Title

Prediagnosis aspirin use, DNA methylation, and mortality after breast cancer: A population-based study

Journal

Cancer, 125(21)

ISSN

0008-543X

Authors

Wang, Tengteng
McCullough, Lauren E
White, Alexandra J
et al.

Publication Date

2019-11-01

DOI

10.1002/cncr.32364

Peer reviewed

HHS Public Access

Author manuscript

Cancer. Author manuscript; available in PMC 2020 November 01.

Published in final edited form as:

Cancer. 2019 November 01; 125(21): 3836–3844. doi:10.1002/cncr.32364.

Pre-diagnosis aspirin use, DNA methylation, and mortality after breast cancer: a population-based study

Tengteng Wang, MBBS MSPH[*,1], **Lauren E. McCullough, MSPH PhD**[2], **Alexandra J. White, MSPH PhD**[3], **Patrick T. Bradshaw, PhD**[4], **Xinran Xu, PhD**[5], **Yoon Hee Cho, PhD**[6], **Mary Beth Terry, PhD**[7], **Susan L. Teitelbaum, PhD**[8], **Alfred I. Neugut, MD, PhD**[7,9], **Regina M. Santella, PhD**[7], **Jia Chen, PhD**[8], **Marilie D. Gammon, PhD**[1]

[1]Department of Epidemiology, University of North Carolina, Chapel Hill, NC 27599, USA

[2]Department of Epidemiology, Emory University, Atlanta, GA 30322, USA

[3]Epidemiology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709, USA

[4]Division of Epidemiology, University of California, Berkeley, CA 94720, USA

[5]Departments of Biometrics, Roche Product Development in Asia-Pacific, Shanghai, China

[6]Department of Biomedical and Pharmaceutical Sciences, University of Montana, Missoula, MT 59812, USA

[7]Department of Epidemiology, Columbia University, New York, NY 10032, USA

[8]Environmental Medicine and Public Health, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA

[9]Department of Medicine, Columbia University, New York, NY 10032, USA

Abstract

BACKGROUND: We hypothesized that epigenetic changes may help to clarify the underlying biologic mechanism linking aspirin use to breast cancer prognosis. Ours is the first epidemiologic study to examine whether global methylation and/or tumor promoter methylation of breast cancer-related genes interact with aspirin use to impact mortality after breast cancer.

METHODS: Pre-diagnosis aspirin use was assessed through in-person interviews within the population-based cohort of 1,508 women diagnosed with first primary breast cancer in 1996-1997. Global methylation in peripheral blood was assessed by long interspersed elements-1 (LINE-1) and the luminometric methylation assay. Promoter methylation of thirteen breast cancer-related genes was measured in tumor by methylation-specific PCR and Methyl Light. Vital status was

[*]**Address all correspondence to:** Tengteng Wang, MSPH, MBBS, Department of Epidemiology, UNC, 2101 McGavran-Greenberg Hall, CB #7435, Chapel Hill, NC 27599-7435; Phone: 404-661-8588; tengteng_wang@med.unc.edu.

Authors' contributions: Study concept and design--all authors; data acquisition--MG, PTB, YHC, XX, MBT, SLT, AIN, RMS, and JC; Statistical analysis--TW in consultation with MG, PTB, LEM, and AJW; interpretation of data--all authors; drafting of the manuscript--TW and MG; critical revision of the manuscript for important intellectual content--all authors; study supervision--MG; approved the manuscript--all authors.

Conflict of interest: The authors declare that they have no competing interests.

determined by the National Death Index through December 31, 2014 (N=237/597 breast cancer-specific/all-cause deaths identified). We used Cox proportional hazards regression to estimate hazard ratios (HRs) and 95% confidence intervals (95%CIs), and the likelihood ratio test to evaluate multiplicative interaction.

RESULTS: All-cause mortality was elevated among aspirin users with methylated promotor of *BRCA1* (HR=1.67; 95%CI=1.26–2.22), but not among those with unmethylated *BRCA1* (HR=0.99; 95%CI=0.67–1.45) ($p_{interaction}$≤0.05). Decreased breast cancer-specific mortality was found among aspirin users with unmethylated promotor of *BRCA1* and *PR*, and LINE-1 global hypermethylation (HR=0.60, 0.78, and 0.63, respectively; $p_{interaction}$≤0.05), although the 95%CIs included the null.

CONCLUSIONS: Our current study suggests that the LINE-1 global methylation, and promoter methylation of *BRCA1* and *PR* in tumor may interact with aspirin use to influence mortality following breast cancer.

Keywords

breast cancer; mortality; aspirin; epigenetic; DNA methylation

INTRODUCTION

Breast cancer (BC) is the second leading cause of cancer death among women in the United States (US), with 41,760 BC deaths expected to occur in 2019.[1] Laboratory and epidemiological studies[2–5] have shown that aspirin, reduces the risk of breast cancer development due to its ability to inhibit cyclooxygenase-2 (COX-2)-mediated prostaglandin synthesis, which plays a crucial role in inflammation and estrogen biosynthesis.[6, 7] However, the underlying biological mechanisms and epidemiological findings on aspirin use in relation to prognosis/mortality after BC are limited and inconsistent. We recently reported no association between aspirin use and mortality after breast cancer [8], which is consistent with a recent meta-analysis that failed to find an inverse association between pre-diagnostic aspirin use and either all-cause or BC-specific mortality.[9]

BC progression is most likely a multifactorial condition involving complex interactions among genetic/epigenetic and estrogen- and inflammation related factors. We therefore hypothesized *a priori* that the inconsistent results for aspirin in relation to mortality could be due to differences in the association by DNA methylation status. Epigenetic modifications involve changes in gene function but do not entail a change in DNA sequence.[10] Methylation may occur during embryogenesis, is heritable by somatic cells after cell division,[11] but also can be modified during the life course under environmental stimulation and lifestyle modulation.[12–16] Global and tumor gene-specific aberrant DNA methylation have been associated with breast cancer prognosis in previous reports, including our own.[17–19] Aberrant DNA methylation may lead to whole genomic instability and altered gene transcription,[15] which may further induce increased mutation rates of key genes (including COX-2) that aspirin acts on.[20, 21] Thus, it is biologically plausible that aspirin works in conjunction with DNA methylation state to influence mortality after BC diagnosis.

The objective of our study was to examine whether pre-diagnosis aspirin use interacts with two global methylation markers in peripheral blood DNA and promoter methylation of a panel of 13 breast cancer-related genes in tumors (*APC, BRCA1, CDH1, CYCLIND2, DAPK1, ESR1, GSTP1, HIN, CDKN2A, PR, RARβ RASSF1A, and TWIST1*) to influence mortality after BC. To our knowledge, no study has systematically addressed the interplay between aspirin use and DNA methylation in the context of BC progression. However, our BC-focused hypothesis is supported by a recent study of colon cancer, which found interactions between aspirin use and methylation profiles in association with polyp occurrence.[13] Our study may help to identify women who, may benefit from aspirin use to improve survival after BC diagnosis because of their DNA methylation profile.

METHODS

Study Design.

We used resources from follow-up component of a population-based study, the Long Island Breast Cancer Study Project (LIBCSP). Details of the LIBCSP baseline[22] and follow-up [23] studies have been described in detail elsewhere. Institutional Review Board approval was obtained from participating institutions.

Study Population.

Eligible subjects were English-speaking adult women newly diagnosed with first primary *in situ* or invasive BC between August 1, 1996, and July 31, 1997, and were residents of either Nassau or Suffolk counties on Long Island, NY at the time of diagnosis. There were no age or race restrictions. Women with BC were identified using rapid case ascertainment through daily/weekly contact with the pathology departments of 31 hospitals in Long Island and New York City. Subjects' physicians were contacted to confirm BC diagnoses and obtain permission to contact their patients. Participants who completed the baseline interview included 1,508 women with BC (82.1% of eligible BC patients), of which 1,273 had invasive BC. Respondents ranged in age from 20 to 98 years, and self-reported their race as white (93%), African American (5%), or other (2%), consistent with the race distribution in Nassau and Suffolk counties at the time of data collection.[22]

Assessment of Pre-Diagnosis Aspirin Use.

Trained interviewers administered the 100-minute standardized baseline questionnaire shortly after diagnosis (mean 96 days).[22] All exposure information was truncated to 12 months prior to the date of diagnosis. Participants were shown a card displaying commonly used prescription and over-the-counter medications by brand name; women reporting use of medications containing aspirin for at least once a week for 6 months or longer before diagnosis were defined as ever users, and others were defined as never users (reference group) (aspirin ever/never). Participants with complete responses in this section of the questionnaire included 1,442 women with BC (96%).

Assessment of Other Epidemiologic/Clinical Factors.

At baseline, respondents were also asked about socio-demographic characteristics, medical and medication history, family history of cancer, menstrual history, use of exogenous

hormones, reproductive history, body size, physical activity, cigarette smoking, alcohol intake, and other factors before date of diagnosis. Dietary intake in the year prior to the interview was assessed using a validated, 101-item modified Block food frequency questionnaire.[24] Medical records were abstracted, at baseline and again about five years later, to obtain information on tumor characteristics and first course of treatment for the first primary BC.[22]

Assessment of Gene-Specific Promoter Methylation.

As described previously,[25] BC tissue blocks were successfully retrieved for 975 BC patients (67.2%), and of these, adequate tissue was available for laboratory analyses for 859 subjects (89.3%).[18] Most socio-demographic and clinicopathological features were similar between patients with or without tumor block available for methylation analysis.[25, 26] Tumor DNA was isolated and extracted from the archived formalin-fixed, paraffin-embedded tumor tissue using the method we previously published.[25]

We selected a panel of 13 genes that were known to play a key role in mammary gland carcinogenesis (*APC, BRCA1, CDH1, CYCLIND2, DAPK1, ESR1, GSTP1, HIN, CDKN2A, PR, RARβ, RASSF1A, and TWIST1*).[27–35] Briefly, methylation-specific (MSP) polymerase chain reaction (PCR) was used to determine promoter methylation of *BRCA1*, *ER* and *PR* genes.[25] This assay outputs dichotomous outcomes (methylated vs. unmethylated) directly with DNA considered methylated if a PCR product using methylated-specific primers was visualized while a PCR product using unmethylated-specific primers is absent, and vice versa. The methylation status of the remaining ten genes were measured by MethyLight assay.[36, 37] The percentage of methylation was calculated by the $2^{-\Delta\Delta CT}$ method, where $\Delta\Delta CT = (C_{T,Target} - C_{T,Actin})_{sample} - (C_{T,Target} - C_{T,Actin})_{fully\ methylated\ DNA}$ and multiplying by 100.[38] These continuous values of the ten gene-specific methylation levels were further dichotomized with ≥4% was defined as methylated.[14–16, 18, 26, 37]

Assessment of Global Methylation.

DNA was also isolated from participant blood samples that were collected by nurse/phlebotomists from 1,102 (73.1%) of women with BC at the time of the baseline interview.[22, 26] Approximately two-thirds of the samples were obtained prior to chemotherapy. As previously described,[39] we used two independent but complementary assays, analysis of long interspersed elements-1 (LINE-1) and the luminometric methylation assay (LUMA) to measure “global methylation content”. The first marker of LINE-1 methylation [40] approximates global methylation levels of repetitive elements or transposons, which play key roles in maintaining genomic stability,[41] while LUMA measures levels of 5-methylcytosine (^{m}C) in the C^{m}CGG motif, which is over-represented in gene promoters.[39] LINE-1 levels were expressed directly as an overall percentage 5^{m}C status from system. LUMA levels were expressed as a percentage based on the following equation: methylation (%) = [1 − (HpaII ΣG/ΣT)/(MspI ΣG/ΣT)] *100guanine, where ΣG represents the sum of guanine (G) peak heights and ΣT represents the sum of thymine (T) peak heights in HpaII- and MspI-digested DNA samples, respectively.[15]

Outcome Assessment.

Women with BC who participated in the baseline interview (n=1,508) continue to be followed to determine mortality, including dates and cause of death, using the National Death Index (NDI).[42] The International Statistical Classification of Diseases codes 174.9 and C-50.9 were used to identify breast cancer-related deaths. The median duration of follow-up was 17.6 years (range, 0.2-18.4). Among our cohort of 1,266 BC patients with information on aspirin use available, 476 (37.6%) deaths occurred, of which 202 (15.9%) were related to BC.

Statistical Analysis.

We constructed Kaplan-Meier Survival curves [43] and estimated hazard ratios (HR) and 95% confidence intervals (CI) using Cox proportional hazards regression [44] for the associations with ever use of aspirin in relation to all-cause and BC-specific mortality after diagnosis over ~18 years of follow-up. Interaction terms with exposure variables and log-time were tested to examine the proportional hazards assumptions.[43] No violations of this assumption were observed, indicating that the results between aspirin use and mortality after BC did not vary substantially over time.

Potential confounders of the aspirin-mortality associations were selected based on a directed acyclic graph.[45] The final minimally sufficient set included age at diagnosis (continuous), race (non-white/white), cigarette smoking (current/former/never smokers) and fruit and vegetable intake (≥35/0-34 servings/week). Because pre-diagnosis aspirin use is associated with less aggressive BC,[46] stage and treatment are likely causal mediators between pre-diagnosis aspirin use and mortality among women with BC; consequently, to reduce bias, [45, 47] we did not include stage and treatment in our models.

To examine potential effect measure modification of the aspirin-mortality association by DNA methylation status, unmethylated status of gene-specific methylation was chosen as the reference group. Additionally, global methylation levels were dichotomized at the median. [22, 26] Given LUMA, a global measurement of promoter methylation, was positively associated with overall gene control,[39] we used the level <median as the referent group. In contrast, LINE-1 hypomethylation is hypothesized to represent decreased genomic integrity, and therefore, LINE-1 level ≥median (hypermethylation) was selected as the referent category. We formally evaluated modification on the multiplicative scale using the likelihood ratio test (LRT) by comparing nested models with and without interaction terms with significance criteria of 0.05.[48] All analyses were completed in SAS 9.4 (Cary, NC).

RESULTS

Ever use of aspirin was reported by 301 of 1,442 BC patients (20.9%) (Table 1). Most women in the population-based LIBCSP cohort of BC patients were white (93%), postmenopausal (68%), former or current smokers (55%) and consumed <34 servings/week of fruits and vegetables (64%). Compared with never users, aspirin ever users were more likely to be older, postmenopausal, overweight/obese at diagnosis and consume more fruit/vegetables.

As previously reported,[8] among women with BC, over ~18 years of follow-up the hazard of mortality for aspirin users, compared to never users, was decreased by 13% for BC-specific mortality (HR=0.87, 95% CI=0.59-1.29), but increased by 21% for all-cause mortality (HR=1.21, 95% 0=0.99-1.48) (Supplemental Table 1). When stratified by global methylation status, we observed significant multiplicative interaction between aspirin use, LINE-1 global methylation, and BC-specific mortality among women with BC ($p_{interaction} \geq 0.05$) (Table 2). Specifically, in women with LINE-1 hypomethylation, ever aspirin use was associated with a higher risk of BC-specific mortality (HR=1.45, 95% CI=0.86–2.42; $p_{interaction}=0.05$) whereas in women with LINE-1 hypermethylation, ever aspirin use was associated with a lower risk of BC-specific mortality (HR=0.63, 95% 0=0.33–1.20). There was no apparent interaction with LINE-1 for all-cause mortality ($p_{interaction}=0.44$), and we also found no statistically significant interaction between aspirin use and LUMA methylation in relation to BC-specific ($p_{interaction}=0.38$) or all-cause mortality ($p_{interaction}=0.94$). Similar patterns of association were also identified among women with hormone receptor positive or invasive breast cancer (supplemental tables 2 and 3).

As shown in Table 3, the association between aspirin use and mortality among women with BC was modified significantly by the tumor promoter methylation status of *BRCA1* and *PR* ($p_{interaction} \leq 0.05$), but not with the other 11 gene promoters considered (although effect estimates were not estimated when cell counts were <5). Specifically, in aspirin users with unmethylated tumor *BRCA1* promoter, BC-specific mortality was lower (HR=0.60, 95% CI=0.25–1.45), but the corresponding effect estimate in women with methylated *BRCA1* was above the null value (HR=1.16, 95% CI=0.69–1.95; $p_{interaction}=0.04$). For all-cause mortality, a positive association was observed in women with a methylated *BRCA1* (HR=1.67, 95% CI=1.26–2.22), but not among women with an unmethylated *BRCA1* (HR=0.99, 95% CI=0.67–1.45; $p_{interaction}=0.02$). For tumor methylation of the *PR*, we observed a higher risk of BC-specific mortality among women with a methylated *PR* promoter (HR=1.63, 95% CI=0.68–3.90), whereas the corresponding hazard for an unmethylated *PR* promoter was below the null (HR=0.78, 95% CI=0.46–1.33; $p_{interaction}=0.03$). No statistically significant interaction was observed between aspirin use and *PR* promoter methylation on all-cause mortality ($p_{interaction}=0.19$). Corresponding findings restricted to hormone receptor positive or invasive breast cancer did not vary substantially from those among women overall (supplemental tables 2 and 3). Consistent patterns of the associations were also observed after a sensitivity analysis by adjusting for global methylation (supplemental table 4).

DISCUSSION

In our population-based cohort of women with breast cancer, all-cause mortality after BC was elevated among aspirin ever-users with methylated tumor promotor of *BRCA1*, but not those with unmethylated tumors. BC-specific mortality was lower among aspirin users with unmethylated tumor promotor of *BRCA1* and *PR*, and hypermethylation of LINE-1, although the corresponding 95% CIs included the null value. These interactions between aspirin use and DNA methylation status were statistically significant on a multiplicative scale ($p<0.05$). Our findings suggest that the association between aspirin use and mortality after BC may depend upon methylation profiles and warrant further investigation.

Improved survival with pre-diagnosis aspirin use among women with BC has been observed in several epidemiologic studies,[49, 50] but not others.[8, 51] Explanations for the inconsistent findings between studies are unclear. However, consideration of DNA methylation profiles as potential modifiers of the aspirin-mortality association may provide new biologic insights. Aspirin is an effective analgesic, antipyretic, and anti-inflammatory drug. It targets the COX-2 enzymes to interrupt prostaglandin and estrogen synthesis, which further modulate apoptosis, cell proliferation, angiogenesis, and immune surveillance.[52, 53] Numerous studies, including our own, have demonstrated positive correlations between aberrant DNA methylation of BC-related gene promoters and lower survival rates among BC patients.[18, 19, 25] The epigenetic inactivation of inflammation and hormone related genes by gene-specific promoter methylation and genomic instability by global methylation may be regulated by exogenous factors with overlapping pathways. Thus, it appears biologically plausible that the association between pre-diagnosis aspirin use and mortality may be altered by aberrant methylation changes.

We are first to report the multiplicative interactions between aspirin use and tumor promoter methylation of *BRCA1* and *PR* on BC prognosis. *BRCA1* is a tumor suppressor gene and part of the DNA repair complex, which plays an important role in maintaining genomic stability and controlling cell-cycle checkpoints.[25] *PR* is a member of the nuclear receptor family, which is involved in normal breast development and tumorigenesis by mediating the physiological effects of progesterone and affecting cellular proliferation/differentiation.[54] Inactivation (silencing) of *BRCA1* and *PR* is thought to occur via gene promoter hypermethylation,[28, 55] which appears to impact the efficiency of DNA repair processes, drive dysregulated cell proliferation, and reduce chromosomal stability.[56, 57] Further, laboratory studies have shown the interaction [58–60] of DNA repair pathways with the inflammation and estrogen pathways through which aspirin mainly operates. Collectively, it is plausible that a tumor environment characterized with *BRCA1* and *PR* promotor methylation may contribute to low sensitivity to aspirin exposure for the host, which would result in little benefit from pre-diagnosis aspirin use on breast cancer prognosis and overall survival. Additionally, methylation of *BRCA1* has previously been found to correlate with large tumor size and the presence of axillary node metastases.[25] Thus, it is plausible that tumor promoter methylation can be linked to worse BC prognosis, which pre-diagnostic use of aspirin is not able to reverse.

Regarding the different BC-specific mortality profiles among aspirin users by LINE-1 global methylation level, our findings are consistent with our *a priori* hypothesis. LINE-1 serves as a surrogate for overall cellular DNA methylation status[40, 61] A low level of LINE-1 methylation (hypomethylation) has been associated with increased chromosomal instability that may be associated with poor prognosis in epithelial cancers, including BC.[62–64] Therefore, we could infer that in the presence of LINE-1 hypermethylation (and improved genomic stability), aspirin may be able to function normally or even better in decreasing mortality due to BC. But in an environment of LINE-1 hypomethylation (and genomic instability), the risk reduction benefit from aspirin is not observed.

There are several limitations of this study. First, tumor tissue and blood specimens were not available for all BC patients identified in the parent LIBCSP study, leading to potential

selection bias. However, we did not observe considerable differences in key socio-demographic and clinicopathological features between BC patients with and without available tissue or blood data.[25, 26] Second, the low prevalence of some methylation biomarkers (i.e., low frequency of *CDKN2A* and *CDH1* methylation) in our study sample, as reflected in the wide confidence intervals, may have contributed to our inability to detect modest interactions; thus, these results should be interpreted with caution. Third, our findings are dependent on self-reports of aspirin use, which could result in potential non-differential misclassification bias and recall error. However, our information on aspirin use was based on comprehensive in-person interviews with several memory aids, which likely improve recall. Our interview approach also resulted in similar effect sizes for the aspirin-breast cancer incidence association [46] as reported by other studies [4, 65, 66] Fourth, we did not have information available regarding the dosage of aspirin, and we were unable to consider aspirin use patterns (i.e., frequency and duration), due to small cell sizes after stratification by methylation status. Fifth, our follow-up study was restricted to an examination of pre-diagnosis NSAID use. However, there is substantial uncertainty about the appropriate timing of aspirin use that will largely impact survival.[50] Finally, our LIBCSP study population is comprised primarily of white women and, therefore, generalizability of our findings to more diverse populations must be examined in future studies. However, our study results are still largely generalizable to those at highest risk of developing breast cancer in the US – white, postmenopausal women.[67]

Our study also has multiple strengths including epigenetic biomarker assessments of DNA methylation in both tumor tissue and blood samples. The latter were collected from our population-based sample of U.S women within a few months following diagnosis of their first primary BC. The archived tumor tissue is from the pathology blocks of the first primary BC, during a time period (the mid-1990s) when it was not customary to treat breast cancer patients with chemotherapy prior to surgery. Participants were followed for 18+ years after diagnosis using the NDI, which provides high-quality ascertainment of vital status.[68] To the best of our knowledge, ours is the first study to examine the potential modification by tissue promotor and/or global methylation on the association between pre-diagnosis aspirin use and mortality after BC diagnosis.

In conclusion, among a population-based cohort of women diagnosed with first primary BC, we are first to report significant heterogeneity of the aspirin-mortality association by *BRCA1* and *PR* promoter methylation, and LINE-1 global methylation profiles. These findings, if confirmed: may provide new biological insights on the association between aspirin use and BC prognosis; may impact clinical decision making by identifying a subgroup of BC patients, using epigenetic markers, for whom pre-diagnosis aspirin use impacts subsequent mortality; and may help refine risk reduction strategies to improve survival among women with BC. Future research designed to replicate our findings should include a larger sample size to allow examination of patterns of aspirin use, and an enlarged panel of genes to explore the role of genetic predisposition in driving overall genetic instability on survival after breast cancer diagnosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding: Supported in part by grants from the National Institutes Health (U01CA/ES66572, R01CA66572, R01CA109753, 3R01CA109753-04S, ES009089, and intramural Z99 ES999999).

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA: a cancer journal for clinicians 2019;69(l):7–34. [PubMed: 30620402]
2. Carter CA, Milholland RJ, Shea W, Ip MM. Effect of the prostaglandin synthetase inhibitor indomethacin on 7,12-dimethylbenz(a)anthracene-induced mammary tumorigenesis in rats fed different levels of fat. Cancer Res. 1983;43: 3559–3562. [PubMed: 6407750]
3. Johnson TW, Anderson KE, Lazovich D, Folsom AR. Association of aspirin and nonsteroidal anti-inflammatory drug use with breast cancer. Cancer Epidemiol Biomarkers Prev. 2002; 11: 1586–1591. [PubMed: 12496048]
4. Harris RE, Chlebowski RT, Jackson RD, et al. Breast cancer and nonsteroidal anti-inflammatory drugs: prospective results from the Women's Health Initiative. Cancer Res. 2003;63: 6096–6101. [PubMed: 14522941]
5. Zhong S, Chen L, Zhang X, Yu D, Tang J, Zhao J. Aspirin use and risk of breast cancer: systematic review and meta-analysis of observational studies. Cancer Epidemiol Biomarkers Prev. 2015;24: 1645–1655. [PubMed: 26315555]
6. Dannenberg AJ, Lippman SM, Mann JR, Subbaramaiah K, DuBois RN. Cyclooxygenase-2 and epidermal growth factor receptor: pharmacologic targets for chemoprevention. J Clin Oncol. 2005;23: 254–266. [PubMed: 15637389]
7. Holmes MD, Chen WY, Schnitt SJ, et al. COX-2 expression predicts worse breast cancer prognosis and does not modify the association with aspirin. Breast Cancer Res Treat. 2011; 130: 657–662. [PubMed: 21728052]
8. Wang T, Parada H, McClain KM, et al. Pre-diagnostic aspirin use and mortality after breast cancer. Cancer Causes Control. 2018.
9. Zhong S, Zhang X, Chen L, Ma T, Tang J, Zhao J. Association between aspirin use and mortality in breast cancer patients: a meta-analysis of observational studies. Breast Cancer Res Treat. 2015;150: 199–207. [PubMed: 25677744]
10. Wu C, Morris JR. Genes, genetics, and epigenetics: a correspondence. Science. 2001;293: 1103–1105. [PubMed: 11498582]
11. Alegria-Torres JA, Baccarelli A, Bollati V. Epigenetics and lifestyle. Epigenomics. 2011;3: 267–277. [PubMed: 22122337]
12. Feil R, Fraga MF. Epigenetics and the environment: emerging patterns and implications. Nat Rev Genet. 2012;13: 97–109. [PubMed: 22215131]
13. Noreen F, Roosli M, Gaj P, et al. Modulation of age- and cancer-associated DNA methylation change in the healthy colon by aspirin and lifestyle. J Natl Cancer Inst. 2014; 106.
14. McCullough LE, Chen J, White AJ, et al. Gene-Specific Promoter Methylation Status in Hormone-Receptor-Positive Breast Cancer Associates with Postmenopausal Body Size and Recreational Physical Activity. Int J Cancer Clin Res. 2015;2.
15. McCullough LE, Chen J, White AJ, et al. Global DNA Methylation, Measured by the Luminometric Methylation Assay (LUMA), Associates with Postmenopausal Breast Cancer in Non-Obese and Physically Active Women. J Cancer. 2015;6: 548–554. [PubMed: 26000047]
16. White AJ, Chen J, Teitelbaum SL, et al. Sources of polycyclic aromatic hydrocarbons are associated with gene-specific promoter methylation in women with breast cancer. Environ Res. 2016;145: 93–100. [PubMed: 26671626]

17. Soares J, Pinto AE, Cunha CV, et al. Global DNA hypomethylation in breast carcinoma: correlation with prognostic factors and tumor progression. Cancer. 1999;85: 112–118. [PubMed: 9921982]

18. Xu X, Gammon MD, Zhang Y, et al. Gene promoter methylation is associated with increased mortality among women with breast cancer. Breast Cancer Res Treat. 2010; 121: 685–692. [PubMed: 19921426]

19. Cho YH, Shen J, Gammon MD, et al. Prognostic significance of gene-specific promoter hypermethylation in breast cancer patients. Breast Cancer Res Treat. 2012; 131: 197–205. [PubMed: 21837480]

20. Yiannakopoulou E Targeting epigenetic mechanisms and microRNAs by aspirin and other non steroidal anti-inflammatory agents--implications for cancer treatment and chemoprevention. Cell Oncol (Dordr). 2014;37: 167–178. [PubMed: 24996792]

21. Domingo-Gonzalez R, Huang SK, Laouar Y, Wilke CA, Moore BB. COX-2 expression is upregulated by DNA hypomethylation after hematopoietic stem cell transplantation. J Immunol. 2012;189: 4528–4536. [PubMed: 23008450]

22. Gammon MD, Neugut AI, Santella RM, et al. The Long Island Breast Cancer Study Project: description of a multi-institutional collaboration to identify environmental risk factors for breast cancer. Breast Cancer Res Treat. 2002;74: 235–254. [PubMed: 12206514]

23. Cleveland RJ, North KE, Stevens J, Teitelbaum SL, Neugut AI, Gammon MD. The association of diabetes with breast cancer incidence and mortality in the Long Island Breast Cancer Study Project. Cancer Causes Control. 2012;23: 1193–1203. [PubMed: 22674293]

24. Block G, Hartman AM, Dresser CM, Carroll MD, Gannon J, Gardner L. A data-based approach to diet questionnaire design and testing. Am J Epidemiol. 1986;124: 453–469. [PubMed: 3740045]

25. Xu X, Gammon MD, Zhang Y, et al. BRCA1 promoter methylation is associated with increased mortality among women with breast cancer. Breast Cancer Res Treat. 2009; 115: 397–404. [PubMed: 18521744]

26. McCullough LE, Chen J, Cho YH, et al. DNA methylation modifies the association between obesity and survival after breast cancer diagnosis. Breast Cancer Res Treat. 2016;156: 183–194. [PubMed: 26945992]

27. Chappuis PO, Kapusta L, Begin LR, et al. Germline BRCA1/2 mutations and p27(Kipl) protein levels independently predict outcome after breast cancer. J Clin Oncol. 2000;18: 4045–4052. [PubMed: 11118465]

28. Yang X, Yan L, Davidson NE. DNA methylation in breast cancer. Endocr Relat Cancer. 2001;8: 115–127. [PubMed: 11446343]

29. Lehmann U, Langer F, Feist H, Glockner S, Hasemeier B, Kreipe H. Quantitative assessment of promoter hypermethylation during breast cancer development. Am J Pathol. 2002; 160: 605–612. [PubMed: 11839581]

30. Robson ME, Chappuis PO, Satagopan J, et al. A combined analysis of outcome following breast cancer: differences in survival based on BRCA1/BRCA2 mutation status and administration of adjuvant treatment. Breast Cancer Res. 2004;6: R8–r17. [PubMed: 14680495]

31. Couch FJ, Shimelis H, Hu C, et al. Associations Between Cancer Predisposition Testing Panel Genes and Breast Cancer. JAMA Oncol. 2017;3: 1190–1196. [PubMed: 28418444]

32. Debouki-Joudi S, Trifa F, Khabir A, et al. CpG methylation of APC promoter 1A in sporadic and familial breast cancer patients. Cancer Biomark. 2017;18: 133–141. [PubMed: 27983523]

33. Huang Y, Lin M, Chen X, et al. Evaluation of the prognostic and physiological functions of death associated protein kinase 1 in breast cancer. Oncol Lett. 2018;15: 8261–8268. [PubMed: 29805560]

34. Michailidou K, Hall P, Gonzalez-Neira A, et al. Large-scale genotyping identifies 41 new loci associated with breast cancer risk. Nat Genet. 2013;45: 353–361, 361e351–352. [PubMed: 23535729]

35. Maxwell KN, Nathanson KL. Common breast cancer risk variants in the post-COGS era: a comprehensive review. Breast Cancer Res. 2013;15: 212. [PubMed: 24359602]

36. Eads CA, Danenberg KD, Kawakami K, Saltz LB, Danenberg PV, Laird PW. CpG island hypermethylation in human colorectal tumors is not associated with DNA methyltransferase overexpression. Cancer Res. 1999;59: 2302–2306. [PubMed: 10344733]
37. Eads CA, Lord RV, Kurumboor SK, et al. Fields of aberrant CpG island hypermethylation in Barrett's esophagus and associated adenocarcinoma. Cancer Res. 2000;60: 5021–5026. [PubMed: 11016622]
38. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001;25: 402–408. [PubMed: 11846609]
39. Xu X, Gammon MD, Hernandez-Vargas H, et al. DNA methylation in peripheral blood measured by LUMA is associated with breast cancer in a population-based study. Faseb j. 2012;26: 2657–2666. [PubMed: 22371529]
40. Weisenberger DJ, Campan M, Long TI, et al. Analysis of repetitive element DNA methylation by MethyLight. Nucleic Acids Res. 2005;33: 6823–6836. [PubMed: 16326863]
41. Yang AS, Estecio MR, Doshi K, Kondo Y, Tajara EH, Issa JP. A simple method for estimating global DNA methylation using bisulfite PCR of repetitive DNA elements. Nucleic Acids Res. 2004;32: e38. [PubMed: 14973332]
42. CDC. National Death Index [Internet], 2014; Available at: http://www.cdc.gov/nchs/ndi.htm. (accessed on April 12, 2016).
43. Allison P Survival analysis using SAS: a practical guide. 2010.
44. Collett D Modelling Survival Data in Medical Research. Boca Raton, FL: Chapman & Hall/CRC Press LLC, 2003.
45. Greenland S, Pearl J, Robins JM. Causal diagrams for epidemiologic research. Epidemiology. 1999; 10: 37–48. [PubMed: 9888278]
46. Terry MB, Gammon MD, Zhang FF, et al. Association of frequency and duration of aspirin use and hormone receptor status with breast cancer risk. Jama. 2004;291: 2433–2440. [PubMed: 15161893]
47. Schisterman EF, Cole SR, Platt RW. Overadjustment bias and unnecessary adjustment in epidemiologic studies. Epidemiology. 2009;20: 488–495. [PubMed: 19525685]
48. Hosmer DW LS. Applied logistic regression. New York: Wiley, 2000.
49. Barron TI, Flahavan EM, Sharp L, Bennett K, Visvanathan K. Recent prediagnostic aspirin use, lymph node involvement, and 5-year mortality in women with stage I-III breast cancer: a nationwide population-based cohort study. Cancer Res. 2014;74: 4065–4077. [PubMed: 25085874]
50. Li Y, Brasky TM, Nie J, et al. Use of nonsteroidal anti-inflammatory drugs and survival following breast cancer diagnosis. Cancer Epidemiol Biomarkers Prev. 2012;21: 239–242. [PubMed: 22068285]
51. Fraser DM, Sullivan FM, Thompson AM, McCowan C. Aspirin use and survival after the diagnosis of breast cancer: a population-based cohort study. Br J Cancer. 2014;111: 623–627. [PubMed: 24945997]
52. Elwood PC, Morgan G, Pickering JE, et al. Aspirin in the Treatment of Cancer: Reductions in Metastatic Spread and in Mortality: A Systematic Review and Meta-Analyses of Published Studies. PLoS One. 2016;11: e0152402. [PubMed: 27096951]
53. DuBois RN. Aspirin and breast cancer prevention: the estrogen connection. Jama. 2004;291: 2488–2489. [PubMed: 15161901]
54. Grimm SL, Hartig SM, Edwards DP. Progesterone Receptor Signaling Mechanisms. J Mol Biol. 2016;428: 3831–3849. [PubMed: 27380738]
55. Widschwendter M, Jones PA. DNA methylation and breast carcinogenesis. Oncogene. 2002;21: 5462–5482. [PubMed: 12154408]
56. Chiyoda T, Hart PC, Eckert MA, et al. Loss of BRCA1 in the Cells of Origin of Ovarian Cancer Induces Glycolysis: A Window of Opportunity for Ovarian Cancer Chemoprevention. Cancer Prev Res (Phila). 2017; 10: 255–266. [PubMed: 28264838]
57. Jahandoost S, Farhanghian P, Abbasi S. The Effects of Sex Protein Receptors and Sex Steroid Hormone Gene Polymorphisms on Breast Cancer Risk. J Natl Med Assoc. 2017; 109: 126–138. [PubMed: 28599754]

58. Mohan HM, Aherne CM, Rogers AC, Baird AW, Winter DC, Murphy EP. Molecular pathways: the role of NR4A orphan nuclear receptors in cancer. Clin Cancer Res. 2012;18: 3223–3228. [PubMed: 22566377]

59. Ames BN, Gold LS, Willett WC. The causes and prevention of cancer. Proc Natl Acad Sci U S A. 1995;92: 5258–5265. [PubMed: 7777494]

60. Jaamaa S, Laiho M. Maintenance of genomic integrity after DNA double strand breaks in the human prostate and seminal vesicle epithelium: the best and the worst. Mol Oncol. 2012;6: 473–483. [PubMed: 22762987]

61. Mima K, Nowak JA, Qian ZR, et al. Tumor LINE-1 methylation level and colorectal cancer location in relation to patient survival. Oncotarget. 2016;7: 55098–55109. [PubMed: 27391152]

62. Baba Y, Huttenhower C, Nosho K, et al. Epigenomic diversity of colorectal cancer indicated by LINE-1 methylation in a database of 869 tumors. Mol Cancer. 2010;9: 125. [PubMed: 20507599]

63. Saito K, Kawakami K, Matsumoto I, Oda M, Watanabe G, Minamoto T. Long interspersed nuclear element 1 hypomethylation is a marker of poor prognosis in stage IA non-small cell lung cancer. Clin Cancer Res. 2010;16: 2418–2426. [PubMed: 20371677]

64. van Hoesel AQ, van de Velde CJ, Kuppen PJ, et al. Hypomethylation of LINE-1 in primary tumor has poor prognosis in young breast cancer patients: a retrospective cohort study. Breast Cancer Res Treat. 2012; 134: 1103–1114. [PubMed: 22476853]

65. Rahme E, Ghosn J, Dasgupta K, Raj an R, Hudson M. Association between frequent use of nonsteroidal anti-inflammatory drugs and breast cancer. BMC Cancer. 2005;5: 159. [PubMed: 16343343]

66. Bardia A, Olson JE, Vachon CM, et al. Effect of aspirin and other NSAIDs on postmenopausal breast cancer incidence by hormone receptor status: results from a prospective cohort study. Breast Cancer Res Treat. 2011; 126: 149–155. [PubMed: 20669045]

67. DeSantis CE, Fedewa SA, Goding Sauer A, Kramer JL, Smith RA, Jemal A. Breast cancer statistics, 2015: Convergence of incidence rates between black and white women. CA Cancer J Clin. 2016;66: 31–42. [PubMed: 26513636]

68. Cowper DC, Kubal JD, Maynard C, Hynes DM. A primer and comparative review of major US mortality databases. Ann Epidemiol. 2002; 12: 462–468. [PubMed: 12377423]

Precis for use in the Table of Contents:

Our findings suggest that the association between aspirin use and mortality after BC may depend upon patients' DNA methylation profiles. Our study may help to identify women who, may benefit from aspirin use to improve survival after BC diagnosis because of their DNA methylation profile.

Table 1.

Distribution of selected participant characteristics, overall and stratified by pre-diagnosis aspirin use among women with breast cancer, LIBCSP, 1996-1997

Characteristics	No. of Women with Breast Cancer (%)		
	Overall (N=1,442)	Aspirin users (N=301)	Aspirin non-users (N=1,441)
	n (%)	n (%)	n (%)
Age at diagnosis (years)			
<35	39 (2.6)	5 (1.7)	34 (3.0)
35-44	173 (12.0)	21 (7.0)	152 (13.3)
45-54	379 (26.3)	67 (22.3)	312 (27.3)
55-64	356 (24.7)	75 (25.2)	281 (24.6)
65-74	345 (23.9)	95 (31.6)	250 (21.9)
≥75	150 (10.4)	38 (7.1)	112 (9.8)
Race			
White	1,354 (94.0)	289 (96.0)	1,065 (93.5)
Black	62 (4.3)	11 (3.7)	51 (4.5)
Other	24 (1.7)	11 (0.3)	23 (2.0)
Menopausal status			
Premenopausal	451 (31.9)	73 (24.9)	378 (33.8)
Postmenopausal	962 (68.1)	220 (75.1)	742 (66.3)
BMI at diagnosis (kg/m2)			
<25	655 (45.9)	120 (40.4)	535 (47.4)
25-30	454 (31.8)	97 (32.7)	257 (31.6)
>30	317 (22.2)	80 (26.9)	237 (21.0)
Smoking			
Never	645 (44.7)	137 (45.5)	508 (44.5)
Current	276 (19.1)	52 (17.3)	224 (19.6)
Past/former	521 (36.1)	112 (37.2)	409 (35.9)
Fruits and vegetables intake			
0-34 servings/week	910 (64.3)	178 (59.5)	732 (65.5)

Characteristics	No. of Women with Breast Cancer (%)		
	Overall (N=1,442)	Aspirin users (N=301)	Aspirin non-users (N=1,441)
	n (%)	n (%)	n (%)
35+ servings/week	506 (35.7)	121 (40.5)	385 (34.5)

Table 2.

Multivariable-adjusted[a] hazard ratios (HRs) and 95% confidence intervals (CIs) for the association between pre-diagnostic aspirin use and mortality (breast cancer-specific and all-cause) stratified by global methylation status (measured by LINE-1 and LUMA[b]) among all women (with blood sample), in the Long Island Breast Cancer Study Project (N=1,026), 1996-1997.

	Breast Cancer-Specific Mortality								All-Cause Mortality							
Global marker Aspirin categories	**Deaths**	**PY[b]**	**HR**	**95% CI**	**Deaths**	**PY[b]**	**HR**	**95% CI**	**Deaths**	**PY[b]**	**HR**	**95% CI**	**Deaths**	**PY[b]**	**HR**	**95% CI**
LINE-1 methylation		**< Median (78.65)**				**≥ Median**				**< Median (78.65)**				**≥ Median**		
Never users	62	5877	1.00	reference	61	5875	1.00	reference	156	5877	1.00	reference	144	5875	1.00	reference
Ever users	21	1429	1.45	(0.86, 2.42)	11	1670	0.63	(0.33, 1.20)	54	1429	1.32	(0.98, 1.79)	48	1670	1.06	(0.77, 1.47)
p interaction				0.05								0.44				
LUMA methylation		**< Median (0.56)**				**≥ Median**				**< Median (0.56)**				**≥ Median**		
Never users	47	3905	1.00	reference	75	7717	1.00	reference	107	3905	1.00	reference	189	7717	1.00	reference
Ever users	16	1122	1.10	(0.62, 1.96)	16	1946	0.88	(0.51, 1.53)	39	1122	1.08	(0.76, 1.54)	61	1946	1.27	(0.96, 1.68)
p interaction				0.38								0.94				

[a]Adjusted for baseline age, race, fruit and vegetable intake, cigarette smoking

[b]LINE-1, long interspersed elements-1; LUMA, luminometric methylation assay

[c]PY, person-years

Table 3.

Multivariable-adjusted[a] hazard ratios (HRs) and 95% confidence intervals (CIs) for the association between pre-diagnostic aspirin use and mortality (breast cancer-specific and all-cause) stratified by gene methylation status (methylated vs. unmethylated tumors) among all women (with tissue sample), in the Long Island Breast Cancer Study Project (N=821), 1996-1997.

	Breast Cancer-Specific Mortality								All-Cause Mortality							
	Unmethylated				Methylated				Unmethylated				Methylated			
Gene promoter Aspirin categories	Deaths	PY[b]	HR	95% CI	Deaths	PY[b]	HR	95% CI	Deaths	PY[b]	HR	95% CI	Deaths	PY[b]	HR	95% CI
APC																
Never users	45	4607	1.00	reference	62	4049	1.00	reference	121	4607	1.00	reference	127	4049	1.00	reference
Ever users	8	1074	0.79	(0.37, 1.67)	15	1006	1.03	(0.58, 1.84)	38	1074	1.19	(0.85, 1.68)	47	1006	1.48	(1.08, 2.04)
p interaction				0.12								0.39				
BRCA1																
Never users	34	3601	1.00	reference	76	5548	1.00	reference	104	3601	1.00	reference	156	5548	1.00	reference
Ever users	6	1157	0.60	(0.25, 1.45)	19	1147	1.16	(0.69, 1.95)	32	1157	0.99	(0.67, 1.45)	60	1147	1.67	(1.26, 2.22)
p interaction				0.04								0.02				
CDH1																
Never users	92	7753	1.00	reference	7	413	1.00	reference	217	7753	1.00	reference	18	413	1.00	reference
Ever users	21	1986	0.89	(0.55, 1.44)	N.A.	N.A.	N.A.	N.A.	81	1986	1.35	(1.06, 1.72)	N.A.	1.21	N.A.	N.A.
p interaction				N.A.								N.A.				
CYCLIND2																
Never users	75	6272	1.00	reference	24	1444	1.00	reference	183	6272	1.00	reference	52	1444	1.00	reference
Ever users	16	1641	0.85	(0.49, 1.47)	5	466	0.73	(0.27, 1.96)	58	1641	1.21	(0.91, 1.61)	26	466	1.56	(1.02, 2.39)
p interaction				0.99								0.49				
DAPK																
Never users	81	7157	1.00	reference	18	1009	1.00	reference	196	7157	1.00	reference	39	1009	1.00	reference
Ever users	15	1715	0.76	(0.44, 1.32)	6	391	0.89	(0.33, 2.40)	68	1715	1.32	(1.01, 1.72)	16	391	1.25	(0.73, 2.15)
p interaction				0.54								0.99				
ESR1																
Never users	58	5033	1.00	reference	52	4017	1.00	reference	141	5033	1.00	reference	118	4017	1.00	reference
Ever users	12	1220	0.87	(0.47, 1.62)	13	1084	0.98	(0.52, 1.83)	46	1220	1.37	(0.99, 1.90)	46	1084	1.27	(0.92, 1.76)

	Breast Cancer-Specific Mortality								All-Cause Mortality							
	Unmethylated				Methylated				Unmethylated				Methylated			
Gene promoter **Aspirin categories**	**Deaths**	**PY[b]**	**HR**	**95% CI**	**Deaths**	**PY[b]**	**HR**	**95% CI**	**Deaths**	**PY[b]**	**HR**	**95% CI**	**Deaths**	**PY[b]**	**HR**	**95% CI**
p interaction				0.91								0.99				
GSTP1																
Never users	53	6084	1.00	reference	46	2082	1.00	reference	155	6084	1.00	reference	80	2082	1.00	reference
Ever users	18	1508	1.30	(0.76, 2.23)	N.A.	N.A.	N.A.	N.A.	59	1508	1.47	(1.09, 1.98)	25	599	0.97	(0.65, 1.45)
p interaction				N.A.								0.98				
HIN																
Never users	29	3095	1.00	reference	70	5071	1.00	reference	82	3095	1.00	reference	153	5071	1.00	reference
Ever users	11	763	1.58	(0.77, 3.27)	10	1343	0.53	(0.27, 1.02)	33	763	1.63	(1.09, 2.44)	51	1343	1.14	(0.85, 1.53)
p interaction				0.32								0.86				
P16																
Never users	96	8090	1.00	reference	6	317	1.00	reference	231	8090	1.00	reference	8	317	1.00	reference
Ever users	20	1954	0.88	(0.54, 1.43)	N.A.	N.A.	N.A.	N.A.	80	1954	1.35	(1.06, 1.72)	4	50	2.76	(0.82, 9.27)
p interaction				N.A.								0.52				
PR																
Never users	92	8042	1.00	reference	18	1107	1.00	reference	232	8042	1.00	reference	28	1107	1.00	reference
Ever users	17	2038	0.78	(0.46, 1.33)	8	266	1.63	(0.68, 3.90)	75	2038	1.25	(0.97, 1.60)	17	266	2.14	(1.18, 3.90)
p interaction				0.03								0.19				
RARB																
Never users	60	5957	1.00	reference	39	2209	1.00	reference	161	5957	1.00	reference	74	2209	1.00	reference
Ever users	16	1500	0.98	(0.56, 1.72)	5	556	0.61	(0.24, 1.52)	64	1500	1.43	(1.07, 1.89)	20	556	1.12	(0.71, 1.75)
p interaction				0.97								0.83				
RASSF1A																
Never users	9	1259	1.00	reference	90	6907	1.00	reference	26	1259	1.00	reference	209	6907	1.00	reference
Ever users	3	279	1.76	(0.49, 6.33)	18	1828	0.75	(0.45, 1.26)	11	279	1.91	(0.99, 3.69)	73	1828	1.22	(0.95, 1.58)
p interaction				0.99								0.96				
TWIST1																
Never users	82	7083	1.00	reference	17	1084	1.00	reference	196	7083	1.00	reference	39	1084	1.00	reference
Ever users	14	1743	0.65	(0.37, 1.15)	7	364	1.71	(0.67, 4.34)	68	1743	1.34	(1.03, 1.73)	16	364	1.20	(0.67, 2.15)

	Breast Cancer-Specific Mortality								All-Cause Mortality							
	Unmethylated				Methylated				Unmethylated				Methylated			
Gene promoter **Aspirin categories**	**Deaths**	**PY[b]**	**HR**	**95% CI**	**Deaths**	**PY[b]**	**HR**	**95% CI**	**Deaths**	**PY[b]**	**HR**	**95% CI**	**Deaths**	**PY[b]**	**HR**	**95% CI**
p interaction				0.23								0.99				

[a] Adjusted for baseline age, race, fruit and vegetable intake, cigarette smoking

[b] PY, person-years; N.A. not applicable because cell sizes were less than 5