Published OnlineFirst July 10, 2018; DOI: 10.1158/1055-9965.EPI-18-0175

Review

Cancer Epidemiology, Biomarkers & Prevention

Physical Activity, Global DNA Methylation, and Breast Cancer Risk: A Systematic Literature Review and Meta-analysis

Devon J. Boyne[1,2], Dylan E. O'Sullivan[3], Branko F. Olij[1,4], Will D. King[3], Christine M. Friedenreich[1,2,5], and Darren R. Brenner[1,2,5]

Abstract

The extent to which physical activity reduces breast cancer risk through changes in global DNA methylation is unknown. We systematically identified studies that investigated the association between: (i) physical activity and global DNA methylation; or (ii) global DNA methylation and breast cancer risk. Associations were quantified using random-effects models. Heterogeneity was investigated through subgroup analyses and the *Q*-test and I^2 statistics. Twenty-four studies were reviewed. We observed a trend between higher levels of physical activity and higher levels of global DNA methylation [pooled standardized mean difference = 0.19; 95% confidence interval (CI), −0.03–0.40; $P = 0.09$] which, in turn, had a suggestive association with a reduced breast cancer risk (pooled relative risk = 0.70; 95% CI, 0.49–1.02; $P = 0.06$). In subgroup analyses, a positive association between physical activity and global DNA methylation was observed among studies assessing physical activity over long periods of time ($P = 0.02$). Similarly, the association between global DNA methylation and breast cancer was statistically significant for prospective cohort studies ($P = 0.007$). Despite the heterogeneous evidence base, the literature suggests that physical activity reduces the risk of breast cancer through increased global DNA methylation. This study is the first to systematically overview the complete biologic pathway between physical activity, global DNA methylation, and breast cancer. *Cancer Epidemiol Biomarkers Prev; 27(11); 1320–31. ©2018 AACR.*

Introduction

Breast cancer is the most common cancer (excluding nonmelanoma skin cancer) among women worldwide, and the incidence is increasing (1). Several lifestyle factors have been linked to breast cancer risk, which include poor-quality diet (2), alcohol intake (2), smoking (3), and inadequate levels of physical activity (4, 5). In observational settings, regular physical activity has been shown to reduce breast cancer risk by 20% to 25% when comparing the most to least physically active (4, 5). This protective effect may be explained by several hypothesized mechanisms (6), including changes in adiposity (7), sex hormones (8, 9), growth factors (9), insulin resistance (10, 11), and immune function (12).

Global DNA hypomethylation is a molecular event that has been implicated in the carcinogenic process (13). We use the term global DNA hypomethylation to refer to the systematic loss of DNA methylation across large genomic regions that are normally methylated. The functional consequence of hypomethylation is known to be dependent on the genomic context in which it takes place (14). For example, hypomethylation of repetitive elements leads to transposable element reactivation and genomic instability, whereas hypomethylation within gene bodies is associated with aberrant gene expression (15, 16).

The relation between physical activity and DNA methylation has been previously reviewed (17–22). These reviews provide a narrative synthesis of the association between physical activity and both global and gene-specific measures of DNA methylation. No study to date has quantitatively synthesized reports of the association between physical activity and global DNA methylation, particularly within the context of a mechanism for breast cancer prevention, and sources of statistical heterogeneity in this body of literature have yet to be identified.

Previous meta-analyses assessing the association between global DNA methylation and cancer risk have found increased risk associated with lower levels of DNA methylation (23, 24). However, these meta-analyses pooled estimates across all cancer types and primarily assessed the association within case–control studies, a study design in which temporality is difficult to establish for this particular relationship. The association between global DNA methylation and breast cancer risk has yet to be quantified, particularly among prospective cohort studies where temporality can be inferred.

The aim of this is to determine whether the current literature supports the hypothesis that global DNA methylation is a biologic mechanism through which physical activity reduces breast cancer

[1]Department of Cancer Epidemiology and Prevention Research, CancerControl Alberta, Alberta Health Services, Calgary, Alberta, Canada. [2]Department of Community Health Sciences, Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada. [3]Department of Public Health Sciences, Queen's University, Kingston, Ontario, Canada. [4]Department of Public Health, Erasmus MC—University Medical Center Rotterdam, the Netherlands. [5]Department of Oncology, Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada.

Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

D.J. Boyne and D.E. O'Sullivan are co-first authors and contributed equally to this article.

Corresponding Author: Darren R. Brenner, Alberta Healthy Services, 2210 2nd St SW, Room 513, Calgary, AB T2S 3C3, Canada. Phone: 403-698-8178; Fax: 403-264-2654; E-mail: darren.brenner@ucalgary.ca

doi: 10.1158/1055-9965.EPI-18-0175

Published OnlineFirst July 10, 2018; DOI: 10.1158/1055-9965.EPI-18-0175

risk. This aim will be accomplished by reviewing two separate yet related bodies of literature: (i) studies examining the association between physical activity and global DNA methylation; and (ii) studies assessing the association between global DNA methylation and subsequent breast cancer risk. In addition to synthesizing and identifying sources of heterogeneity in this literature, we also highlight methodologic and conceptual issues related to the use of global DNA methylation biomarkers in molecular epidemiologic studies and provide recommendations for future research.

Materials and Methods

Literature search and study inclusion criteria

We conducted a systematic review using the guidelines of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA; ref. 25). Two separate literature searches were conducted in PubMed, including all studies published up to August 2017 on: (i) physical activity and DNA methylation; and (ii) DNA methylation and breast cancer. The Medical Subject Headings (MeSH) used for each search were: (i) "Motor Activity," "Exercise," "Sports," "Physical Fitness," "Lifestyle," "Sedentary Lifestyle," "Movement," "DNA Methylation," "Methylation," and "Epigenomics"; (ii) "DNA Methylation," "Methylation," "Epigenomics," and "Breast Neoplasms." Reference lists and other secondary sources were examined for additional relevant articles. In the initial screening process, articles were deemed suitable for a full-text review if they were performed in adult humans, involved DNA methylation, and were of relevance to the research question. In the full-text review, studies were included if they: (i) were an observational or interventional study of physical activity; or (ii) were an observational study of breast cancer; (iii) included a global measure of DNA methylation; and (iv) reported an effect estimate or the values necessary to derive one. If two studies reported on the same study population and methylation measure, only the study with the largest sample size was included. Reviews, meta-analyses, and studies measuring DNA methylation in tumor tissue were excluded from this review. We made no restrictions on language or on the healthy target tissue in which DNA methylation was assessed.

Data extraction and synthesis

Information on author, year of publication, country, study design, number of participants, study demographics (age and sex), type of DNA methylation measure (assay and measure type), physical activity exposure (duration and type of intervention or measure), and timing of the DNA methylation assessment with respect to cancer diagnosis were extracted from the studies included in this review. We extracted effect estimates and corresponding measures of dispersion within study-defined exposure categories. When multiple effect estimates were reported, we extracted the estimate that was adjusted for the largest number of confounders. Data reported in graphical form (e.g., means and SEs in bar graphs or medians and interquartile ranges in box plots) were extracted using the WebPlotDigitizer where appropriate (26).

Meta-analyses

Two separate analyses were performed: (i) a meta-analysis describing the association between physical activity and global DNA methylation; and (ii) a meta-analysis of the relation between global DNA methylation and breast cancer risk. DerSimonian and Laird random-effect models were used in both analyses given the anticipated heterogeneity among the studies included in this review. Heterogeneity was investigated through subgroup analyses and meta-regression and was quantified using the *Q*-test and the I^2 statistics. To assess publication bias, we visually reviewed funnel plots and employed both Egger weighted linear regression and Begg rank correlation tests.

For the investigation of physical activity and global DNA methylation, we estimated the standardized mean difference (SMD), or Cohen *d*, comparing the mean global DNA methylation in the highest and lowest study-defined categories of physical activity (27). When the range of values, SE, interquartile range (IQR), or 95% confidence intervals (CI) were reported, the group-specific SD was estimated as follows: $\mathrm{SD} = \frac{\mathrm{range}}{4} = \frac{\mathrm{IQR}}{1.35} = \mathrm{SE} \times \sqrt{n} = \frac{\text{upper CI} - \text{lower CI}}{3.92} \times \sqrt{n}$ (28). The median was used as a proxy for the mean when necessary. The median should approximate the mean in this context because global DNA methylation can be assumed to follow an approximately normal distribution. We estimated the SMD in studies reporting a correlation coefficient (r) using a previously published method whereby $\mathrm{SMD} = \frac{2r}{\sqrt{1-r^2}}$ (27). In settings where studies reported pre- and postexercise global DNA methylation scores within the same group of individuals, we assumed a correlation of 0.70 between the pre- and posttest scores as done in previous meta-analyses and estimated the SMD using methods for matched data (27, 29). In a sensitivity analysis, we repeated these analyses where we varied the correlation from 0.10 to 0.90 in increments of 0.10 to assess the robustness of our results to violations this assumption.

For the quantitative synthesis of studies on global DNA methylation and breast cancer, we pooled the study-specific estimates of the relative risk of breast cancer comparing the highest with the lowest study-defined cut-off points of DNA methylation. For the purposes of this study, HRs and ORs were treated as estimates of the relative risk. All estimates were log-transformed and the SE of the log relative risk ($\mathrm{SE}_{\mathrm{logRR}}$) was estimated using the 95% CIs as follows: $\mathrm{SE}_{\mathrm{logRR}} = \frac{\log(\text{upper CI}) - \log(\text{lower CI})}{3.92}$ (28). All analyses were conducted in R Studio v.1.0.153 with the *metafor* package.

Results

Study inclusion

In total, 788 studies on physical activity and DNA methylation, and 1,882 studies on DNA methylation and breast cancer risk were screened for inclusion (Fig. 1). After full-text screening, 12 studies examining the association between physical activity and global DNA methylation and 12 studies on global DNA methylation and breast cancer risk were included in this review. We found no studies examining the mediating effect of global DNA methylation with respect to physical activity and breast cancer risk. There was a high degree of agreement between the two reviewers with respect to study inclusion for both systematic reviews. The percent agreement and the kappa statistics were 89.5% and 0.68 for the physical activity and DNA methylation review, and 94.6% and 0.87 for the DNA methylation and breast cancer review, respectively.

The association between physical activity and global DNA methylation

Characteristics of the 12 studies investigating physical activity and global DNA methylation are presented in Table 1 (30–41). A

Published OnlineFirst July 10, 2018; DOI: 10.1158/1055-9965.EPI-18-0175

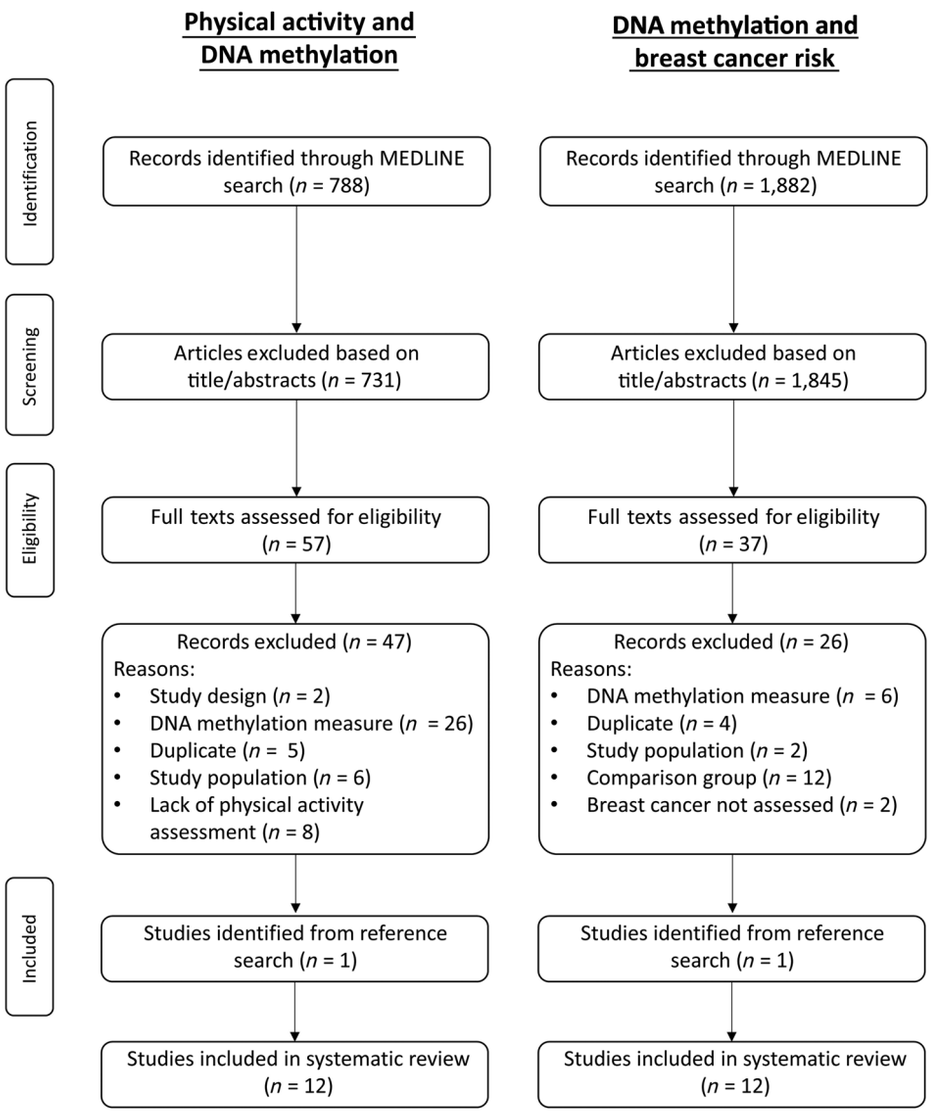

Figure 1.
Flow diagram of the selection procedure of studies of the two literature searches. This figure contains a PRISMA flow diagram that describes the inclusion and exclusion of studies included in this review.

total of 3,880 individuals were examined in these reports. Most of the studies were observational ($n = 8$) and assessed physical activity over periods longer than 6 months ($n = 8$). Half of the studies were conducted in the United States ($n = 6$). All studies measured DNA methylation in white blood cells apart from two investigations, one of which relied on whole blood and the other relied on muscle tissue. The most commonly used methylation measure was LINE1 methylation ($n = 8$). Global DNA methylation was directly assessed (percent methylation of total CpG sites in the genome) in only two reports.

A meta-analysis was conducted on 14 estimates reported from 12 studies (Fig. 2). The study by Marques-Rocha and colleagues (2016) was excluded from this quantitative synthesis as it reported mean levels of physical activity between categories of DNA methylation rather than mean levels of DNA methylation between categories of physical activity (36). Overall, we found that higher levels of physical activity trended toward higher levels of DNA methylation, but this difference was not statistically significant (pooled SMD = 0.19; 95% CI, −0.03–0.40; $P = 0.09$). In sensitivity analyses, these findings were found to be robust to the misspecification of the correlation between measurements taken from the same individual (Supplementary Table S1). There was no evidence of publication bias according to the funnel plot (Supplementary Fig. S1), the Begg test ($P = 0.45$), or the Egger test ($P = 0.11$).

This body of literature was heterogeneous ($Q < 0.01$; $I^2 = 86.1\%$). In subgroup analyses (Table 2), we found that the tissue in which DNA methylation was assessed was a statistically significant source of heterogeneity (meta-regression $P = 0.01$). Specifically, physical activity had a statistically significant positive association with global DNA methylation measured in blood (pooled SMD = 0.25; 95% CI, 0.05–0.45; $P = 0.02$; $I^2 = 84.2\%$). The direction of this association was reversed in the single study that assessed the impact of physical activity on global DNA methylation in muscle tissue ($P < 0.01$). Although not statistically significant, study demographics (mean age, percent female, and country), the duration of the physical activity exposure, and the genomic context in which the DNA methylation was assessed

Published OnlineFirst July 10, 2018; DOI: 10.1158/1055-9965.EPI-18-0175

Table 1. Characteristics of studies investigating the association between physical activity and global DNA methylation ($n = 12$)

Intervention studies ($n = 4$)

Study	Country	Study design	Study population	Sample size (n)	Mean age (years)	Female (%)	Duration of intervention	Intervention	Methylation measure	Tissue
Barres (2012; ref. 30)	Ireland	Crossover	Healthy individuals	14	25.0	N/A	Acute (single bout)	Aerobic	LUMA	Muscle
Duggan (2014; ref. 32)	USA	Randomized controlled trial	Postmenopausal overweight women	300	57.9	100.0	Chronic (12 months)	Aerobic	LINE1	WBC
Delgada-Cruzata (2015; ref. 31)	USA	Crossover	Minority breast cancer survivors	24	52.2	100.0	Chronic (6 months)	Aerobic + resistance + diet	LINE1, Sat2, LUMA	WBC
King-Himmelreich (2016; ref. 34)	Germany	Nonrandomized controlled trial	Untrained individuals with a leg injury	30	46.1	66.7	Acute (1 month)	Aerobic + resistance	LINE1	WBC

Observational studies ($n = 8$)

Study	Country	Study design	Study population	Sample size (n)	Mean age (years)	Female (%)	Duration of exposure	Exposure self-reported	Methylation measure	Tissue
McGuinness (2011; ref. 37)	Scotland	Cross-sectional	Healthy individuals	239	49.8	51.0	Chronic	Yes	Global	WBC
Zhang (2011; ref. 41)	USA	Cross-sectional	Healthy individuals	161	53.0	62.6	Acute	No	LINE1	WBC
Gomes (2012; ref. 33)	Brazil	Cross-sectional	Healthy older adults	126	70.8	52.4	Acute	No	Global	WBC
Morabia (2012; ref. 38)	USA	Case–control	Healthy individuals	180	22.7	36.0	Chronic	Yes	LINE1	WBC
Luttropp (2013; ref. 35)	Sweden	Cross-sectional	Healthy older individuals	1016	70+	N/A	Chronic	Yes	LUMA	WBC
White (2013; ref. 40)	USA and Puerto Rico	Cross-sectional	Women with a family history of breast cancer	647	54.8	100.0	Chronic	Yes	LINE1	Whole Blood
Perng (2014; ref. 39)	USA	Cross-sectional	Healthy individuals	987	62.4	52.4	Chronic	Yes	LINE1, Alu	WBC
Marques-Rocha (2016; ref. 36)	Brazil	Cross-sectional	Healthy individuals	156	23.1	58.3	Chronic	Yes	LINE1	WBC

Published OnlineFirst July 10, 2018; DOI: 10.1158/1055-9965.EPI-18-0175

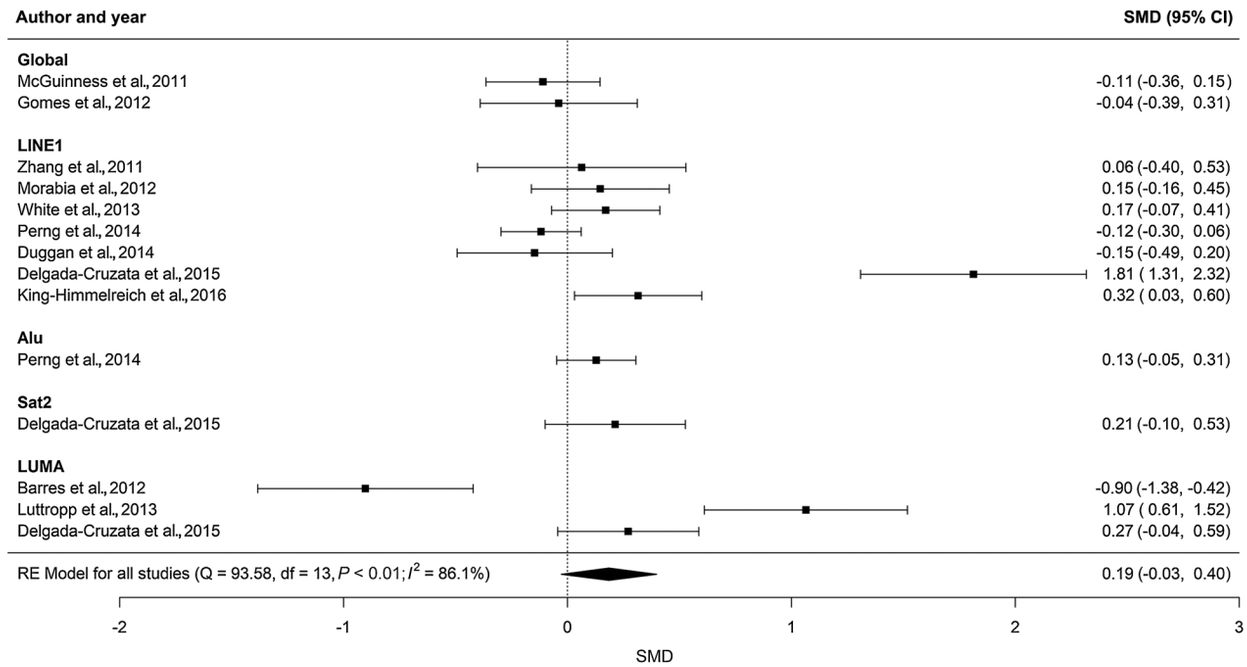

Figure 2.

Estimated SMD comparing study-defined categories of high and low levels of physical activity. This figure presents a forest plot that describes the pooled SMD in DNA methylation between high and low study-defined categories of physical activity.

appeared to be meaningful sources of heterogeneity. With respect to the duration of physical activity, a positive association between physical activity and global DNA methylation was observed among studies that assessed long-term levels of physical activity (pooled SMD = 0.29; 95% CI, 0.04–0.54; $P = 0.02$), but not among those that assessed more acute bouts of physical activity ($P = 0.63$). A similar association was observed when analyses were restricted to studies targeting DNA methylation within repetitive elements (pooled SMD = 0.25; 95% CI, 0.00–0.49; $P = 0.048$; $I^2 = 85.4\%$) but not among those focused on levels of DNA methylation within the entire genome ($P = 0.42$) or within CCGG sequences as measured by the LUMA assay ($P = 0.77$). It should be noted that the subgroup estimate specific to studies assessing physical activity over long periods of time and to studies measuring DNA methylation in blood was robust to the overestimation of the correlation among matched samples but the estimate specific to studies of repetitive DNA methylation was not (Supplementary Table S1).

The association between global DNA methylation and breast cancer

Characteristics of the 12 studies investigating the association of global DNA methylation and breast cancer are presented in Table 3 (42–53). When accounting for subject overlap among studies, a total of 4,035 patients with breast cancer and 4,140 healthy controls were examined in these reports. Most studies were case–control designs ($n = 7$), and all studies measured DNA methylation in whole blood cells, aside from two studies that measured DNA methylation in whole blood. Half of the studies were conducted in the United States, and most studies consisted of study populations with an average age under 60 ($n = 9$). Only four studies reported on menopausal status and only two studies reported on the pathologic stage or ER status of the included patients with breast cancer. Three studies (42, 48, 51) and one study population from van Veldhoven and colleagues (2015) were excluded from the quantitative analysis because they did not present a measure of relative risk associated with a category of DNA methylation (45).

The association between global DNA methylation and breast cancer risk is graphically displayed in Fig. 3. A random effects meta-analysis of 14 effect estimates from nine different studies yielded a pooled relative risk of 0.70 (95% CI, 0.49–1.02; $P = 0.06$) comparing the risk of breast cancer in the highest category of methylation to the lowest. There was no evidence of publication bias in these results (Supplementary Fig. S2; Begg test: $P = 0.83$; Egger test: $P = 0.28$).

There was heterogeneity among the included studies ($Q < 0.01$; $I^2 = 87.3\%$). Potential sources of heterogeneity include study design (meta-regression $P = 0.49$), timing of the blood draw (meta-regression $P = 0.37$), the measurement of methylation (meta-regression $P = 0.18$), the country in which the study took place (meta-regression $P = 0.14$), and the average age of the study population (meta-regression $P = 0.08$). Subgroup analyses suggested that the protective effects of higher levels of global DNA methylation were specific to measures of the 450k Illumina assay, global measures, and repetitive elements, but not to LUMA, although none of the estimates were statistically significant (Table 4). When removing studies that measured DNA methylation with the LUMA assay, a statistically significant decreased risk of breast cancer for the highest category of DNA methylation compared with the lowest was observed, 0.69 (95% CI, 0.53–0.89; $P < 0.001$; $I^2 = 67.1\%$). In addition, prospective studies and

Published OnlineFirst July 10, 2018; DOI: 10.1158/1055-9965.EPI-18-0175

Table 2. Assessment of heterogeneity among reports of the association between physical activity and global DNA methylation

Subgroup	Estimates (*n*)	SMD (95% CI); *P*	I^2	Meta-regression *P*
Study demographics				
Mean age				
<50	4	−0.10 (−0.51–0.31); $P = 0.50$	84.9%	0.25
50–<60	6	0.37 (−0.04–0.78); $P = 0.08$	88.4%	
60+	4	0.21 (−0.15–0.57); = 0.25	87.2%	
% Female				
<60	5	0.36 (0.02–0.70); $P = 0.04$	86.1%	0.07
60+	7	0.00 (−0.12–0.12); $P = 0.99$	24.6%	
Country				
North American	8	0.26 (−0.03–0.55); $P = 0.08$	85.9%	0.73
Other	6	0.09 (−0.28–0.46); $P = 0.64$	87.4%	
Physical activity measure				
Study design				
Intervention	6	0.25 (−0.27–0.77); $P = 0.34$	92.2%	0.66
Observational	8	0.12 (−0.06–0.31); $P = 0.19$	73.4%	
Measurement type				
Self-reported	6	0.16 (−0.07–0.39); $P = 0.17$	80.7%	0.99
Other[a]	8	0.19 (−0.21–0.59); $P = 0.34$	89.4%	
Duration of physical activity[b]				
Long-term	10	0.29 (0.04–0.54); $P = 0.02$	87.8%	0.11
Acute	4	−0.12 (−0.59–0.36); $P = 0.63$	83.7%	
DNA methylation assessment				
Genomic context				
Global	2	−0.09 (−0.29–0.12); $P = 0.42$	0.00%	0.61
Repetitive elements	9	0.25 (0.00–0.49); $P = 0.048$	85.4%	
LUMA	3	0.15 (−0.84–1.14); $P = 0.77$	94.2%	
Assay type				
Non-LUMA	11	0.18 (−0.02–0.39); $P = 0.08$	82.9%	0.92
LUMA	3	0.15 (−0.84–1.14); $P = 0.77$	94.2%	
Tissue				
Blood	13	0.25 (0.05–0.45); $P = 0.02$	84.2%	0.01
Muscle	1	−0.90 (−1.38 to −0.42); $P < 0.01$	—	

[a]Other includes intervention studies and observational studies with objective assessments via accelerometry.
[b]Long-term physical activity exposure was defined as a physical activity intervention that lasted 6 or more months in duration or an observational study employing self-reported physical activity levels reflective of typical levels in the past year. Acute physical activity exposure was defined as a physical activity intervention lasting less than 6 months or an observational study that assessed levels of physical activity over periods shorter than 2 months using objective measures.

studies that conducted blood sampling greater than 3 years prior to diagnosis on average had a significantly decreased risk of breast cancer comparing the highest category of global DNA methylation with the lowest: pooled relative risk = 0.62 (0.43–0.87; $P = 0.01$; $I^2 = 58.1\%$) and 0.52 (0.28–0.97; $P = 0.04$; $I^2 = 71.5\%$), respectively. Finally, studies conducted outside of North America and on study populations with an average age between 50 and 60 were at significantly decreased risk for breast cancer when in the highest category of global DNA methylation with a pooled relative risk of 0.47 (0.28–0.79; $P < 0.01$; $I^2 = 67.9\%$) and 0.47 (0.32–0.69; $P < 0.001$; $I^2 = 64.7\%$), respectively.

Discussion

Despite the presence of heterogeneity, the evidence base provides modest support for the hypothesis that global DNA methylation is one of the mechanisms whereby physical activity prevents breast cancer. Overall, we found suggestive associations between high levels of physical activity and high levels of global DNA methylation as well as between high levels of global DNA methylation and lower breast cancer risk. It is important to note that the association between physical activity and global DNA methylation was statistically significant when restricting to studies that assessed physical activity over long periods of time. The magnitude of the estimated effect was consistent with a small to moderate effect size. Among studies of global DNA methylation in relation to breast cancer, we observed a statistically significant association in prospective studies and studies that collected blood samples more than 3 years before diagnosis. The estimated pooled risk reduction of 40% to 50% observed in these studies is clinically meaningful. Studies assessing the relation between global DNA methylation and breast cancer risk did not report a mean difference between the highest and lowest categories of global DNA methylation. Consequently, it is difficult to determine the clinical relevance of the pooled standardized mean difference estimated for investigations of physical activity and global DNA methylation. Large prospective studies that measure both physical activity and DNA methylation and follow for development of breast cancer are needed to assess the extent to which global DNA methylation mediates the association between physical activity and breast cancer risk.

In our quantitative analysis, we were unable to include five studies because they did not report an effect estimate that could be pooled with other studies, and the effect of the exclusion of these studies on our results warrant consideration. Of these studies, one assessed the association between physical activity and global DNA methylation and four investigated the relation between global DNA methylation and breast cancer risk. Marques-Rocha and colleagues (2016) found that the mean MET-hours per day of self-reported physical activity among individuals with higher levels of LINE-1 methylation was significantly greater than that of individuals with lower levels of LINE-1 methylation ($P = 0.047$; ref. 36).

Published OnlineFirst July 10, 2018; DOI: 10.1158/1055-9965.EPI-18-0175

Table 3. Characteristics of studies investigating the association between global DNA methylation and breast cancer risk ($n = 12$)

Prospective studies ($n = 5$)

Study	Country	Study design	Study population	Sample size (cases/controls)	Age (years)[a]	Blood timing	Methylation assay	Methylation measure	Tissue
Brennan (2012; ref. 42)	United Kingdom	Nested case–control	BGS	242/241	54/54	N/R	Pyrosequencing	LINE1	WBC
	Italy		EPIC	263/232	52/52				
	Australia and New Zealand		KConFab	218/153	50/60				
Deroo (2014; ref. 43)	USA and Puerto Rico	Case–cohort	Sister study	294/646	<60	1.3 years avg	Pyrosequencing	LINE1	Whole blood
Severi (2014; ref. 44)	Australia	Nested case–control	MCCS	420/420	64/64	50% > 8.9 years	Illumina 450k	Betas	Whole blood
van Veldhoven (2015; ref. 45)	Italy	Nested case–control	EPIC	166/166	54.4/54.2	50% > 3.8 years	Illumina 450k	Betas	WBC
	Norway		NOWAC	192/192	55.4/55.4	50% > 2.1 years	Illumina 450k		
	United Kingdom		BGS	548/548	52.0/52.0	N/R	WGBS		
Sturgeon (2017; ref. 46)	USA	Nested case–control	PLCO (control arm of intervention study)	428/419	60+	68.6% > = 4 years	%5-mdC	Global	WBC

Nonprospective studies ($n = 7$)

Study	Country	Study design	Study population	Sample size (cases/controls)	Age (years)[a]	Blood timing	Methylation assay	Methylation measure	Tissue
Choi (2009; ref. 47)	USA	Family-based case–control	American women	176/173	< 60	At diagnosis	5-mdC	Global	WBC
Cho (2010; ref. 48)	Turkey	Case–control	Turkish women	40/40	50.8/48.3	At diagnosis	MethylLight (%)	LINE1 ALU SAT2	WBC
Wu (2012; ref. 49)	USA	Family-based case–control	Sisters discordant for breast cancer	266/334	49.5/48.0	At diagnosis	MethylLight (%)	LINE1 ALU SAT2	WBC
Xu (2012; ref. 50)	USA	Population based case–control	American women (LIBCSP)	1064/1101	60+	At diagnosis	LUMA (% methylation) Pyrosequencing	LUMA LINE1	WBC
Kitkumthorn (2012; ref. 51)	Thailand	Population-based case–control	Women from Thailand	36/144	50.28/47.72	At diagnosis	COBRA (%)	LINE1	WBC
Delgado-Cruzata (2012; ref. 52)	USA	Family-based case–control	Sisters discordant for breast cancer	263/321	49.6/48.2	At diagnosis	LUMA (% methylation) 3H-methyl	LUMA Global	WBC
Kuchiba (2014; ref. 53)	Japan	Hospital-based case–control	Japanese women	384/384	53.9/54.1	At diagnosis	LUMA (% methylation)	LUMA	WBC

[a]Average age for cases/controls if available and age range if not available.

Published OnlineFirst July 10, 2018; DOI: 10.1158/1055-9965.EPI-18-0175

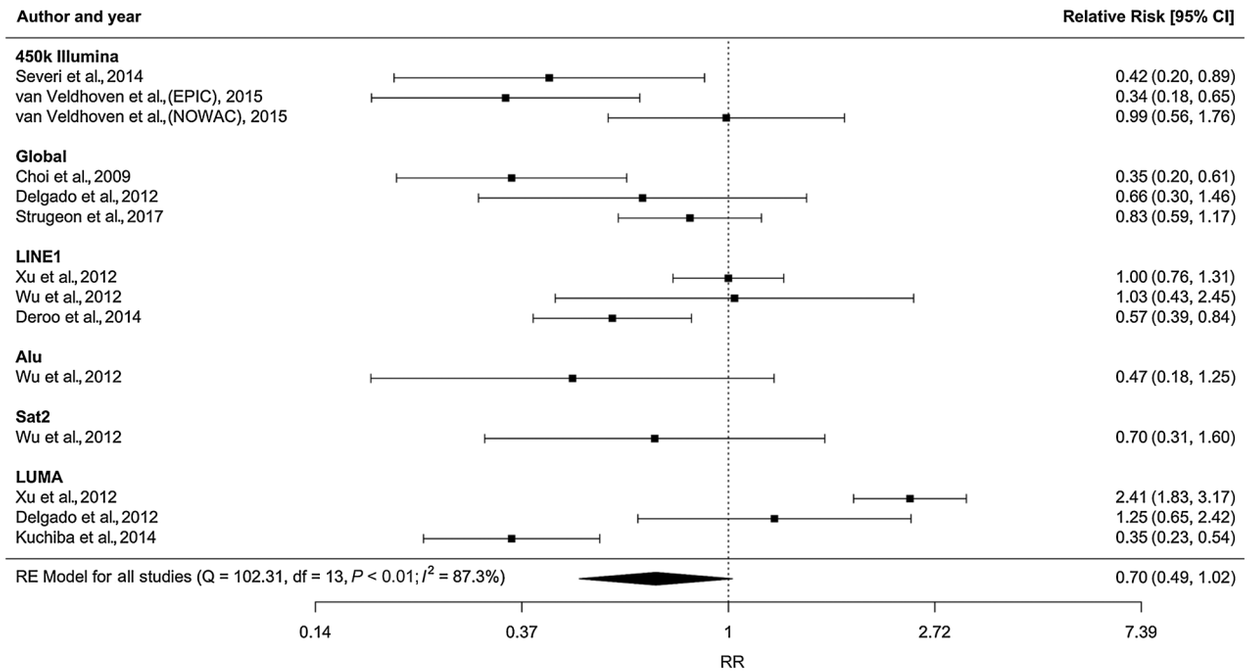

Figure 3.

Estimated relative risk of breast cancer comparing study-defined categories of high and low levels of global DNA methylation. This figure presents a forest plot that describes the pooled relative risk of breast cancer between study-defined categories of DNA methylation.

This result supports our suggested finding of a positive association between physical activity and global DNA methylation. Brennan and colleagues (2012) reported no difference in mean LINE-1 methylation between cases and controls in three nested case–control studies (42). In contrast, van Veldhoven and colleagues (2015) reported significantly higher global DNA methylation among controls compared with breast cancer cases in the British Generations Study (45). A higher level of global DNA methylation comparing controls to cases was also observed in the investigations by Cho and colleagues (2010) and Kitkumthorn and colleagues (2012), but neither of these results were statistically significant (48, 51). It is noteworthy that none of the excluded

Table 4. Assessment of heterogeneity among studies examining the association between global DNA methylation and breast cancer risk

Subgroup	Estimates (*n*)	RR (95% CI); *P*	I^2	Meta-regression *P*
Study demographics				
Mean age				
<50	5	0.83 (0.58–1.19); $P = 0.31$	0.0%	0.08
50–<60	5	0.47 (0.32–0.69); $P = 0.02$	64.7%	
60+	4	1.01 (0.54–1.90); $P = 0.97$	92.0%	
Country				
North American	10	0.83 (0.55–1.25); $P = 0.37$	86.6%	0.14
Other	4	0.47 (0.28–0.79); $P = 0.004$	67.9%	
Study design				
Type				
Prospective[a]	5	0.62 (0.43–0.87); $P = 0.007$	58.1%	0.49
Case–control	9	0.77 (0.45–1.32); $P = 0.34$	90.1%	
Blood draw type				
>3 years prior to diagnosis	3	0.52 (0.28–0.97); $P = 0.04$	71.5%	0.37
<3 years prior to diagnosis	11	0.77 (0.49–1.20); $P = 0.24$	88.8%	
DNA methylation assessment				
Genomic context				
Illumina 450k	3	0.53 (0.27–1.06); $P = 0.07$	69.8%	0.26
Global	3	0.59 (0.33–1.04); $P = 0.07$	69.9%	
Repetitive elements	6	0.84 (0.63–1.13); $P = 0.25$	54.0%	
LUMA	4	1.02 (0.28–3.76); $P = 0.98$	96.4%	
Assay type				
Non-LUMA	12	0.64 (0.50–0.83); $P < 0.001$	58.3%	0.25
LUMA	3	1.02 (0.28–3.76); $P = 0.98$	96.4%	

[a]Includes nested case–control studies.

Published OnlineFirst July 10, 2018; DOI: 10.1158/1055-9965.EPI-18-0175

studies reported findings in the opposite direction of our pooled effect estimates. As such, the exclusion of these studies is unlikely to have had a major impact on the results or qualitative findings of this investigation.

Physical activity and global DNA methylation

With respect to the association between physical activity and global DNA methylation, sources of heterogeneity included the duration of the exposure, the target tissue of interest, the genomic context, and study demographics. Of relevance to the underlying hypothesis, the pooled effect estimate was statistically significant when examining studies that measured physical activity over long periods of time, which suggests that short-term changes in physical activity may have little impact on global DNA methylation. This speculation is supported by studies reporting a high degree of stability in levels of DNA methylation within LINE-1 repeats over time (54, 55). It should be noted that the study by White and colleagues (2013) was the only study to assess lifetime (childhood, teenage, and adulthood) levels of physical activity (40). Additional research assessing lifetime levels of physical activity is warranted and should be accomplished by nesting epigenetic studies within existing cohort investigations with long durations of follow-up.

It is important to highlight two notable outliers in the meta-analysis of the association between physical activity and global DNA methylation. The study by Barres and colleagues (2012) observed a significant negative association between physical activity and global DNA methylation, which is in the opposite direction of the hypothesized effect (30). There were several noteworthy differences between this study with respect to the intervention and assessment of DNA methylation. Specifically, the study assessed LUMA methylation within muscle tissue immediately before and after a single acute bout of exercise. It is not clear whether the observed effect would also reflect chronic changes in DNA methylation months or years after this initial change. Similar disparities with respect to the intervention and study population can also be found in the study by Delgado-Cruzata and colleagues (2015), which reported a relatively large effect on LINE-1 methylation that was in the hypothesized direction (31). These investigators assessed the effects of a combined physical activity and caloric restriction dietary weight-loss intervention among ethnic minority breast cancer survivors. It should be mentioned that Duggan and colleagues (2014) assessed the effects of a physical activity intervention and a combined physical activity and dietary intervention but found that neither intervention had a significant impact on levels of LINE-1 methylation (32).

Global DNA methylation and breast cancer risk

Regarding the suggested association between global DNA methylation and breast cancer risk, sources of heterogeneity include population characteristics, the timing of the assessment of DNA methylation, and the genomic context. Given the potential for reverse causality and potentially long latency period between the initiation and diagnosis of breast cancer, the most reliable source of evidence regarding the association of interest would arise from prospective studies or investigations whereby DNA methylation was assessed years prior to the diagnosis of cancer. Case–control studies are susceptible to reverse causality, because the active disease may influence cell type distribution, deplete required enzymes, and contribute circulation of tumor cells in the blood. Moreover, depending on when the blood was sampled, DNA methylation may be altered in response to cancer treatment. Therefore, our result of a statistically significant association between global DNA methylation and breast cancer risk among prospectively collected blood samples is important for establishing an etiologic relationship. However, this analysis is still limited, because the majority of studies have a short period between blood collection and diagnosis. It is also important to note that the majority of the prospective studies measured DNA methylation with the 450k array. More studies assessing DNA methylation in repetitive elements and other genomic contexts in prospective studies are required to determine which genomic contexts are most associated with breast cancer risk.

It is important to discuss one notable outlier in the meta-analysis of global DNA methylation and breast cancer risk. Xu and colleagues (2012) found an increased risk of breast cancer associated with higher levels of global DNA methylation as measured by the LUMA assay, which is in the opposite direction of the hypothesized effect. One potential explanation for this result is that the targeted sequences for their assay largely represented genomic contexts for which the direction of dysregulation was increased methylation as described in further detail below. This speculation is supported by the authors' finding that their measure of LUMA methylation was positively correlated with promoter hypermethylation and was not associated with LINE-1 methylation. Moreover, the average global DNA methylation levels of this study population were much lower than other studies using the LUMA assay, providing further evidence of variable targeted sequences.

Limitations of the literature on global DNA methylation

There are several limitations involved in the measurement, conceptualization, and reporting of the global DNA methylation measures included in this review. Identified as an important source of heterogeneity, the studies included in this review used different measures of global DNA methylation that target different CpG sites within different genomic regions that have different functional implications (14). For example, the biologic consequences of DNA methylation across the entire genome would differ depending on the extent to which it occurs within repetitive element regions or within and around genes involved in the carcinogenic process (14). Measures focused on repetitive element hypomethylation could be missing potentially important losses of methylation within the promoter, gene bodies, and other gene regions distal of promoters. Measures focused on DNA methylation across the entire genome or measures that capture both intra- and intergene methylation (e.g., 450k Illumina array) would capture these potentially important losses of DNA methylation. Such differences may explain why the pooled effect estimate for the association between global DNA methylation and breast cancer risk was larger among studies using true global or the 450k Illumina array DNA methylation measure. However, there is evidence that physical inactivity and the development of breast cancer are associated with the increased methylation of certain promoter regions (22, 56). Unlike repetitive element DNA methylation, measures that capture aspects of gene-specific DNA methylation may therefore introduce additional measurement error by pooling levels of DNA methylation across regions that are normally unmethylated with those that are normally methylated in healthy cells. Such pooling would attenuate the magnitude of

Published OnlineFirst July 10, 2018; DOI: 10.1158/1055-9965.EPI-18-0175

the estimated effect, and this source of error may explain some heterogeneity across studies investigating the association between physical activity and global DNA methylation.

In both meta-analyses, the pooled effect estimates were statistically significant when excluding measures of global DNA methylation measured with the LUMA assay, which could be explained by several factors. First, the LUMA assay has been found to be the least valid surrogate measure of DNA methylation across the entire genome because in comparison with other assays, it is particularly sensitive to the quality of the original DNA isolate (57). More importantly, the distribution of targeted sequences for the LUMA assay, CCGG sequences across the genome, can vary across studies (57). That is, some studies can be targeting sequences that are vulnerable to loss of methylation, whereas others are targeting sequences that are vulnerable to gain of methylation, which may explain the divergent results among studies investigating the associations of interest with the LUMA assay.

The majority of studies did not measure or control for cell-type distribution. Specifically, only one study investigating physical activity (32) and two studies on breast cancer (44, 45) adjusted for the distribution of cell types. Although it has yet to be conclusively demonstrated, there is some suggestion that measures of global DNA methylation differ across blood cell types (58, 59). Therefore, the observed associations measured in samples with heterogeneous mixtures of cell types (e.g., leukocytes) could reflect changes in cell-type composition that are a product of already established mechanisms, such as changes in immune function or levels of inflammation that arise from changes in physical activity or the initiation of breast cancer. The extent to which these associations can be explained by failure to control for the distribution of cell-type is difficult to assess and future research is needed.

One of the methodologic issues related to the use of circulating measures of DNA methylation is the fundamental assumption that epigenetic changes occurring in breast tissue are reflected in the blood. Despite the proliferation of studies assessing global DNA methylation in peripheral blood, this fundamental assumption has yet to be widely assessed and preliminary evidence is conflicting. A number of differences between the methylation of specific loci in blood and breast tissue have been identified (60). More crucial to the interpretation of the body of literature reviewed herein, however, is the extent to which there is agreement between global DNA methylation measures taken from the breast and blood. Sturgeon and colleagues (2014) assessed levels of LINE1, Alu, and Sat2 methylation in matched breast epithelial and white blood cells among 22 healthy women and found no correlation (61). Similarly, Guo and colleagues (2015) reported a Spearman correlation coefficient of 0.35 when comparing matched healthy breast tissue and blood samples with respect to global DNA methylation measured by the 450K Illumina Array among 31 patients with breast cancer (62). In contrast, Cho and colleagues (2010) reported a strong correlation ($r = 0.67$) between levels of Sat2 methylation in healthy breast tissue and white blood cells among 40 patients with breast cancer (48). The extent to which levels of global DNA methylation in the blood correspond to that of breast tissue is therefore questionable. However, the lack of correlation between blood and tissue methylation may, in part, be attributable to differences in cell-type distribution. It should also be noted that all of these studies were relatively small and used correlation coefficients and/or *P* values to assess agreement, statistics that have long been recognized as inappropriate measures of agreement (63–65). Future studies with larger samples sizes that use more appropriate measures of agreement such as the intraclass correlation coefficient or limits of agreement are needed to better understand the extent to which results from epigenetic studies carried out in blood are generalizable to breast tissue.

Finally, many investigations included in this review were nested within existing studies using previously collected blood samples. It is unclear whether the investigators conducted an *a priori* power calculation. These investigations may have, therefore, been underpowered to detect the effect of interest, which would have inflated the risk of a type II error in this body of evidence.

Strengths of investigation

To the best of our knowledge, this review is the first to provide an overview of the complete biological pathway between physical activity, global DNA methylation, and breast cancer risk. Moreover, it is the first study to quantitatively assess the association of physical activity or breast cancer risk with measures of global DNA methylation using meta-analytic techniques. In addition, our use of meta-regression and subgroup analyses identified key factors that contribute to the heterogeneity in this body of literature, which will help to guide future research and provide some clues about the nature of the underlying associations of interest.

Recommendations for future research

Future studies on global DNA methylation should adjust for the distribution of cell-types and should assess modification by study demographics, particularly age and ethnicity. In addition, the associations of interest within healthy breast tissue should be assessed and the agreement between blood and breast tissue measures of global DNA methylation using large sample sizes and appropriate statistical measures should be examined. Power calculations are needed before conducting a nested epigenetic study to ensure that the investigation is adequately powered. With respect to the association between physical activity and global DNA methylation, studies should consider assessing interactions with dietary intake and mediation by weight loss. Ideally, these types of investigations would be carried out within long-term prospective cohort studies, which estimate lifetime levels of physical activity using both objective and self-reported measures. Within cross-sectional settings, assessments of physical activity using accelerometers should be augmented with historical and long-term questionnaire-based measures. Authors should also aim to report the average levels of physical activity within study-defined categories such that the presence of a dose–response relationship can be assessed in future meta-analyses. With respect to the association between global DNA methylation and breast cancer, the assessment of DNA methylation should use samples taken years before the cancer diagnosis to ensure temporality. Measures that summarize levels of methylation across regions with similar levels of methylation in healthy tissues are needed to produce more appropriate global methylation estimates. For example, one could conceptualize a global DNA methylation measure that quantifies the degree of methylation across the entire genome within regions that are normally methylated and one could similarly conceptualize a separate measure for regions that are normally unmethylated. Such measures would avoid the issue we previously described regarding global DNA

Published OnlineFirst July 10, 2018; DOI: 10.1158/1055-9965.EPI-18-0175

methylation measures that combine regions that are normally methylated with regions that are normally unmethylated.

Conclusion

In conclusion, this systematic review provides preliminary evidence that long-term physical activity is associated with the maintenance of global DNA methylation, which, in turn, is associated with a decreased risk of breast cancer. A framework for standardizing the assessment, conceptualization, and reporting of global DNA methylation measures is needed and could be developed as an extension to existing STROBE guidelines as previously done for genetic and molecular epidemiologic studies (66–68). Large prospective studies that assess lifetime physical activity levels, control for changes in cell-type distribution, and measure global DNA methylation years prior to the onset of breast cancer are required to confirm these suggested findings.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

C.M. Friedenreich was supported by an Alberta Innovates Health Solutions Health Senior Scholar Award and by the Alberta Cancer Foundation Weekend to End Women's Cancers Breast Cancer Chair. D.R. Brenner holds a Canadian Cancer Society Cancer Prevention Capacity Development Award (#703917). D.J. Boyne and D.E. O'Sullivan are supported by Queen Elizabeth II Scholarships.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received February 18, 2018; revised April 16, 2018; accepted July 4, 2018; published first July 10, 2018.

References

1. Global Burden of Disease Cancer Collaboration. The global burden of cancer 2013. JAMA Oncol 2015;1:505–27.
2. Brennan SF, Cantwell MM, Cardwell CR, Velentzis LS, Woodside JV. Dietary patterns and breast cancer risk: a systematic review and meta-analysis. Am J Clin Nutr 2010;91:1294–302.
3. Gaudet MM, Gapstur SM, Sun J, Diver WR, Hannan LM, Thun MJ. Active smoking and breast cancer risk: original cohort data and meta-analysis. J Natl Cancer Inst 2013;105:515–25.
4. Friedenreich CM, Neilson HK, Lynch BM. State of the epidemiological evidence on physical activity and cancer prevention. Eur J Cancer 2010;46:2593–604.
5. Lynch BM, Neilson HK, Friedenreich CM. Physical activity and breast cancer prevention. Recent Results Cancer Res 2011;186:13–42.
6. Neilson HK, Friedenreich CM, Brockton NT, Millikan RC. Physical activity and postmenopausal breast cancer: proposed biologic mechanisms and areas for future research. Cancer Epidemiol Biomarkers Prev 2009;18:11–27.
7. Friedenreich CM, Woolcott CG, McTiernan A, Terry T, Brant R, Ballard-Barbash R, et al. Adiposity changes after a 1-year aerobic exercise intervention among postmenopausal women: a randomized controlled trial. Int J Obes 2011;35:427–35.
8. Friedenreich CM, Woolcott CG, McTiernan A, Ballard-Barbash R, Brant RF, Stanczyk FZ, et al. Alberta physical activity and breast cancer prevention trial: sex hormone changes in a year-long exercise intervention among postmenopausal women. J Clin Oncol 2010;28:1458–66.
9. Rinaldi S, Kaaks R, Friedenreich CM, Key TJ, Travis R, Biessy C, et al. Physical activity, sex steroid, and growth factor concentrations in pre- and postmenopausal women: a cross-sectional study within the EPIC cohort. Cancer Causes Control 2014;25:111–24.
10. Albright A, Franz M, Hornsby G, Kriska A, Marrero D, Ullrich I, et al. American College of Sports Medicine position stand: Exercise and type 2 diabetes. Med Sci Sports Exerc 2000;32:1345–60.
11. Larsson SC, Mantzoros CS, Wolk A. Diabetes mellitus and risk of breast cancer: a meta-analysis. Int J Cancer 2007;121:856–62.
12. Shephard RJ, Rhind S, Shek PN. The impact of exercise on the immune system: NK cells, Interleukins 1 and 2, and related responses. Exerc Sport Sci Rev 1995;23:215–42.
13. Lim U, Song MA. Dietary and lifestyle factors of DNA methylation. Methods Mol Biol 2012;863:359–76.
14. Jones PA. Functions of DNA methylation: islands, start sites, gene bodies and beyond. Nat Rev Genet 2012;13:484–92.
15. Esteller M. Epigenetics in cancer. N Engl J Med 2008;358:1148–59.
16. Terry MB, Delgado-Cruzata L, Vin-Raviv N, Wu HC, Santella RM. DNA methylation in white blood cells. Epigenetics 2011;6:828–37.
17. Denham J, Marques FZ, O'Brien BJ, Charchar FJ. Exercise: putting action into our epigenome. Sports Med 2014;44:189–209.
18. Ehlert T, Simon P, Moser DA. Epigenetics in sports. Sports Med 2013;43:93–110.
19. Horsburgh S, Robson-Ansley P, Adams R, Smith C. Exercise and inflammation-related epigenetic modifications: focus on DNA methylation. Exerc Immunol Rev 2015;21:26–41.
20. Ling C, Rönn T. Epigenetic adaptation to regular exercise in humans. Drug Discov Today 2014;19:1015–8.
21. Soci UPR, Melo SFS, Gomes JLP, Silveira AC, Nóbrega C, de Oliveira EM. Exercise training and epigenetic regulation: multilevel modification and regulation of gene expression. In: Xiao J. Exercise for cardiovascular disease prevention and treatment. Singapore: Springer; 2017, p. 281–322.
22. Voisin S, Eynon N, Yan X, Bishop D. Exercise training and DNA methylation in humans. Acta Physiol 2015;213:39–59.
23. Woo HD, Kim J. Global DNA hypomethylation in peripheral blood leukocytes as a biomarker for cancer risk: a meta-analysis. PLoS One 2012;7:e34615.
24. Brennan K, Flanagan JM. Is there a link between genome-wide hypomethylation in blood and cancer risk? Cancer Prev Res 2012;5:1345–57.
25. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med 2009;6:e1000097.
26. Rohatgi A. WebPlotDigitizer. 2011. Available from: https://automeris.io/WebPlotDigitizer/.
27. Cooper H, Hedges LV, Valentine JC. The handbook of research synthesis and meta-analysis. New York, NY: Russell Sage Foundation; 2009.
28. Higgins JP, Green S. Cochrane handbook for systematic reviews of interventions. Hoboken, NJ: John Wiley & Sons; 2011.
29. Johnsen TJ, Friborg O. The effects of cognitive behavioral therapy as an antidepressive treatment is falling: A meta-analysis. Washington, DC: American Psychological Association; 2015.
30. Barres R, Yan J, Egan B, Treebak JT, Rasmussen M, Fritz T, et al. Acute exercise remodels promoter methylation in human skeletal muscle. Cell Metab 2012;15:405–11.
31. Delgado-Cruzata L, Zhang W, McDonald JA, Tsai WY, Valdovinos C, Falci L, et al. Dietary modifications, weight loss, and changes in metabolic markers affect global DNA methylation in Hispanic, African American, and Afro-Caribbean breast cancer survivors. J Nutr 2015;145:783–90.
32. Duggan C, Xiao L, Terry MB, McTiernan A. No effect of weight loss on LINE-1 methylation levels in peripheral blood leukocytes from postmenopausal overweight women. Obesity 2014;22:2091–6.
33. Gomes MV, Toffoli LV, Arruda DW, Soldera LM, Pelosi GG, Neves-Souza RD, et al. Age-related changes in the global DNA methylation profile of leukocytes are linked to nutrition but are not associated with the MTHFR C677T genotype or to functional capacities. PLoS One 2012;7:e52570.
34. King-Himmelreich TS, Schramm S, Wolters MC, Schmetzer J, Moser CV, Knothe C, et al. The impact of endurance exercise on global and AMPK gene-specific DNA methylation. Biochem Biophys Res Commun 2016;474:284–90.

35. Luttropp K, Nordfors L, Ekstrom TJ, Lind L. Physical activity is associated with decreased global DNA methylation in Swedish older individuals. Scand J Clin Lab Invest 2013;73:184–5.
36. Marques-Rocha JL, Milagro FI, Mansego ML, Mourao DM, Martinez JA, Bressan J. LINE-1 methylation is positively associated with healthier lifestyle but inversely related to body fat mass in healthy young individuals. Epigenetics 2016;11:49–60.
37. McGuinness D, McGlynn LM, Johnson PC, MacIntyre A, Batty GD, Burns H, et al. Socio-economic status is associated with epigenetic differences in the pSoBid cohort. Int J Epidemiol 2012;41:151–60.
38. Morabia A, Zhang FF, Kappil MA, Flory J, Mirer FE, Santella RM, et al. Biologic and epigenetic impact of commuting to work by car or using public transportation: a case-control study. Prev Med 2012;54:229–33.
39. Perng W, Villamor E, Shroff MR, Nettleton JA, Pilsner JR, Liu Y, et al. Dietary intake, plasma homocysteine, and repetitive element DNA methylation in the Multi-Ethnic Study of Atherosclerosis (MESA). Nutr Metab Cardiovasc Dis 2014;24:614–22.
40. White AJ, Sandler DP, Bolick SC, Xu Z, Taylor JA, DeRoo LA. Recreational and household physical activity at different time points and DNA global methylation. Eur J Cancer 2013;49:2199–206.
41. Zhang FF, Cardarelli R, Carroll J, Zhang S, Fulda KG, Gonzalez K, et al. Physical activity and global genomic DNA methylation in a cancer-free population. Epigenetics 2011;6:293–9.
42. Brennan K, Garcias-Closas M, Orr N, Fletcher O, Jones M, Ashworth A, et al. Intragenic ATM methylation in peripheral blood DNA as a biomarker of breast cancer risk. Cancer Res 2012;72:2304–13.
43. DeRoo LA, Bolick SC, Xu Z, Umbach DM, Shore D, Weinberg CR, et al. Global DNA methylation and one-carbon metabolism gene polymorphisms and the risk of breast cancer in the Sister Study. Carcinogenesis 2013;35:333–8.
44. Severi G, Southey MC, English DR, Jung C-h, Lonie A, McLean C, et al. Epigenome-wide methylation in DNA from peripheral blood as a marker of risk for breast cancer. Breast Cancer Res Treat 2014;148:665–73.
45. van Veldhoven K, Polidoro S, Baglietto L, Severi G, Sacerdote C, Panico S, et al. Epigenome-wide association study reveals decreased average methylation levels years before breast cancer diagnosis. Clin Epigenetics 2015;7:67.
46. Sturgeon SR, Pilsner JR, Arcaro KF, Ikuma K, Wu H, Kim S-M, et al. White blood cell DNA methylation and risk of breast cancer in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO). Breast Cancer Res 2017;19:94.
47. Choi J-Y, James SR, Link PA, McCann SE, Hong C-C, Davis W, et al. Association between global DNA hypomethylation in leukocytes and risk of breast cancer. Carcinogenesis 2009;30:1889–97.
48. Cho YH, Yazici H, Wu H-C, Terry MB, Gonzalez K, Qu M, et al. Aberrant promoter hypermethylation and genomic hypomethylation in tumor, adjacent normal tissues and blood from breast cancer patients. Anticancer Res 2010;30:2489–96.
49. Wu H-C, Delgado-Cruzata L, Flom JD, Perrin M, Liao Y, Ferris JS, et al. Repetitive element DNA methylation levels in white blood cell DNA from sisters discordant for breast cancer from the New York site of the Breast Cancer Family Registry. Carcinogenesis 2012;33:1946–52.
50. Xu X, Gammon MD, Hernandez-Vargas H, Herceg Z, Wetmur JG, Teitelbaum SL, et al. DNA methylation in peripheral blood measured by LUMA is associated with breast cancer in a population-based study. FASEB J 2012;26:2657–66.
51. Kitkumthorn N, Tuangsintanakul T, Rattanatanyong P, Tiwawech D, Mutirangura A. LINE-1 methylation in the peripheral blood mononuclear cells of cancer patients. Clin Chim Acta 2012;413:869–74.
52. Delgado-Cruzata L, Wu H-C, Perrin M, Liao Y, Kappil MA, Ferris JS, et al. Global DNA methylation levels in white blood cell DNA from sisters discordant for breast cancer from the New York site of the Breast Cancer Family Registry. Epigenetics 2012;7:868–74.
53. Kuchiba A, Iwasaki M, Ono H, Kasuga Y, Yokoyama S, Onuma H, et al. Global methylation levels in peripheral blood leukocyte DNA by LUMA and breast cancer: a case–control study in Japanese women. Br J Cancer 2014;110:2765.
54. Bollati V, Schwartz J, Wright R, Litonjua A, Tarantini L, Suh H, et al. Decline in genomic DNA methylation through aging in a cohort of elderly subjects. Mech Ageing Dev 2009;130:234–9.
55. Wu H-C, Wang Q, Delgado-Cruzata L, Santella RM, Terry MB. Genomic methylation changes over time in peripheral blood mononuclear cell DNA: differences by assay type and baseline values. Cancer Epidemiol Prev Biomarkers 2012;21:1314–8.
56. Tang Q, Cheng J, Cao X, Surowy H, Burwinkel B. Blood-based DNA methylation as biomarker for breast cancer: a systematic review. Clin Epigenet 2016;8:115.
57. Lisanti S, Omar WA, Tomaszewski B, De Prins S, Jacobs G, Koppen G, et al. Comparison of methods for quantification of global DNA methylation in human cells and tissues. PLoS One 2013;8:e79044.
58. Wu H-C, Delgado-Cruzata L, Flom JD, Kappil M, Ferris JS, Liao Y, et al. Global methylation profiles in DNA from different blood cell types. Epigenetics 2011;6:76–85.
59. Zhu Z-Z, Hou L, Bollati V, Tarantini L, Marinelli B, Cantone L, et al. Predictors of global methylation levels in blood DNA of healthy subjects: a combined analysis. Int J Epidemiol 2010;41:126–39.
60. Lowe R, Slodkowicz G, Goldman N, Rakyan VK. The human blood DNA methylome displays a highly distinctive profile compared with other somatic tissues. Epigenetics 2015;10:274–81.
61. Sturgeon SR, Arcaro KF, Johnson MA, Balasubramanian R, Zorn M, Jerry DJ, et al. DNA methylation in paired breast epithelial and white blood cells from women undergoing reduction mammoplasty. Anticancer Res 2014;34:2985–90.
62. Guo X, Shu X-O, Li C, Long J, Li B, Gao Y-T, et al. Correlation of DNA methylation pattern between peripheral blood cell and normal breast tissue [abstract]. In: Proceedings of the 106th Annual Meeting of the American Association for Cancer Research; 2015 Apr 18–22; Philadelphia, PA: 2015. Abstract nr 1068.
63. Bunce C. Correlation, agreement, and Bland–Altman analysis: statistical analysis of method comparison studies. Am J Ophthalmol 2009;148:4–6.
64. Altman DG, Bland JM. Measurement in medicine: the analysis of method comparison studies. J R Stat Soc Series B 1983;32:307–17.
65. Bland JM, Altman D. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet North Am Ed 1986;327:307–10.
66. Field N, Cohen T, Struelens MJ, Palm D, Cookson B, Glynn JR, et al. Strengthening the Reporting of Molecular Epidemiology for Infectious Diseases (STROME-ID): an extension of the STROBE statement. Lancet Infect Dis 2014;14:341–52.
67. Gallo V, Egger M, McCormack V, Farmer PB, Ioannidis JP, Kirsch-Volders M, et al. STrengthening the Reporting of OBservational studies in Epidemiology–Molecular Epidemiology (STROBE-ME): an extension of the STROBE Statement. Mutagenesis 2011;27:17–29.
68. Little J, Higgins JP, Ioannidis JP, Moher D, Gagnon F, Von Elm E, et al. STrengthening the REporting of Genetic Association Studies (STREGA): an extension of the STROBE statement. Hum Genet 2009;125:131–51.

Published OnlineFirst July 10, 2018; DOI: 10.1158/1055-9965.EPI-18-0175

Cancer Epidemiology, Biomarkers & Prevention

AACR American Association for Cancer Research

Physical Activity, Global DNA Methylation, and Breast Cancer Risk: A Systematic Literature Review and Meta-analysis

Devon J. Boyne, Dylan E. O'Sullivan, Branko F. Olij, et al.

Cancer Epidemiol Biomarkers Prev 2018;27:1320-1331. Published OnlineFirst July 10, 2018.