HHS Public Access

Author manuscript

Breast Cancer Res Treat. Author manuscript; available in PMC 2024 May 01.

Published in final edited form as:

Breast Cancer Res Treat. 2023 May ; 199(1): 137–146. doi:10.1007/s10549-023-06902-6.

mTOR pathway candidate genes and physical activity interaction on breast cancer risk in Black women from the Women's Circle of Health Study

Mmadili N. Ilozumba[1,2], **Lusine Yaghjyan**[1], **Susmita Datta**[3], **Jinying Zhao**[1], **Zhihong Gong**[4], **Chi-Chen Hong**[4], **Kathryn L. Lunetta**[5], **Gary Zirpoli**[6], **Elisa V. Bandera**[7], **Julie R. Palmer**[6], **Song Yao**[4], **Christine B. Ambrosone**[4], **Ting-Yuan David Cheng**[1,4,8]

[1]Department of Epidemiology, University of Florida, Gainesville, FL

[2]Department of Population Health Sciences, Huntsman Cancer Institute, University of Utah, Salt Lake City, UT

[3]Department of Biostatistics, University of Florida, Gainesville, FL

[4]Department of Cancer Prevention and Control, Roswell Park Comprehensive Cancer Center, Buffalo, NY

[5]Department of Biostatistics, Boston University School of Public Health, Boston, MA

[6]Slone Epidemiology Center, Boston University, Boston, MA

[7]Cancer Epidemiology and Health Outcomes, Rutgers Cancer Institute of New Jersey, New Brunswick, NJ

[8]Division of Cancer Prevention and Control, Department of Internal Medicine, The Ohio State University, Columbus, OH

Abstract

Background: Physical activity has been shown to affect the mammalian target of rapamycin (mTOR) signaling pathway and consequently breast carcinogenesis. Given that Black women in the US are less physically active, it is not well understood whether there are gene-environment

Corresponding authors Mmadili N. Ilozumba, PhD, Department of Population Health Sciences, Huntsman Cancer Institute, University of Utah, 2000 Circle of Hope, Salt Lake City, UT 84112, Ph: (813)551-8777, Mmadili.Ilozumba@hci.utah.edu, Ting-Yuan David Cheng, PhD, Division of Cancer Prevention and Control, Department of Internal Medicine, The Ohio State University, Suite 525, 1590 North High Street, Columbus, OH, 43201, ting-yuan.cheng@osumc.edu.

Author contributions

Study conception and design: MNI, TYC. Data acquisition: ZG, JRP, SY, CCH, EVB, CBA. Writing—initial draft: MNI. Data analysis: MNI. Data interpretation: MNI, TYC, LY. Contributed to the statistical methods: KLL, TYC, LY, SD, JZ. Revised the paper: MNI, TYC, LY, SD, JZ, ZG, GZ, SY, EB. Writing—final review and approval: all authors. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest

Ethics approval

The WCHS protocol was approved by the Institutional Review Boards at Roswell Park Cancer Institute, the Rutgers Cancer Institute of New Jersey, Mount Sinai School of Medicine, and participating hospitals in New York. The current study was approved by the University of Florida's institutional review board.

Consent to participate

Signed informed consent was obtained from all individual participants included in the study.

interactions between mTOR pathway genes and physical activity in relation to breast cancer risk in Black women.

Methods: The study included 1398 Black women (567 incident breast cancer cases and 831 controls) from the Women's Circle of Health Study (WCHS). We examined interactions between 43 candidate single-nucleotide polymorphisms (SNPs) in 20 mTOR pathway genes with levels of vigorous physical activity in relation to breast cancer risk overall and by ER-defined subtypes using Wald test with 2-way interaction term and multivariable logistic regression.

Results: *AKT1* rs10138227 (C>T) and *AKT1* rs1130214 (C>A) were only associated with a decreased risk of ER+ breast cancer among women with vigorous physical activity, (odds ratio [OR] = 0.15, 95% confidence interval (CI) 0.04, 0.56, for each copy of the T allele, p-interaction = 0.007 and OR = 0.52, 95% CI 0.27, 0.96, for each copy of the A allele, p-interaction = 0.045, respectively). *MTOR* rs2295080 (G>T) was only associated with an increased risk of ER+ breast cancer among women with vigorous physical activity (OR = 2.24, 95% CI 1.16, 4.34, for each copy of the G allele; p-interaction= 0.043). *EIF4E* rs141689493 (G>A) was only associated with an increased risk of ER− breast cancer among women with vigorous physical activity (OR = 20.54, 95% CI 2.29, 184.17, for each copy of the A allele; p-interaction = 0.003). These interactions became non-significant after correction for multiple testing (FDR-adjusted p-value >0.05).

Conclusion: Our findings suggest that mTOR genetic variants may interact with physical activity in relation to breast cancer risk in Black women. Future studies should confirm these findings.

Keywords

physical activity; mTOR pathway; breast cancer; Black women; effect modification

Introduction

Physical inactivity may promote the development of obesity, induce insulin resistance, and subsequently enhance breast cancer risk (1). The mammalian target of rapamycin (mTOR) pathway receives signaling from insulin and insulin-like growth factors and its aberrant activation has been implicated in breast cancer etiology (2), mTOR is a part of phosphatidylinositol 3-kinase (PI3K) pathway generally involved in cell growth, differentiation, and survival (2,3), and may be influenced by various factors, including nutrients, growth factors, and hormones (2,3). It has two functionally distinct complexes: mTOR complex 1 (mTORC1) controls protein translation and plays a functional role in the mechanism of tumor cell growth (4), by phosphorylating mTOR downstream effector molecules including the p70 ribosomal S6 kinase 1 (S6K1) and the eukaryotic initiation factor 4E (EIF4E)-binding protein 1 (4E-BP1) (5–7); mTOR complex 2 (mTORC2) stimulates cell survival which is dependent on the activation of AKT kinase (8–11). In previous epidemiological studies, genetic variants in the mTOR pathway were associated with breast cancer risk (12–22).

Physical activity, a modifiable behavioral factor may reduce breast cancer risk (23–33), potentially through the inhibition of the mTOR signaling pathway (34,35). The relationship

between physical activity and breast cancer risk has been extensively investigated with studies mostly reporting an inverse association in White women (23,24). In Black women, an inverse association was also reported (25–33), specifically for ER+ breast cancer (33). Black women are disproportionately obese compared to other races in the US with over 70% of Black women physically inactive (33,36,37). Physical activity may also be associated with decreased breast cancer risk through biological pathways involving chronic inflammation, adiposity and insulin resistance (23,38,39). In animal models, physical activity is associated with decreased signaling of mTOR and its downstream targets in mammary cancer (35), and the influence may be independent of weight reduction (40). However, it is unknown how physical inactivity interacts with the mTOR signaling pathway or its etiological implications for breast cancer in Black women.

The primary objective of this study was to investigate potential gene-environment interactions between mTOR pathway candidate genes and physical activity in relation to breast cancer risk in Black women, overall and by ER-defined subtypes. We hypothesized stronger inverse associations of mTOR candidate polymorphisms with breast cancer in women with physical activity not reaching the vigorous level (metabolic equivalent of energy expenditure [MET] value of less than 6.0) than women with physical activity reaching the vigorous level (MET values of 6.0 or greater).

Methods

Study population

Women for these analyses were selected from participants of the Women's Circle of Health Study (WCHS), described in detail elsewhere (41,42). WCHS is a case-control study comprised of two recruitment bases, a hospital-based case ascertainment in New York City and a population-based case ascertainment in New Jersey (41). Both study sites had identical eligibility criteria. The hospital-based case ascertainment in New York City started in 2003 and included women who were between the ages of 20 to 75 years old, with no previous history of cancer other than nonmelanoma skin cancer, who were diagnosed within 9 months with primary, histologically confirmed invasive breast cancer or ductal carcinoma in situ and were English-speaking. In the population-based case ascertainment in New Jersey, cases were identified through rapid case ascertainment by the New Jersey State Cancer Registry. Black women who were less than 75 years of age, diagnosed within 9 months with primary, histologically confirmed invasive breast cancer or ductal carcinoma in situ were eligible for participation. Control eligibility and identification was similar for New York City and New Jersey study bases as women who were between the ages of 20 to 75 years without a history of any cancer diagnosis other than non-melanoma skin cancer were eligible to be controls. Random digit dialing was used to generate controls in New York City while community-based recruitment was used to supplement random digit dialing for sampling controls in New Jersey (42). Controls were frequency matched to cases by race and 5-year age groups. The in-person interview comprised the informed consent procedure and administration of extensive behavioral questionnaires, and collection of saliva samples and anthropometric measurements.

Participants reported any activities they participated in for at least one hour per week for at least three months, the number of years in total for the activity, the number of months per year, and the average hours per week. Vigorous physical activity variables have been derived as part of effort in African American Breast Cancer Epidemiology and Risk (AMBER) Consortium and categorized as vigorous physical activity (Yes and No) during the recent past and the duration of vigorous physical activity (0, <2, 2+ hours/week). Vigorous-intensity activity was defined as activities with a metabolic equivalent of energy expenditure (MET) value of 6.0 or greater (43). Consent for medical records release, pathology data and tumor tissue release was obtained from cases. The current study included 1398 Black women (567 incident breast cancer cases with invasive breast cancer or ductal carcinoma in situ and 831 controls) with available questionnaire, anthropometric, and genetic data. The WCHS protocol was approved by the Institutional Review Boards at Roswell Park Cancer Institute, Rutgers Cancer Institute of New Jersey, Mount Sinai School of Medicine, and participating hospitals in New York. Signed informed consent was obtained from each participant prior to interview and biospecimen collection. The current study was approved by the University of Florida's institutional review board.

Anthropometric Data Collection

Anthropometric measurements were taken at the end of the interview by trained research staff using standardized protocols (44); participants were asked to wear light clothing. Weight was measured in kilograms (kg) while standing height was measured to the nearest 0.1 cm. Body mass index (BMI) was calculated as the measured weight (kg) divided by height $(m)^2$.

SNP Selection

We performed a computerized literature search of the PubMed database (2000–2021) and Google search engine to identify all the relevant studies of mTOR candidate polymorphisms and cancer risk. The search strategy included the following key words: "Candidate polymorphisms in the mTOR pathway and breast cancer risk", "mTOR genetic variants and breast cancer", "mTOR genetic variants and breast cancer risk", "mTOR genetic variants and cancer", "Candidate polymorphisms in the mTOR pathway and cancer". The studies selected were required to meet the following criteria: 1) evaluate the associations between mTOR genetic variants and breast cancer risk in Black and/or Non-Black population; 2) evaluate the associations between mTOR genetic variants and risk of other cancer types in Black and/or Non-Black population. The following information was extracted from each of the included publications: the first author's name, publication date, gene/SNP name, sample population, cancer type, study type and sample size. We identified 86 SNPs in 38 genes in the mTOR pathway that were significantly associated with breast cancer risk, as well as risk for other cancer types from the literature studying Black or Non-Black women (Supplemental Table 1). Candidate SNPs in this present study are defined as SNPs that were statistically significantly associated with breast cancer risk and other cancers. Out of 86 identified SNPs, we selected a total of 43 candidate SNPs in the mTOR pathway (43 SNPs in 20 genes) that were available and already genotyped in WCHS for statistical analyses (Supplemental Table 2). WCHS is a member of the AMBER consortium and genotyping, imputation and quality control procedures have been previously reported (16,26,45–47).

Statistical Analyses

Descriptive characteristics of the cases and controls were compared using t-tests for continuous variables and chi-square tests for categorical variables. Multivariable logistic regression was used to test the associations between selected candidate SNPs and breast cancer risk while adjusting for the following known and potential confounders: age (18–39, 40–49, 50–59 [reference] and 60–79), BMI (<25 [reference], 25-<30, and ≥30 kg/m^2), geographic location (New Jersey [reference] and New York City), DNA source (blood [reference], mouth wash and saliva) and principal components (PC) of the genotypes (PC5, PC6 and PC8). Since menopausal status and educational status did not change the estimates, they were not included in the final models. The associations were presented as odds ratios (OR) and corresponding 95% confidence intervals (CI). The genetic association analysis tested for an additive model; the genetic variants, i.e., the independent variables, were modeled as 0, 1, or 2 alleles. We examined associations for all breast cancer cases combined as well as separately for ER+ and ER− tumors. Among cases with known ER status, case-only analyses were conducted comparing ER− tumors to ER+ tumors.

To explore whether associations between mTOR genetic variants and breast cancer were modified by vigorous physical activity, we conducted stratified analyses by vigorous physical activity defined as vigorous physical activity (Yes and No) during the recent past. The Wald test was used to evaluate effect modification, including a 2-way interaction term between the SNPs and vigorous physical activity. We further conducted a stratification analysis by menopausal status for the gene-environment interactions in association with overall breast cancer risk. Statistical significance was defined as nominal $p < 0.05$ for selected candidate SNPs and all statistical tests were 2-sided. To control for the inflation of false-positive rates from multiple comparisons, we controlled the false discovery rate (FDR). The adjusted p-value with a significance threshold of 0.05 was applied. We calculated aggregated genetic risk scores only for mTOR candidate SNPs associated with overall breast cancer with nominal p-values ($p <0.05$) and ($p<0.10$) (48), and evaluated whether their associations with overall breast cancer were modified by physical activity variable. The scores for risk alleles were modeled as 0, 1, or 2 alleles and imputed values were rounded up to the nearest whole number. The scores of all the SNPs were summed and the distribution of the total SNP score was divided into quartiles in multivariable logistic regressions. Statistical analyses were performed using SAS 9.4 software (SAS Institute Inc.).

Results

This study included 567 cases and 831 controls. Descriptive characteristics of study participants were presented in Table 1. Among cases with known ER status, 26.46 % were ER− and 60.14% were ER+ tumors. The distribution of age, BMI, vigorous physical activity as well as menopausal status did not differ by case-control status.

Table 2 shows stratified results for the associations between mTOR candidate SNPs and overall breast cancer risk by vigorous physical activity. A borderline interaction was found between *EIF4E* rs141689493 (G>A) and vigorous physical activity (p-interaction= 0.094). The variant was associated with borderline decreased risk of overall breast cancer among women with no vigorous physical activity (OR = 0.52, 95% CI 0.24, 1.11, for each copy of

the A allele) but not among women with vigorous physical activity. The interaction however became non-significant after correction for multiple testing (FDR-adjusted p-value >0.05).

Table 3 shows stratified results for the associations between mTOR candidate SNPs and ER+ breast cancer risk by vigorous physical. Vigorous physical activity was an effect modifier for the association of *AKT1* rs1130214 (C>A) and *AKT1* rs10138227 (C>T) with ER+ breast cancer risk (p-interaction = 0.045 and 0.007, respectively). The variants were associated with a decreased risk of ER+ breast cancer among women with vigorous physical activity (OR = 0.52, 95% CI 0.27, 0.96, for each copy of the A allele and OR = 0.15, 95% CI 0.04, 0.56, for each copy of the T allele, respectively) but not among women with no vigorous physical activity. These interactions, however, became non-significant after correction for multiple testing (FDR-adjusted p-value>0.05).

Table 4 shows stratified results for the associations between mTOR candidate SNPs and ER− breast cancer risk by vigorous physical activity. Vigorous physical activity was an effect modifier for the association of *EIF4E* rs141689493 (G>A) with ER− breast cancer risk (p-interaction = 0.003). The variant was associated with an increased risk of ER− breast cancer among women with vigorous physical activity (OR = 20.54, 95% CI 2.29, 184.17, for each copy of the A allele) but not among women with no vigorous physical activity. These interactions became non-significant after correction for multiple testing (FDR-adjusted p-value >0.05).

Table 5 shows stratified results for the associations between mTOR candidate SNPs and breast cancer risk by vigorous physical activity. in case-only analysis comparing ER− cases to ER+ cases. An interaction existed between *AKT1* rs10138227 (C>T) and vigorous physical activity (p-interaction = 0.001). The interaction remained statistically significant after correction for multiple testing (FDR-adjusted p-value = 0.030). Interactions also existed between vigorous physical activity and *EIF4E* rs141689493 (G>A) (p-interaction = 0.044) and *MTOR* rs2536 (T>C) (p-interaction = 0.023), which however, became non-significant after correction for multiple testing (FDR-adjusted p-value >0.05).

Supplemental Table 3 provides the aggregated genetic risk score estimation for the associations of mTOR candidate SNPs with overall breast cancer risk stratified by vigorous physical activity. Although, we observed no gene-environment interaction, quartile (Q)4 vs Q1 of aggregated genetic risk score was associated with an increased overall breast cancer risk among women with no vigorous physical activity but not among women with vigorous physical activity.

Supplemental Table 4 provides the results of the stratified analysis by menopausal status for the gene-environment interaction in relation to overall breast cancer risk. *EIF4E* rs141689493 (G>A) was associated with a borderline increased overall breast cancer risk in premenopausal women with vigorous physical activity but not in postmenopausal women.

Discussion

In Black women enrolled in the WCHS, we found significant interactions of several of the mTOR genetic variants with vigorous physical activity in relation to breast cancer

risk, overall and in ER+ and ER− tumors separately. These interactions did not remain statistically significant after correction for multiple testing and thus should be interpreted with caution.

The interactions of mTOR genetic variants and physical activity on breast cancer risk have not been investigated in epidemiological studies and the evidence for other cancer risk is limited. In a large White population-based case-control study, physical activity, energy intake, and genetic variants in the mTOR pathway including *AKT3*, *RAPTOR* and *TSC2* jointly influenced bladder cancer risk (49). In another case-control study of renal cell carcinoma risk in Non-Hispanic White participants, a potential joint effect of low physical activity and mTOR genetic variants (six SNPs in *AKT3*) on renal cell carcinoma risk was reported. An increased renal cell carcinoma risk was observed only in participants who were physically inactive but not in participants with intensive physical activity (50). However, in the recent Netherlands Cohort study for colorectal cancer risk, no modifying effects of mTOR genes including *AKT2*, *AKT3*, *MTOR* and *TSC2* were observed on the association of physical activity with colorectal cancer risk (51).

In the present study, we observed that vigorous physical activity was an effect modifier for the associations of *AKT1* rs1130214 (C>A) and *AKT1* rs10138227 (C>T) with ER+ breast cancer risk. The variants were associated with a decreased risk of ER+ breast cancer among women with vigorous physical activity, findings that supported our study hypothesis. These two genetic variants in *AKT1* may interact with vigorous physical activity to confer a protective effect on ER+ breast cancer risk but our findings warrant validation. *AKT1* rs1130214 and *AKT1* rs10138227 have been mapped in regions with active transcriptional enhancers in breast myoepithelial cells (52). In Sri-Lankan women, *AKT1* rs1130214 was associated with HER2-positive breast cancer (14), whether the observed association is specific to HER2-positive tumors in Black women needs further research.

We observed that *EIF4E* rs141689493 (G>A) variant was associated with a borderline decreased risk of overall breast cancer among women with no vigorous physical activity and an increased risk of ER− breast cancer among women with vigorous physical activity. Our stratified analysis by menopausal status also showed that *EIF4E* rs141689493 (G>A) was associated with a borderline increased overall breast cancer risk among premenopausal women with vigorous physical activity but not in postmenopausal women. These findings did not support our study hypothesis. The reason behind this is not clear but a potential hypothesis is that other biological mechanisms independent of vigorous physical activity that were not measured in the present study may be implicated. *EIF4E* rs141689493 (G>A) is an intronic SNP found in region with active transcriptional promoters in mammary epithelial cells (52). Although, we cannot dismiss the chances for a false positive result, an important next step is to investigate whether the functional impact of *EIF4E* rs141689493 (G>A) on the mTOR pathway differs in Black women.

Substantive epidemiological evidence suggests that moderate to vigorous physical activity is associated with a decreased breast cancer risk (43). There is still however, limited understanding of the impact of the mechanisms of physical activity as well as the heterogeneous measures of physical activity exposures on breast cancer risk (38,43). Given

that maintaining a lifestyle that only involves reducing energy intake has been challenging, a feasible strategy to maintain energy balance and reduce breast cancer risk is to engage in physical activity and reduce energy intake (49). Physical activity has been reported to confer several anticancer benefits such as decrease in inflammation, enhanced immune function and carcinogen detoxification and DNA repair mechanism as well as altered cell proliferation and apoptosis (53). Although the present study did not examine these anticancer benefits of physical activity, the modifying effects of physical activity on mTOR pathway and breast cancer in our study warrants further investigation.

To our knowledge, this is the first study to comprehensively evaluate gene-environment interactions between mTOR pathway candidate genes and physical activity among Black women. It had a relatively large sample size of Black women which enabled the analysis of risk for overall breast cancer, as well as for ER+ and ER− cancer separately. The large panel of genes used in the study covered a detailed mTOR signaling pathway and genes were available for the selected candidate SNPs that were reported in the literature.

A few limitations in our study should be noted. Our findings require validation, as some of the gene-environment interactions were not significant after correction for multiple tests. Significant results from this study also require validation. Thus, interpretation of the study findings with caution is warranted. There is lack of generalization of study findings to other racial and ethnic groups as the study only used Black women participants. There is still a possibility for residual confounding potentially due to unmeasured variables despite the study aiming to adjust for important confounders in the statistical analysis. We did not have information on comorbidities, whether the residual confounding is large enough to affect result estimates need to be determined in future studies. There is lack of objective measurement of physical activity because physical activity was self-reported which may introduce measurement errors. Physical activity is a complex behavioral activity, and its quantification is multifaceted involving varying measures of type, frequency, intensity, and duration of physical activity. In the present study, vigorous intensity activity was defined as activities with MET value of 6.0 or greater, indicating the absolute rate of energy expenditure and the recommended descriptor for the specific range of intensity measured in our study (43). These variables were analyzed as vigorous physical activity (Yes and No) during the recent past. We also considered the duration of vigorous physical activity (0, <2, 2+ hours/week) but the number of breast cancer cases were small within strata of physical activity measurements, which resulted in wide 95% confidence intervals and potentially inflated risk estimates (data not shown). Since this is a case-control study, recall bias cannot be ruled out as cases and controls may have varying recall of physical activity. Therefore, a prospective cohort study with an objective measure of physical activity is required to estimate the causal relationship of physical activity and breast cancer considering the mTOR signaling pathway. The difference in ascertainment of controls in New York City and New Jersey may raise concerns regarding bias due to systematic over-enumeration of controls in New Jersey however, the sampled controls were representative of the same populations from which the cases were derived (42).

In conclusion, our findings suggest that mTOR genetic variants may interact with physical activity in relation to breast cancer risk in Black women. Studies with larger sample size of Black women are needed to validate our findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Funding

This work was supported by grants from the US National Institutes of Health, The National Cancer Institute (grant number P01 CA151135 J.R.P. and C.B.A, R01CA098663 to J.R.P.; R01 CA100598 to C.B.A. and E.V.B; R01 CA185623, P30 CA016056, P30 CA072720, K07 CA201334, R37 CA248371); the Breast Cancer Research Foundation (C.B.A., C-CH).

Data availability

Data will be made available on request.

References

1. Hursting SD, Lashinger LM, Wheatley KW, Rogers CJ, Colbert LH, Perkins SN. Reducing the weight of cancer: mechanistic targets for breaking the obesity – carcinogenesis link. Best Pract Res Clin Endocrinol Metab [Internet]. 2008;22(4):659–69. Available from: 10.1016/j.beem.2008.08.009 [PubMed: 18971125]
2. Liu P, Cheng H, Roberts TM, Zhao JJ. Targeting the phosphoinositide 3-kinase (PI3K) pathway in cancer. Nat Rev Drug Discov. 2011;8(8):627–44.
3. Altomare DA, Khaled AR. Homeostasis and the importance for a balance between AKT/mTOR activity and intracellular signaling. Curr Med Chem [Internet]. 2012;19(22):3748–62. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22680924%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC3414727 [PubMed: 22680924]
4. Ma B, Shan M-H, Sun G, Ren G-H, Dong C, Yao X, et al. Immunohistochemical analysis of phosphorylated mammalian target of rapamycin and its downstream signaling components in invasive breast cancer. Mol Med Rep. 2015;12:5246–54. [PubMed: 26151180]
5. Wang X, Beugnet A, Murakami M, Yamanaka S, Proud CG. Distinct signaling events downstream of mTOR cooperate to mediate the effects of amino acids and insulin on initiation factor 4E-binding proteins. Mol Cell Biol. 2005;25(7):2558–72. [PubMed: 15767663]
6. Gingras A, Gygi SP, Raught B, Polakiewicz RD, Abraham RT, Hoekstra MF, et al. Regulation of 4E-BP1 phosphorylation : a novel two-step mechanism. Genes Dev. 1999;13(11):1422–37. [PubMed: 10364159]
7. Gingras A, Raught B, Gygi SP, Niedzwiecka A, Miron M, Burley SK, et al. Hierarchical phosphorylation of the translation inhibitor 4E-BP1. Genes Dev. 2001;15(21):2852–64. [PubMed: 11691836]
8. Laplante M, David M. mTOR signaling at a glance. Cell Sci. 2009;122:3589–94.
9. Stephens L, Anderson K, Stokoe D, Erdjument-bromage H, Painter GF, Holmes AB, et al. Protein kinase B kinases that mediate phosphatidylinositol 3,4,5-trisphosphate-dependent activation of protein kinase B. Science (80-). 1998;279(5351):710–4.
10. Manning B, Cantley L. AKT/PKB signaling: Navigating downstream. Cell. 2007;129(7):1261–74. [PubMed: 17604717]
11. Zarogoulidis P, Lampaki S, Francis Turner J, Huang H, Kakolyris S, Syrigos K, et al. mTOR pathway: A current, up-to-date mini-review. Oncol Lett. 2014;8(6):2367–70. [PubMed: 25360163]

12. Araujo ME De, Erhart G, Buck K, Mu E, Hubalek M, Fiegl H, et al. Polymorphisms in the gene regions of the adaptor complex LAMTOR2 / LAMTOR3 and their association with breast cancer risk. PLoS One. 2013;8(1):1–8.
13. Mehta MS, Vazquez A, Kulkarni DA, Kerrigan JE, Atwal G, Metsugi S, et al. Polymorphic variants in TSC1 and TSC2 and their association with breast cancer phenotypes. Breast Cancer Res Treat. 2013;125(3):1–15.
14. Sirisena ND, Adeyemo A, Kuruppu AI, Samaranayake N, Dissanayake VHW. Genetic variants associated with clinicopathological profiles in sporadic breast cancer in Sri Lankan women. J Breast Cancer. 2018;21(2):165–72. [PubMed: 29963112]
15. Cheng T-YD, Shankar J, Zirpoli G, Roberts MR, Hong C-C, Bandera EV, et al. Genetic variants in the mTOR pathway and interaction with body size and weight gain on breast cancer risk in African-American and European-American women. Cancer Causes Control. 2017;27(8):965–76.
16. Cheng TD, Ambrosone CB, Hong C, Lunetta KL, Liu S, Hu Q, et al. Genetic variants in the mTOR pathway and breast cancer risk in African American women. Carcinogenesis. 2015;00(2):1–7.
17. Wang S, Huo D, Ogundiran TO, Ojengbede O, Nathanson KL, Nemesure B, et al. Association of breast cancer risk and the mTOR pathway in women of African ancestry in 'The Root ' Consortium. Carcinogenesis. 2017;38(8):789–96. [PubMed: 28582508]
18. Campa D, Claus R, Dostal L, Stein A, Karina JC, Benetou V, et al. Variation in genes coding for AMP-activated protein kinase (AMPK) and breast cancer risk in the European Prospective Investigation on Cancer (EPIC). Breast Cancer Res Treat. 2011;127:761–7. [PubMed: 21116708]
19. Zhao Y, Diao Y, Wang X, Lin S, Wang M, Kang H. Impacts of the mTOR gene polymorphisms rs2536 and rs2295080 on breast cancer risk in the Chinese population. Oncotarget. 2016;7(36):58174–80. [PubMed: 27533457]
20. Ren H, Wang X, Lin S. Associations between C1772T polymorphism in hypoxia-inducible factor-1a gene and breast Cancer: A meta-analysis. Med Sci Monit. 2014;20:2578–83. [PubMed: 25484025]
21. Li X, Zhang R, Liu Z, Li S, Xu H. The genetic variants in the PTEN/PI3K/AKT pathway predict susceptibility and CE(A)F chemotherapy response to breast cancer and clinical outcomes. Oncotarget. 2017;8(12):20252–65. [PubMed: 28423632]
22. Wang Y, Zhang H, Lin M, Wang Y. Association of FGFR2 and PI3KCA genetic variants with the risk of breast cancer in a Chinese population. Cancer Manag Res. 2018;10:1305–11. [PubMed: 29872343]
23. Lynch BM, Neilson HK FC. Physical activity and breast cancer prevention. Recent Results Cancer Res. 2011;186:13–42. [PubMed: 21113759]
24. Friedenreich C The role of physical activity in breast cancer etiology. Semin Oncol. 2010;37(3):297–302. [PubMed: 20709211]
25. Cohen S, Matthews C, Bradshaw P, Lipworth L, Buchowski M, Signorello L, et al. Sedentary behavior, physical activity, and likelihood of breast cancer among black and white women: a report from the Southern Community Cohort Stu. Cancer Prev Res. 2014;6(6):566–76.
26. Sanderson M, Lipworth L, Shen-miller D, Nechuta S. Energy-related indicators and breast cancer risk among White and Black women. PLoS One. 2015;10(4):8–9.
27. Rosenberg L, Palmer J, Bethea T, Ban Y, Kipping-Ruane K, Adams-Campbell L. A prospective study of physical activity and breast cancer incidence in African-American women. Cancer Epidemiol Biomarkers Prev. 2015;23(11):2522–31.
28. Sheppard V, Makambi K, Taylor T, Wallington S, Sween J, Adams-Campbell L. Physical activity reduces breast cancer risk in African American women. Ethn Dis. 2013;21(4):406–11.
29. Adams-campbell LL, Rosenberg L, Rao RS, Palmer JR. Strenuous physical activity and breast cancer risk in African-American women. J Natl Med Assoc. 2001;93(7):267–75. [PubMed: 11491277]
30. Bernstein L, Patel AV, Ursin G, Sullivan-halley J, Press F, Deapen D, et al. Lifetime recreational exercise activity and breast cancer risk among Black women and White women. J Natl Cancer Inst. 2005;97(22):1671–9. [PubMed: 16288120]
31. John EM, Horn-ross PL, Koo J. Lifetime Physical Activity and Breast Cancer Risk in a Multiethnic Population : The San Francisco Bay Area Breast Cancer Study. 2003;12(November):1143–52.

32. Ratnasinghe LD, Modali RV, Seddon MB LT. Physical activity and reduced breast cancer risk: a multinational study. Nutr Cancer [Internet]. 2010;62(4):425–35. Available from: 10.1080/01635580903441295 [PubMed: 20432163]
33. Gong Z, Elisa CH, Lucile VB, Troester MA, Kathryn SP, Gary AM, et al. Vigorous physical activity and risk of breast cancer in the African American breast cancer epidemiology and risk consortium. Breast Cancer Res Treat. 2016;159(2):347–56. [PubMed: 27514396]
34. Agostini D, Natalucci V, Baldelli G, Santi M De, Zeppa SD, Vallorani L, et al. New Insights into the Role of Exercise in Inhibiting mTOR Signaling in Triple-Negative Breast Cancer. Oxid Med Cell Longev. 2018;2018(9):5896786. [PubMed: 30363988]
35. Jiang W, Zhu Z, Thompson HJ. Effects of limiting energy availability via diet and physical activity on mammalian target of rapamycin-related signaling in rat mammary carcinomas. Carcinogenesis. 2013;34(2):378–87. [PubMed: 23125225]
36. Center Office of Minority Health Resource. Obesity and African Americans [Internet]. 2020. Available from: https://minorityhealth.hhs.gov/omh/browse.aspx?lvl=4&lvlid=25
37. Bandera E V, Chandran U, Hong C-C, Troester MA, Bethea TN, Adams-Campbell LL, et al. Obesity, body fat distribution, and risk of breast cancer subtypes in African American women participating in the AMBER consortium. Breast Cancer Res Treat. 2015;150(3):655–66. [PubMed: 25809092]
38. World Cancer Research Fund/American Institute for Cancer Research. Diet, nutrition, physical activity and cancer: a global perspective [Internet]. 2018. Available from: dietandcancerreport.org
39. World Cancer Research Fund/American Institute for Cancer Research. Diet, nutrition, physical activity and breast cancer [Internet]. 2018. Available from: dietandcancerreport.org
40. Thompson HJ, Jiang W, Zhu Z, Collins F. Candidate mechanisms accounting for effects of physical activity on breast carcinogenesis. IUBMB Life. 2015;61(9):895–901.
41. Ambrosone CB, Ciupak GL, Bandera EV, Jandorf L, Bovbjerg DH, Zirpoli G, et al. Conducting molecular epidemiological research in the age of HIPAA: A multi-institutional case-control study of breast cancer in African-American and European-American women. J Oncol. 2009;2009:871250. [PubMed: 19865486]
42. Bandera EV, Chandran U, Zirpoli G, Mccann SE, Ciupak G, Ambrosone CB. Rethinking sources of representative controls for the conduct of case – control studies in minority populations. BMC Med Res Methodol [Internet]. 2013;13(1):1. Available from: BMC Medical Research Methodology [PubMed: 23297754]
43. 2018 Physical Activity Guidelines Advisory Committee. Physical Activity Guidelines Advisory Committee Scientific Report. Washington, DC:U.S. Department of Health and Human Services,; 2018.
44. Bandera EV, Chandran U, Zirpoli G, Gong Z, Mccann SE, Hong C, et al. Body fatness and breast cancer risk in women of African ancestry. BMC Cancer. 2013;13(475).
45. Stolk L, Perry JR, Chasman DI, He C, Mangino M, Sulem P, Barbalic M, Broer L, Byrne EM, Ernst F, Esko T, Franceschini N, Gudbjartsson DF, Hottenga JJ, Kraft P, McArdle PF, Porcu E, Shin SY, Smith AV, van Wingerden S, Zhai G, Zhuang WV, Albrecht E, Alizade LK. Meta-analyses identify 13 novel loci associated with age at menopause and highlights DNA repair and immune pathways. Nat Genet. 2012;44(3):260–8. [PubMed: 22267201]
46. Elks CE, Perry JR, Sulem P, Chasman DI, Franceschini N, He C, Lunetta KL, Visser JA, Byrne EM, Cousminer DL, Gudbjartsson DF, Esko T, Feenstra B, Hottenga JJ, Koller DL, Kutalik Z, Lin P, Mangino M, Marongiu M, McArdle PF, Smith AV, Stolk L, van Wingerden MA. Thirty new loci for age at menarche identified by a meta-analysis of genome-wide association studies. Nat Genet. 2011;42(12):1077–85.
47. Patterson N, Price AL, Reich D. Population structure and eigenanalysis. PLoS Genet. 2006;2(12).
48. Ilozumba MN, Yaghjyan L, Datta S, Zhao J, Hong C-C, Lunetta KL, et al. mTOR pathway candidate genes and obesity interaction on breast cancer risk in black women from the Women ' s Circle of Health Study. Cancer Causes Control [Internet]. 2023; Available from: 10.1007/s10552-022-01657-9

49. Lin J, Wang J, Greisinger AJ, Grossman HB, Michele R, Dinney CP, et al. Energy balance, the PI3K-AKT-mTOR pathway genes and the risk of bladder cancer. Cancer Prev Res (Phila). 2011;3(4):505–17.
50. Shu X, Lin J, Wood CG, Tannir NM, Wu X. Energy balance, polymorphisms in the mTOR pathway, and renal cell carcinoma risk. J Natl Cancer Inst. 2013;105(6):424–432. [PubMed: 23378641]
51. Simons CCJM, Schouten LJ, Godschalk RWL, van Schooten FJ, Stoll M, Van Steen K, et al. Polymorphisms in the mTOR-PI3K-Akt pathway, energy balance-related exposures and colorectal cancer risk in the Netherlands Cohort Study. BioData Min. 2022;15(1):1–20. [PubMed: 34980200]
52. Broad Institute. HaploReg v3 [Internet]. 2023 [cited 2023 Apr 22]. Available from: https://pubs.broadinstitute.org/mammals/haploreg/haploreg_v3.php
53. Rogers CJ, Colbert LH, Greiner JW, Perkins SN, Hursting SD. Physical activity and cancer prevention pathways and targets for intervention. Sport Med. 2008;38(4):271–96.

Table 1:

Descriptive characteristics of study participants

	Cases N = 567	Control N = 831	p-value
Age, years mean ± SD	52.44 ±10.86	51.00±10.00	
Age group, n (%)			0.101
18–39	69 (12.17)	116 (13.96)	
40–49	146 (25.75)	228 (27.44)	
50–59	205 (36.16)	318 (38.27)	
60–79	147 (25.93)	169 (20.34)	
Geographical Location, n (%)			0.0001
New Jersey	443 (78.13)	571 (68.71)	
New York City	124 (21.87)	260 (31.29)	
DNA source, n (%)			0.001
Blood	101 (17.81)	212 (25.51)	
Mouthwash	11 (1.94)	8 (0.96)	
Saliva	455 (80.25)	611 (73.53)	
BMI (kg/m^2), n (%)			0.435
<25	97 (17.11)	162 (19.49)	
25–30	168 (29.63)	248 (29.84)	
≥30	293 (51.68)	404 (48.62)	
Vigorous Physical Activity, n (%)			0.870
Yes	72 (12.70)	108 (13.00)	
No	495 (87.30)	723 (87.00)	
Menopausal Status n (%)			0.355
Pre-menopausal	253 (44.78)	393 (47.29)	
Post-menopausal	312 (55.22)	438 (52.71)	
ER Status, n (%)			-
Negative	150 (26.46)	NA	
Positive	341 (60.14)	NA	
Unknown	76 (13.40)		

Abbreviations: ER− estrogen receptor, NA- not applicable

Table 2:

Associations of mTOR candidate SNPs with overall breast cancer risk by vigorous physical activity

Gene	SNPs	Vigorous physical activity				P-interaction	FDR-adjusted p-value
		No (487 cases/706 controls)		Yes (71 cases/108 controls)			
		OR [a,b] (95% CI)	p-value	OR [a,b] (95% CI)	p-value		
EIF4E	rs141689493 (G>A)	0.52 (0.24, 1.11)	**0.089**	2.95 (0.44, 19.67)	0.265	**0.094**	0.805
TSC2	rs181088346 (G>A)	0.68 (0.46, 1.00)	**0.050**	1.48 (0.60, 3.66)	0.396	0.108	0.805
AKT1	rs2494732 (T>C)	1.05 (0.88, 1.25)	0.611	1.57 (0.98. 2.52)	**0.060**	0.117	0.805
AKT	rs2498801 (T>C)	1.06 (0.90, 1.26)	0.487	1.58 (0.98, 2.53)	**0.059**	0.125	0.805
MTOR	rs2295080 (G>T)	1.03 (0.83, 1.28)	0.794	1.64 (0.96, 2.79)	**0.068**	0.126	0.805
RHEB	rs717775 (A>C)	0.90 (0.61, 1.32)	0.583	2.23 (0.88, 5.67)	**0.091**	0.145	0.805
BRAF	rs114729114 (C>T)	2.26 (1.27, 4.03)	**0.006**	1.03 (0.22, 4.80)	0.971	0.164	0.805
MTOR	rs1883965 (A>G)	1.06 (0.90, 1.26)	0.469	1.58 (0.96, 2.60)	**0.070**	0.187	0.805
MTOR	rs1064261 (G>A)	1.07 (0.91, 1.27)	0.420	1.55 (0.95, 2.52)	**0.078**	0.207	0.810
PRKAG3	rs190843378 (C>T)	0.73 (0.45, 1.17)	0.186	0.29 (0.08, 1.10)	**0.071**	0.350	0.941
AKT1	rs2494752 (A>G)	0.83 (0.68, 1.03)	**0.084**	0.65 (0.37, 1.14)	0.133	0.423	0.981
MTOR	rs7784818 (A>G)	0.86 (0.72, 1.02)	**0.074**	1.00 (0.62, 1.61)	0.993	0.574	0.981
MTOR	rs4128396 (A>C)	0.68 (0.45, 1.01)	**0.058**	0.83 (0.33, 2.04)	0.676	0.858	0.981
MTOR	rs7651265 (A>G)	1.25 (0.98, 1.60)	**0.073**	1.19 (0.64, 2.21)	0.584	0.912	0.981
FRAP1	rs12125777 (C>T)	1.33 (1.00, 1.77)	**0.047**	1.45 (0.67, 3.15)	0.343	0.957	0.981

Abbreviations: OR odds ratio; 95% CI, 95% confidence interval

[a]Adjusting for age, geographic location, DNA source, principal components of the genotypes, and body mass index

[b]The genetic association analysis tested for an additive model and the genetic variants, i.e., the independent variables, were modeled as (0, 1, or 2 alleles)

Bolded p-value: Nominal p-values ($p<0.05$) and ($p<0.10$)

Table 3:

Associations of mTOR candidate SNPs with ER+ breast cancer risk by vigorous physical activity

Gene	SNPs	Vigorous physical activity				P-interaction	FDR-adjusted p-value
		No (295 cases/706 controls)		Yes (40 cases/108 controls)			
		OR [a,b] (95% CI)	p-value	OR [a,b] (95% CI)	p-value		
AKT1	rs10138227 (C>T)	0.99 (0.79, 1.24)	0.912	0.15 (0.04, 0.56)	**0.004**	**0.007**	0.288
MTOR	rs2295080 (G>T)	1.01 (0.78, 1.30)	0.939	2.24 (1.16, 4.34)	**0.017**	**0.043**	0.511
AKT1	rs1130214 (C>A)	1.00 (0.82, 1.22)	0.996	0.51 (0.27, 0.96)	**0.036**	**0.045**	0.511
MTOR	rs1883965 (A>G)	1.00 (0.82, 1.23)	0.974	2.19 (1.15, 4.19)	**0.018**	**0.054**	0.511
MTOR	rs1064261 (G>A)	1.01 (0.83, 1.24)	0.903	2.12 (1.13, 3.99)	**0.020**	**0.059**	0.511

Abbreviations: OR odds ratio; 95% CI, 95% confidence interval

[a]Adjusting for age, geographic location, DNA source, principal components of the genotypes, and body mass index

[b]The genetic association analysis tested for an additive model and the genetic variants, i.e., the independent variables, were modeled as (0, 1, or 2 alleles)

Bolded p-value: Nominal p-values ($p<0.05$) and ($p<0.10$)

Table 4:

Associations of mTOR candidate SNPs with ER− breast cancer risk by vigorous physical activity

Gene	SNPs	Vigorous physical activity				P-interaction	FDR-adjusted p-value
		No (129 cases/706 controls)		Yes (19 cases/108 controls)			
		OR [a,b] (95% CI)	p-value	OR [a,b] (95% CI)	p-value		
EIF4E	rs141689493 (G>A)	0.55 (0.16, 1.90)	0.344	20.54 (2.29, 184.17)	**0.007**	**0.003**	0.120
PRKAG2	rs7784818 (A>G)	0.73 (0.55, 0.97)	**0.030**	1.51 (0.69, 3.31)	0.308	**0.054**	0.996
AKT1	rs10138227 (C>T)	0.82 (0.59, 1.13)	0.221	2.17 (0.99, 4.79)	**0.055**	**0.071**	0.996

Abbreviations: OR odds ratio; 95% CI, 95% confidence interval

[a]Adjusting for age, geographic location, DNA source, principal components of the genotypes, and body mass index

[b]The genetic association analysis tested for an additive model and the genetic variants, i.e., the independent variables, were modeled as (0, 1, or 2 alleles)

Bolded p-value: Nominal p-values ($p<0.05$) and ($p<0.10$)

Table 5:

Associations of mTOR candidate SNPs with breast cancer risk in case-only analysis (ER− vs. ER+ tumors), by vigorous physical activity

Gene	SNPs	Vigorous physical activity				P-interaction	FDR-adjusted p-value
		No (129 ER− cases/295 ER+ cases)		Yes (19 ER− cases/40 ER+ cases)			
		OR [a,b] (95% CI)	p-value	OR [a,b] (95% CI)	p-value		
AKT1	rs10138227 (C>T)	0.82 (0.56, 1.21)	0.320	23.00 (2.92, 181.07)	**0.003**	**0.001**	**0.030**
MTOR	rs2536 (T>C)	1.37 (0.86, 2.20)	0.188	0.11 (0.01, 0.85)	**0.034**	**0.023**	0.485
EIF4E	rs141689493 (G>A)	0.90 (01.19, 4.16)	0.889	285.37 (0.85, >999.99)	**0.057**	**0.044**	0.485
AKT1	rs1130214 (C>A)	0.84 (0.62, 1.16)	0.294	2.37 (0.75, 7.42)	0.140	**0.059**	0.485
FRAP1	rs12125777 (C>T)	1.33 (0.81, 2.18)	0.261	0.13 (0.01, 1.17)	**0.069**	**0.086**	0.485
MTOR	rs1064261 (G>A)	1.18 (0.87, 1.59)	0.283	0.46 (0.17, 1.20)	0.113	**0.093**	0.485
MTOR	rs1883965 (A>G)	1.19 (0.88, 1.62)	0.252	0.47 (0.18, 1.23)	0.124	**0.093**	0.485

Abbreviations: OR odds ratio; 95% CI, 95% confidence interval

[a]Adjusting for age, geographic location, DNA source, principal components of the genotypes, and body mass index

[b]The genetic association analysis tested for an additive model and the genetic variants, i.e., the independent variables, were modeled as (0, 1, or 2 alleles)

Bolded p-value: Nominal p-values ($p<0.05$) and ($p<0.10$)