW), MR-Egger, weighted median, penalized weighted median, and maximum likelihood methods. IVW, MR-Egger, and MR-PRESSO methods were used to perform sensitivity analysis to assess the accuracy of the results.

Results: Telomere length was negatively associated with the prognosis of total breast cancer (odds ratio [OR]=1.84, 95% confidence interval [CI]=1.08-3.14, IVW method), especially with ER- breast cancer (OR=1.89, 95% CI=1.11-3.22, IVW method). No similar relationship was found between telomere length and the prognosis of ER+ breast cancer (OR=0.99, 95% CI=0.62-1.58, IVW method). The findings from other methods were consistent with the results shown by the IVW method. The Mendelian randomization assumptions did not appear to be violated. Sensitivity analysis indicated that the result was robust, and no bias was observed in the study.

Conclusion: Telomere length is associated with the prognosis of total breast cancer, especially with ER- breast cancer. There is no significant correlation between telomere length and the prognosis of ER+ breast cancer. These findings add to the evidence that long telomere could predict a poor prognosis of ER- breast cancer.

KEYWORDS

telomere length, breast cancer, estrogen receptor (ER), ER status, Mendelian randomization study

Introduction

Breast cancer is one of the most common cancers in women worldwide. An estimated 287,850 American women were diagnosed with breast cancer in 2022, resulting in 61,360 deaths (15% of women's cancer mortality) (1, 2).

Several factors affect the risk and mortality rate of breast cancer, such as first-degree family history of breast cancer, early age at menarche, late age at first birth, late age at menopause, overweight or obesity, use of oral contraceptive, and exogenous hormone (3). These factors account for 70% of postmenopausal women with breast cancer in the USA (4, 5). The high incidence and mortality rate of breast cancer threaten women's physical and mental health. Therefore, more predictors are required to identify patients with breast cancer and help doctors formulate personalized breast cancer treatment plans.

The estrogen receptor (ER) plays an important role in breast cancer. About 70% of breast cancer cases could be detected in the expression of ER (6). Its biological characteristics and prognosis are distinctly different from other subtypes, which show sensitivity to anti-hormone therapy (7). Compared to patients with estrogen receptor-negative (ER-, ER<1% is considered ER-) breast cancer, patients with estrogen receptor-positive (ER+, ER>=1% is considered ER+) breast cancer had a better prognosis (8). European Society for Medical Oncology (ESMO) and National Comprehensive Cancer Network (NCCN) guidelines also include ER as an important prognostic indicator for breast cancer (9, 10).

The telomere is a tandem repeat sequence of TTAGGG located at the distal end of the linear chromosome (11, 12). It plays a vital role in maintaining structural integrity and regulating cell replication by preventing DNA double-strand breaks, end-to-end chromosome fusion, and degradation (13). Telomeres shorten with the cell division cycle and are generally considered a marker of aging at the cellular level in organisms (14). Thus, telomeres have been extensively studied as biomarkers for aging and age-related diseases, such as cardiovascular diseases, cancer, and diabetes (15).

The relationship between telomere length and the incidence and prognosis of breast cancer is still unclear. Several studies have shown a positive relationship between telomere length and the risk of breast cancer (16–18), some have reported a negative correlation (19, 20), while other studies show a null association (21–23). Regarding the prognosis of breast cancer, one study shows that telomere length is negatively correlated with breast cancer prognosis (24), while another study reports a positive correlation (25). Several studies did not find any association between breast cancer prognosis and telomere length (26, 27). Furthermore, only a few studies have investigated the relationship between telomere length and the incidence of breast cancer based on the status of ER (28, 29). There is a lack of studies on the relationship between telomere length and the prognosis of breast cancer with different status of ER.

These inconsistent findings mentioned above can be attributed to several confounding factors. Due to inherent flaws in traditional designs, existing observational studies cannot completely rule out possible factors of reverse causation and confounding, leading to biased associations and conclusions (30). Mendelian randomization (MR) is one approach that can address these limitations (31). MR applies genetic variations associated with environmental exposures as instrumental variables (IVs) to assess associated exposures (e.g., telomere length) and outcomes (e.g., the prognosis of breast cancer with different status of ER) (32). Since alleles are randomly assigned at conception according to Mendel's second law (33), MR analysis can effectively eliminate the effect of confounding factors and identify causal determinants of a certain outcome.

This study aimed to identify the causal association between telomere length and the prognosis of breast cancer with different status of ER. To this end, we used two-sample MR to analyze the effect of telomere length on the prognosis of total breast cancer. Next, we individually evaluated the relationship between telomere length and the prognosis of ER+ and ER- breast cancer.

Material and methods

Data collection

We collected single nucleotide polymorphisms (SNPs) related to exposure and outcome. SNPs associated with telomere length (exposure) were obtained from the MRCIEU GWAS database (https://gwas.mrcieu.ac.uk/). The database includes 472,174 samples, containing 20,134,421 SNPs in the exposure dataset. SNPs related to breast cancer survival with different status of ER were collected from the dataset of a large meta-analysis conducted by the Breast Cancer Association Consortium (BCAC) (34), which included 37,954 samples and 12,940,150 SNPs. Of these, 6,881 samples and 8,828,662 SNPs related to breast cancer survival with ER- status, and 23,059 samples and 8,714,606 SNPs associated with breast cancer survival with ER+ status. All data belonged to the population of Europe. The original data are presented as Supplementary Material (Tables S1–S3).

Abbreviations: ER, Estrogen receptor; ER-, Estrogen receptor-negative; ER+, Estrogen receptor-positive; MR, Mendelian randomization; IVs, Instrumental variables; SNP, Single nucleotide polymorphisms; BCAC, Breast Cancer Association Consortium; BMI, Body mass index; LD, Linkage disequilibrium; IVW, Inverse-variance weighted; OR, Odds ratio; CI, Confidence interval; PR, progesterone receptor; HER2, Human epidermal growth factor receptor 2.

Instrumental variable extraction

SNPs were selected as IVs to evaluate the causal effects of telemore length on the risk of breast cancer in accordance with the following assumptions (1): genetic variants must be strongly associated with exposure ($P<5\times10^{-8}$); (2) genetic variants cannot be associated with any potential confounders; (3) genetic variants affect the outcome only *via* the risk factors (35). The window of linkage disequilibrium (LD) was set $r^2<0.01$ at 10,000 kb to ensure the independence of the selected genetic variation. These SNPs were examined for the potential violations of assumptions (2) and (3) based on the PhenoScanner database (http://www.phenoscanner.medschl.cam.ac.uk/) (36); SNPs closely related to breast cancer survival were excluded (BMI, weight, smoking, cholesterol) (37–39). We also examined the possible pleiotropy of the selected SNPs using the MR Pleiotropy RESidual Sum and Outlier (MR-PRESSO) test, and no SNPs were excluded. Besides, palindromic SNPs with intermediate allele frequencies were also removed. Furthermore, all data were extracted from the European population, which could decrease the influence of population stratification. According to the above inclusion and exclusion criteria, we excluded inappropriate IVs. Besides, multiple methods were used in the study to ensure the accuracy of the results.

Finally, 104 SNPs (total breast cancer survival), 99 SNPs (breast cancer survival with ER+ status), and 100 SNPs (breast cancer survival with ER- status) were included for further study.

Mendelian randomization analysis

Inverse-variance weighted (IVW) method was used for preliminary analysis to assess the causal relationship between telomere length and the prognosis of breast cancer with different status of ER. Inverse variance weighting is a method of aggregating two or more random variables to minimize the variance of the sum, the weighting of each random variable in the sum is inversely proportional to its variance, which is often used to combine results from independent studies (35). The exposure-outcome effect for each SNP was calculated using the Wald ratio method. To ensure the accuracy of results across a wider range of scenarios, multiple methods including MR-Egger regression, weighted median, penalized weighted median, and maximum likelihood were also performed.

Sensitivity analysis

IVW and MR-Egger methods were applied in the leave-one-out analysis to evaluate the combined effect of the remaining SNPs. If the combined effect was consistent with the main effect, this indicated that no single SNP had an excessive influence on MR analysis. Funnel plot and MR-Egger intercept tests were also performed to detect the presence of pleiotropy and assess the robustness of the results. Heterogeneity was evaluated by IVW and MR-Egger tests; *P* value <0.05 indicated the presence of heterogeneity in the study. MR-PRESSO R package was used to assess whether or not there was any difference between the results of MR analysis before and after correction (40).

Statistical analysis

The results of MR estimates were shown as odds ratios (ORs) with corresponding 95% confidence intervals (CIs). We applied R software (Version 4.1.2), using the R package (TwoSampleMR, MR-PRESSO) to perform MR analysis and sensitivity analysis; R package "forestplot" was used to plot figures. A two-sided *P* value <0.05 was considered statistically significant.

Results

Mendelian randomization

MR analysis showed that telomere length was negatively associated with the prognosis of total breast cancer (see Figure 1, OR=1.84, 95% CI=1.08-3.14, IVW method), indicating that telomere length is a risk factor in breast cancer prognosis.

Telomere length was also negatively associated with the prognosis of ER- breast cancer (see Figure 2, OR=1.89, 95% CI=1.11-3.22, IVW method), suggesting that telomere length was a risk factor in the prognosis of breast cancer with ER- status. Interestingly, no similar relationship was found between telomere length and the prognosis of breast cancer with ER+ status (see Figure 3, OR=0.99, 95% CI=0.62-1.58, IVW method).

To ensure the accuracy of the findings, we also evaluated the correlations using other methods, the results of which were consistent considering the prognosis of breast cancer with different ER status (see Figures 1–3).

Sensitivity analysis

We also performed a sensitivity analysis to ensure the accuracy of the results. There was no heterogeneity in the IVW test (Q=100.710, *P*=0.545) and MR-Egger test (Q=99.691, *P*=0.545) (Table 1) with regard to total breast cancer. No significant heterogeneity was observed in both ER- and ER+ breast cancer (Table 1). MR-Egger intercept test showed *P* value >0.05, suggesting the non-existence of horizontal pleiotropy (Table 1). MR-PRESSO test ensured the accuracy of the results (Table 1). Furthermore, no single SNP showed a significant impact on the MR estimation results based on the leave-one-out analysis (Supplementary Figure S1). None of the estimates were violated based on the funnel plots (Supplementary Figure S2).

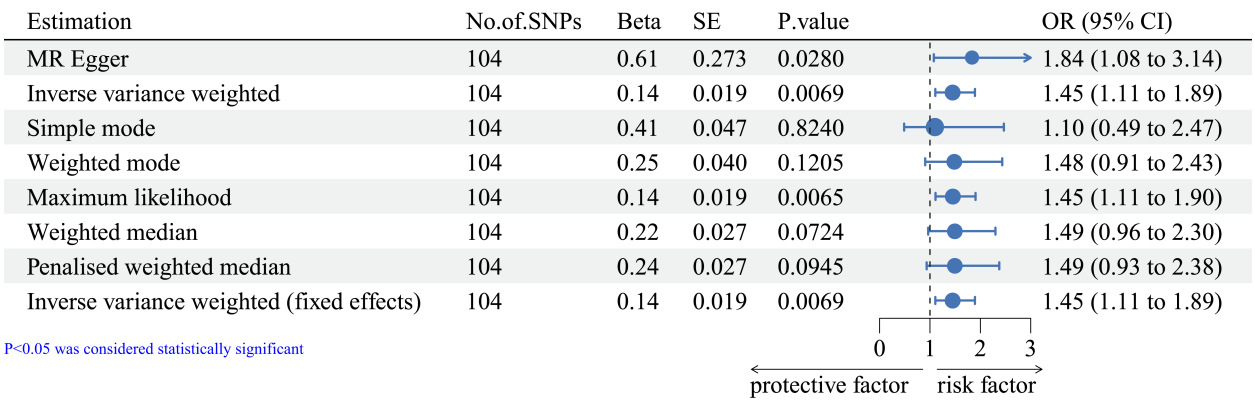

FIGURE 1
Forest plot of MR methods of the effect of telomere length on the prognosis of total breast cancer. MR, Mendelian randomization.

Discussion

The study results show that telomere length is negatively associated with the prognosis of breast cancer, especially in ER- breast cancer, while there is no significant relationship between telomere length and the prognosis of ER+ breast cancer.

As mentioned in the Introduction, research shows that telomere length is negatively correlated with the incidence of breast cancer (19, 20). However, a meta-analysis of prospective studies including approximately 14,000 cases has shown that longer leukocyte telomere length was marginally associated with an increased risk of total breast cancer incidence (41). Another study also found a positive association between longer telomere length and increased risk of breast cancer (18). The possible mechanism is that blood lymphocytes may be stimulated during inflammation and tumorigenesis and regulate telomerase through the NF-κB pathway, thereby regulating telomere length (42). Long telomeres may allow damaged cells to survive longer and continue to divide and acquire additional mutations, resulting in malignant transformation (43).

Telomere length is also associated with the prognosis of breast cancer. Research shows that long telomere predicts a good prognosis in breast cancer (25). However, another study found a negative correlation between telomere length and the prognosis of breast cancer (24). It could be explained by the mechanism that maintaining telomere length is required for the continuous growth of the tumor, especially in advanced tumor (44). Cancer cells can maintain their immortality by reactivating or up-regulating telomerase, another possible mechanism is that cancer cells can reverse telomere attrition in order to bypass

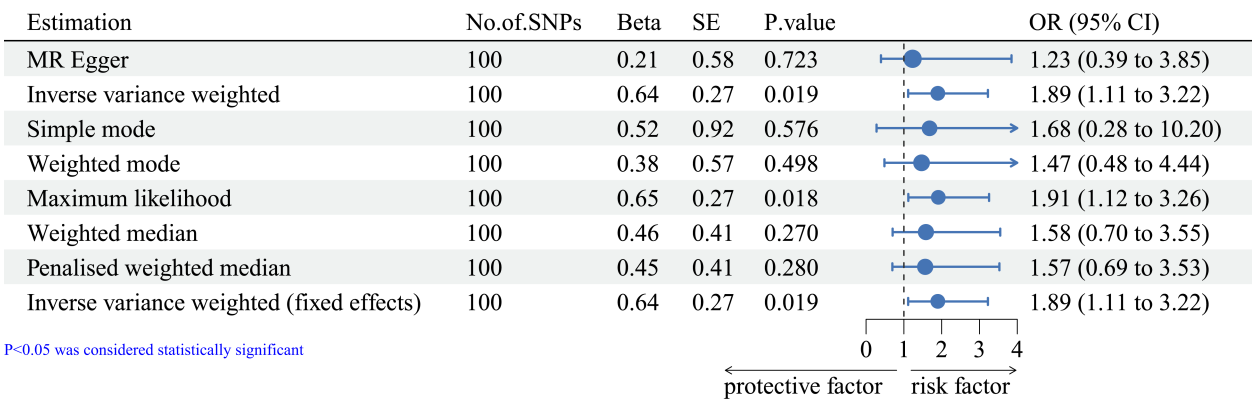

FIGURE 2
Forest plot of MR methods of the effect of telomere length on the prognosis of ER- breast cancer. MR, Mendelian randomization.

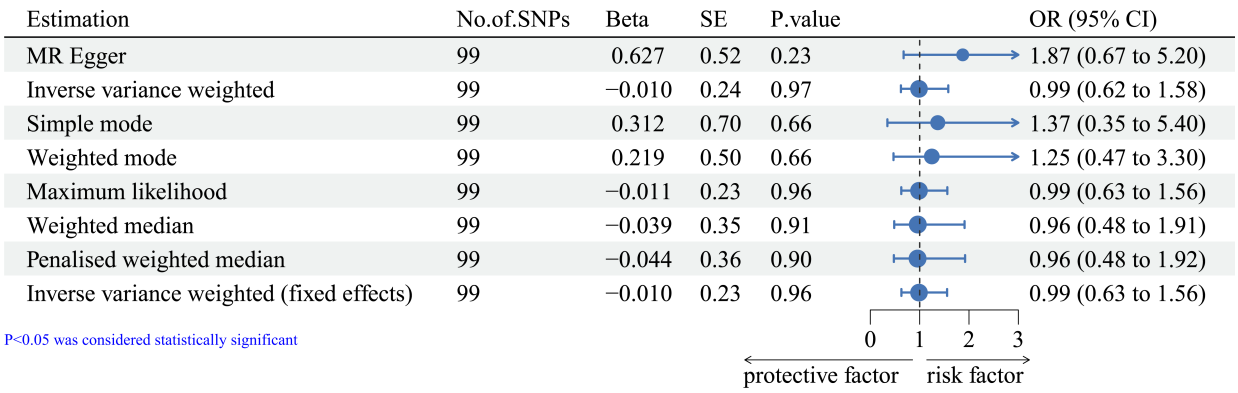

FIGURE 3
Forest plot of MR methods of the effect of telomere length on the prognosis of ER+ breast cancer. MR, Mendelian randomization.

senescence that is termed the alternative lengthening of telomeres pathway that involves DNA recombination between telomeres to achieve the immortality (45). Our results are consistent with the findings of this study. The genetic predisposition to long telomeres may influence cancer mortality through the telomere maintenance pathway (18). One explanation is that the rate of telomere shortening in breast cancer cells is slowed, and apoptosis is reduced when the immune system is suppressed (46). Immune suppression is found to be associated with a bad prognosis of breast cancer (18). Another explanation is that cells with very short telomeres may induce replicative senescence or apoptosis, thereby inhibiting the proliferative potential of the cells and thus supporting tumor suppressor activity (11, 47). The specific functional mechanisms of telomeres in cancer are still unclear. Further studies are needed to identify these mechanisms.

Hormones are also strongly associated with telomere length. The present study shows that long telomere length is related to a poor prognosis of ER- breast cancer. A study evaluating long telomere length of ER expression in 200 breast cancer patients did not find any statistically significant difference in the prognosis between ER+ and ER- patients, but it did find that ER+ cases had longer telomere length compared to control cases (16). This is because estrogen is directly involved in telomerase activation promotion through its action on the effects of human telomerase reverse transcriptase (hTERT) and post-transcriptional modification by AKT-dependent phosphorylation of hTERT (48). However, another study did not find any significant correlation between telomere length and breast cancer with different ER status (26). Further research is required to clarify the specific mechanism of estrogen action on telomeres.

The present study has several limitations. First, because of a lack of secondary data, we were unable to conduct a stratified analysis on progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2). Second, the present study had a small sample size; future studies should include a bigger sample population to improve the universality of the conclusion. Third, this study included data from only the European population. Future research should focus on other population samples.

TABLE 1 Sensitivity analysis of the causal association between telomere length and the prognosis of breast cancer with different status of estrogen receptor (ER).

ER status	Heterogeneity				Pleiotropy		Outlier examination by MR-PRESSO					
	MR-Egger		Inverse variance weighted		MR-Egger		Before correction			After correction (if necessary)		
	Q	*P* value	Q	*P* value	Intercept	*P* value	MR Analysis Causal Estimate	SD	*P* value	MR Analysis Causal Estimate	SD	*P* value
ER+	102.709	0.326	104.708	0.303	-0.017	0.173	0.021	0.237	0.930	NA	NA	NA
ER-	90.291	0.698	90.994	0.704	0.011	0.404	0.639	0.260	0.016	NA	NA	NA
Total	99.691	0.546	100.710	0.545	-0.007	0.315	0.370	0.135	0.007	NA	NA	NA

Conclusion

This study shows that telomere length is associated with the prognosis of breast cancer, especially in ER- breast cancer; however, there is no significant correlation between telomere length and the prognosis of ER+ breast cancer. These findings suggest that long telomere could predict a poor prognosis of ER- breast cancer.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

Conceptualization, LM; methodology, YL; software, YL; validation, LM and YL; formal analysis, YL; investigation, LM; resources, YL; data curation, YL; writing—original draft preparation, YL; writing—review and editing, YL; visualization, YL; supervision, LM; project administration, LM. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.1024772/full#supplementary-material

SUPPLEMENTARY TABLE 1
Characteristics of SNPs associated with telomere length and the prognosis of total breast cancer.

SUPPLEMENTARY TABLE 2
Characteristics of SNPs associated with telomere length and the prognosis of ER- breast cancer.

SUPPLEMENTARY TABLE 3
Characteristics of SNPs associated with telomere length and the prognosis of ER+ breast cancer.

SUPPLEMENTARY FIGURE 1
Sensitivity analysis based on leave-one-out analysis.

SUPPLEMENTARY FIGURE 2
Funnel plot to assess the robustness of results. Scattering points represent the effect estimated using a single SNP as an instrumental variable. Vertical lines denote the overall estimate obtained by the inverse variance weighted estimate and the MR-Egger regression.

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