Review Article

Relationships between Global DNA Methylation in Circulating White Blood Cells and Breast Cancer Risk Factors

Nayha Chopra-Tandon,1 Haotian Wu,2 Kathleen F. Arcaro,3 and Susan R. Sturgeon1

1Department of Biostatistics and Epidemiology, School of Public Health and Health Sciences, University of Massachusetts, Amherst, MA, USA
2Department of Environmental Health Sciences, School of Public Health and Health Sciences, University of Massachusetts, Amherst, MA, USA
3Department of Veterinary and Animal Science, College of Natural Sciences, University of Massachusetts, Amherst, MA, USA

Correspondence should be addressed to Nayha Chopra-Tandon; nchoprat@umass.edu

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It is not yet clear whether white blood cell DNA global methylation is associated with breast cancer risk. In this review we examine the relationships between multiple breast cancer risk factors and three markers of global DNA methylation: LINE-1, 5-mdC, and Alu. A literature search was conducted using Pubmed up to April 1, 2016, using combinations of relevant outcomes such as “WBC methylation,” “blood methylation,” “blood LINE-1 methylation,” and a comprehensive list of known and suspected breast cancer risk factors. Overall, the vast majority of reports in the literature have focused on LINE-1. There was reasonably consistent evidence across the studies examined that males have higher levels of LINE-1 methylation in WBC DNA than females. None of the other demographic, lifestyle, dietary, or health condition risk factors were consistently associated with LINE-1 DNA methylation across studies. With the possible exception of sex, there was also little evidence that the wide range of breast cancer risk factors we examined were associated with either of the other two global DNA methylation markers: 5-mdC and Alu. One possible implication of the observed lack of association between global WBC DNA methylation and known breast cancer risk factors is that the association between global WBC DNA methylation and breast cancer, if it exists, is due to a disease effect.

1. Introduction

A CpG site, a cytosine followed by a guanine, has the potential to be methylated, and measuring 5-methyl-2'-deoxycytidine (5-mdC) content across the genome by liquid chromatography/mass spectrometry (LC/MS) can provide an overall measure of genome-wide DNA methylation levels. Repetitive sequences of the genome such as LINE-1 and Alu contain up to half of all DNA methylation in the genome [1]. Thus, measuring DNA methylation levels in LINE-1 or Alu repetitive elements by pyrosequencing or Methyl Light is often used as a surrogate higher-throughput approach to assess genome-wide methylation [2]. Genome instability has been associated with DNA hypomethylation and such global loss of methylation is common in breast tumor tissue [3–7].

There is some evidence that peripheral white blood cell (WBC) DNA contains epigenetic information that can be used to assess an individual’s risk of breast cancer. In a case-control study of breast cancer of 179 cases and 180 controls, Choi and colleagues observed a nearly threefold increase in risk among women in the lowest tertile of total 5-mdC level in WBC DNA compared to women in the highest tertile [1]. In the NIEHS sister case-cohort study of 294 cases and 646 noncases in which the mean time between blood collection and breast cancer diagnosis was 15 months [8], LINE-1 methylation percentage in WBC DNA was also inversely associated with the risk of breast cancer, with a nearly twofold increased risk observed among women in the lowest quartile compared with those in the highest quartile. However, Brennan and colleagues reported no association between LINE-1 WBC methylation and breast cancer risk in three prospective nested case-control studies [9]. Several other case-control studies, ranging in the number of breast cancer cases from 19 to 1064, found no association between...
**LINE-1 methylation and breast cancer risk** [1, 10–12] or between *Alu* methylation and breast cancer risk [12, 13].

The LUMinometric Methylation Assay (LUMA) measures levels of 5-mdC in a specific CmCGG motif which is found both in promoter regions of the genome and in repetitive elements [11]. Interestingly, one case-control study reported a twofold increase in risk of breast cancer among women with higher 5-mdC content compared to those with lower levels measured by LUMA. Another study reported no association [14] and a third study reported a strong inverse association between increasing tertiles of LUMA methylation and breast cancer risk [15].

It is not yet clear whether WBC DNA global methylation is associated with breast cancer risk [16]. If there is an association, one possible explanation is that the association represents environmental and lifestyle determinants of breast cancer that influence both DNA methylation and breast cancer risk. An alternative possibility is that, in response to very early breast cancer, a new clone of circulating lymphocytes arises that alters white blood cell DNA methylation [17]. If WBC DNA methylation is a marker of exposure associated with breast cancer risk, rather than a marker of early disease, it is reasonable to expect that white blood cell DNA methylation patterns would be more likely to be correlated with hormonal and other established or suspected risk factors for breast cancer. Terry and colleagues [18] reviewed literature up to 2011 on the relation between WBC DNA methylation patterns and a number of cancer risk factors. As the literature has expanded substantially, we updated the review, focusing on four demographic factors (age, sex, race/ethnicity, and education), three lifestyle factors (alcohol, smoking, and physical activity), three dietary factors (BMI, vegetable intake, and fruit intake), and eight health history and reproductive factors (menopause status, fetal birth weight, family history of breast cancer, age at menarche, age at first birth, parity, hormone replacement therapy, and endogenous hormones) that have been associated with breast cancer [19]. We also included folate in our review because although results have been mixed for breast cancer, folate is plausibly linked to DNA methylation [20]. We examined the relationships between these breast cancer risk factors and three markers of global DNA methylation: LINE-1, 5-mdC, and *Alu*. Our review comprises literature published through April 2016 and includes over 30 new studies that were not included in the 2011 review [18].

### 2. Methods

A literature search was conducted using PubMed up to April 1, 2016. Searches were performed using combinations of relevant outcomes such as “WBC methylation,” “blood methylation,” “blood LINE-1 methylation,” and a comprehensive list of known and suspected breast cancer risk factors such as “diet,” “physical activity,” and “menopause.” Boolean operators “and” and “or” were used whenever appropriate. Titles and abstracts were screened to determine relevancy by three independent reviewers. Additionally, bibliographies of select reviews were screened to ensure capture of all relevant information and ideas. If relevancy could not be determined from the abstract, the full text was retrieved to ensure comprehensive capture.

A study was included if it was primary research, published in English, and contained relevant results on any risk factor and blood DNA methylation outcomes. Studies were included that had both men and women due to the limited number of studies performed only in women. Studies were only included if their data were based on populations of nondiseased individuals.

### 3. Results

Table 1 shows the number of studies reporting associations between global WBC DNA methylation and demographic, lifestyle, dietary, and reproductive factors for each of three markers (i.e., LINE-1, *Alu*, and 5-mdC). Overall, the vast majority of reports in the literature have focused on LINE-1. For example, 21 studies examined the association between age and LINE-1 but only four studies examined age and 5-mdC. There were ten or more studies that each examined the association between LINE-1 and alcohol, smoking, body mass index, vegetables, and folate. Fewer studies were available for *Alu* and 5-mdC and for reproductive risk factors.

#### 3.1. Demographic Factors

**3.1.1. Age.** As shown in Table 2, twenty of the twenty-one studies examining LINE-1 reported no significant association between age and LINE-1 methylation levels [11, 21–38]. Only one study reported a significant association between increasing age and higher LINE-1 methylation levels [29]. Three of the six studies examining *Alu* methylation found no significant association with age [38, 41, 42], while the other three studies reported a significant association between increasing age and decreasing *Alu* methylation [22, 39, 40]. Two of four studies examining 5-mdC levels did not find a significant association with age [1, 44], while two other studies reported a statistically significant association between increasing age and decreasing 5-mdC levels [39, 43]. In summary, of the 31 studies with data on age and estimates of global DNA methylation no relationship was reported for 25 of the studies, a significant inverse relationship was reported for five studies, and a positive relationship was found in only one study.

**3.1.2. Sex.** As shown in Table 2, eleven of the seventeen studies reported statistically significant higher LINE-1 levels in males than in females [25, 28–30, 32, 34–36, 42, 45, 46], while the other six studies found no statistically significant association between sex and LINE-1 levels [23, 27, 33, 37, 38, 47]. Two studies found no significant association between sex and *Alu* methylation [38, 40] while two other studies found a significant association for higher *Alu* methylation in males than in females [42, 48]. One study showed a significant association for higher 5-mdC levels in males than in females [43]. In summary, of the 22 studies with data on sex and estimates of global DNA methylation no relationship was reported for eight of the studies, and significant inverse relationship was reported for fourteen studies.
Table 1: Summary of number of reports by risk factor and global methylation measure.

| Factors                        | Total # of reports | LINE-1 | Alu | 5-mdC |
|-------------------------------|--------------------|--------|-----|-------|
| Demographic Factors           |                    |        |     |       |
| Age                           | 31                 | 21     | 6   | 4     |
| Sex                           | 22                 | 17     | 4   | 1     |
| Race/ethnicity                | 6                  | 5      | 0   | 1     |
| Education                     | 7                  | 6      | 0   | 1     |
| Lifestyle factors             |                    |        |     |       |
| Physical activity             | 5                  | 5      | 0   | 0     |
| Alcohol                       | 17                 | 13     | 3   | 1     |
| Smoking                       | 22                 | 16     | 4   | 2     |
| Dietary factors               |                    |        |     |       |
| BMI                           | 17                 | 13     | 3   | 1     |
| Vegetables                    | 11                 | 11     | 0   | 0     |
| Fruit                         | 6                  | 6      | 0   | 0     |
| Folate                        | 13                 | 12     | 0   | 1     |
| Reproductive factors          |                    |        |     |       |
| Menopause status              | 2                  | 1      | 0   | 1     |
| Fetal birthweight             | 1                  | 1      | 0   | 0     |
| Family history of breast cancer | 6                | 4      | 1   | 1     |
| Age at menarche               | 1                  | 0      | 0   | 1     |
| Age at first birth            | 1                  | 0      | 0   | 1     |
| Parity                        | 1                  | 0      | 0   | 1     |
| Hormones                      | 4                  | 3      | 0   | 1     |

3.1.3. Race/Ethnicity. Five studies investigated the association between LINE-1 methylation and race/ethnicity (Table 2). Non-Hispanic Blacks had a significantly lower LINE-1 level compared to non-Hispanic Whites [36] in one study, whereas the reverse was observed in two studies [28, 34]. Two other studies showed no significant association between race/ethnicity and LINE-1 levels [11, 37]. In summary, of the six studies with data on race/ethnicity and estimates of global DNA methylation no relationship was reported for three of the studies, non-Hispanic Blacks had a significantly lower global DNA methylation compared to non-Hispanic Whites in one of the studies, and the inverse relationship was reported for two of the studies.

3.1.4. Education. As shown in Table 2, all six studies examining LINE-1 that included education as a risk factor reported no significant association between the levels of education attained and LINE-1 levels [21, 27, 29, 34, 36]. None of the studies examining Alu methylation included education as a risk factor. Only one 5-mdC study included education and it reported no significant association between the levels of education attained and 5-mdC levels [1]. In summary, of the seven studies with data on education and estimates of global DNA methylation no relationship was reported for any of the seven studies.

3.2. Lifestyle Factors

3.2.1. Physical Activity. Five studies have investigated the association between physical activity and LINE-1 levels (Table 3). Four studies found no significant difference between physical activity and LINE-1 levels [24, 34, 37, 50] whereas one study reported that higher physical activity was associated with higher DNA methylation levels [49]. No studies examined the association between physical activity and Alu or 5-mdC. In summary, of the five studies with data on physical activity and estimates of global DNA methylation no relationship was reported for four of the studies and a positive relationship was found in one study.

3.2.2. Alcohol. As shown in Table 3, thirteen studies examined LINE-1 methylation and alcohol consumption. None of the thirteen studies reported a significant relationship between alcohol and LINE-1 levels [11, 21, 27–29, 32–34, 36–38, 47]. Three studies found no significant association between alcohol and Alu methylation [38, 40, 41]. Additionally, of the only study that examined 5-mdC and alcohol consumption, there was no significant association between alcohol and 5-mdC levels [1]. In summary, of the 17 studies with data on alcohol and estimates of global DNA methylation no relationship was reported for any of the 17 studies.

3.2.3. Smoking. As shown in Table 3, sixteen studies examined the relationship between LINE-1 and smoking and all but one of the studies reported no significant association between LINE-1 level and smoking habits [11, 21, 26–30, 32–36, 38, 47]. All four studies examining Alu found no association between smoking and Alu levels [38, 40–42], and both studies involving 5-mdC found no significant association between smoking and 5-mdC levels [1, 44]. In summary, of the 22 studies with data on smoking and estimates of global DNA methylation no relationship was reported for
| Authors | Methylation Type | Measurement Method | Study Participants | Findings | Comments |
|---------|------------------|--------------------|--------------------|----------|----------|
| Agodi et al., 2015 [21] | LINE-1 | Pyrosequencing | 177 women aged 13–50, Helsinki | No differences | |
| Bollati et al., 2009 [22] | LINE-1 | Pyrosequencing | 718 individuals aged 55–92 from the Boston Area Normative Aging Study | No differences | |
| Chalitchagorn et al., 2004 [23] | LINE-1 | COBRA PCR | 32 individuals ranging in age, Thailand | No differences | |
| Duggan et al., 2014 [24] | LINE-1 | Pyrosequencing | 300 overweight women aged 50–75 in the US | No differences | |
| El-Maarri et al., 2011 [25] | LINE-1 | Pyrosequencing, SIRPH | 500 individuals aged 18–64, Bonn, Germany | No differences | |
| Gomes et al., 2012 [26] | LINE-1 | ELISA | 126 individuals aged 60–88, Brazil | No differences | |
| Hou et al., 2010 [27] | LINE-1 | Pyrosequencing | 421 individuals aged 21–79 in Warsaw, Poland | No differences | |
| Hsiung et al., 2007 [28] | LINE-1 | COBRA PCR | 765 individuals aged 18–75, Greater Boston Metropolitan Area | No differences | |
| Karami et al., 2015 [29] | LINE-1 | Pyrosequencing | PLCO - 436 controls from individuals aged 55–74 in the US, ATBC - 575 controls from individuals aged 55–69 in Finland | PLCO: No differences ATBC: significant difference between age groups ($p < 0.001$) | |
| Liao et al., 2011 [30] | LINE-1 | Pyrosequencing | 654 individuals aged 20–79 from the Central and Eastern European Renal Cancer Study (CEERCC) | No differences | |
| Authors                   | Methylation Type | Measurement method | Study participants                                                                 | Findings                  | Comments                                       |
|---------------------------|------------------|--------------------|-------------------------------------------------------------------------------------|---------------------------|-----------------------------------------------|
| Marques-Rocha et al., 2016 [31] | LINE-1           | MS-HRM             | 156 individuals aged 19–27, Brazil                                                 | No differences            |                                               |
| Mirabello et al., 2010 [32] | LINE-1           | Pyrosequencing     | 314 individuals aged 12–75+ from the NCI Clinical Genetics Branch Familial TGTC Study in the US | No differences            | Adjusted for sex                               |
| Pearce et al., 2012 [33]   | LINE-1           | Pyrosequencing     | 228 individuals aged 49–51 from Newcastle, England                                  | No differences            |                                               |
| Perng et al., 2014 [34]    | LINE-1           | Pyrosequencing     | 987 adults aged 45–84 from the Multi-Ethnic Study of Atherosclerosis (MESA), NY & LA | No differences            |                                               |
| Wilhelm et al., 2010 [35]  | LINE-1           | Pyrosequencing     | 465 individuals aged 25–74, from NH                                                | No differences            |                                               |
| Xu et al., 2012 [11]       | LINE-1           | Pyrosequencing     | 1101 women aged 20–98, from The Long Island Breast Cancer Study Project              | No differences            |                                               |
| Zhang et al., 2011 [36]    | LINE-1           | Pyrosequencing     | 161 individuals aged 45–75 from the North Texas Healthy Heart Study                 | No differences            |                                               |
| Zhang et al., 2012 [37]    | LINE-1           | Pyrosequencing     | 165 individuals aged 18–78 from the COMIR (Commuting Mode and Inflammatory Response) study, NY | No differences            |                                               |
| Zhu et al., 2012 [38]      | LINE-1           | Pyrosequencing     | 1465 individuals total from a combination of 5 individual studies across MA; Warsaw, Poland; Milan, Italy; Brescia, Italy; Trissino, Italy | No differences            |                                               |
| Bollati et al., 2009 [22]  | Alu              | Pyrosequencing     | 718 individuals aged 55–92 from the Boston Area Normative Aging Study               | Significant differences ($p = 0.012$) between age groups | Increased age associated with an average 0.2 5-mdC percentage decrease |
| Fraga et al., 2005 [39]    | Alu              | Total 5-mdC content: HPCE Sequence specific: bisulfite sequencing                 | 80 monozygotic twins aged 3–74, Spain                                           | Significant differences ($p < 0.05$) between age groups | Youngest pairs of MZ twins epigenetically similar, whereas oldest pairs clearly distinct |
| Authors                  | Methylation Type | Measurement method | Study participants                                                                 | Findings                                                                                                                                  | Comments                                                                                                                                       |
|-------------------------|------------------|--------------------|-------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------|
| Kim et al., 2010 [40]   | Alu              | Pyrosequencing     | 86 individuals aged 42–69, South Korea                                               | Significant differences ($p = 0.03$) between age groups                                                                                   | Statistically significant inverse association with DNA methylation. Adjusted for age                                                        |
| Na et al., 2014 [41]    | Alu              | Pyrosequencing     | 244 women aged 20–51, Korea                                                         | No differences                                                                                                                            | No differences                                                                                                                                |
| Rusiecki et al., 2008 [42] | Alu       | Pyrosequencing     | 70 individuals aged 39–67 from Greenland Inuit, Greenland                          | No differences                                                                                                                            | No differences                                                                                                                                |
| Zhu et al., 2012 [38]   | Alu              | Pyrosequencing     | 1465 individuals total from a combination of 5 individual studies across MA; Warsaw, Poland; Milan, Italy; Brescia, Italy; Trissino, Italy | No differences                                                                                                                            | No differences                                                                                                                                |
| Choi et al., 2009 [1]   | 5-mdC            | LC/ESI-MS/MS       | 180 women aged 35–75                                                                | No differences                                                                                                                            | No differences                                                                                                                                |
| Fraga et al., 2005 [39] | 5-mdC            | Total 5-mdC content: HPCE, Sequence specific: bisulfite sequencing                  | 80 monozygotic twins aged 3–74, Spain                                               | Significant differences ($p < 0.05$) between age groups                                                                                   | Youngest pairs of MZ twins epigenetically similar, whereas oldest pairs clearly distinct                                                     |
| Fuke et al., 2004 [43]  | 5-mdC            | HPLC               | 76 individuals aged 4–94                                                             | Significant differences ($p = 0.0002$) between age groups                                                                                  | Increased age associated with decreased methylation levels. Age 4–14 has 4.018% metC/dC + metC, age 16–22 has 4.03%, age 25–41 has 3.977%, and age 51–94 has 3.948% |
| Moore et al., 2008 [44] | 5-mdC            | HPCE, HpaII digest, densitometry                                                   | 397 individuals aged 20–81 from the Spanish Bladder Cancer Study, Spain              | No differences                                                                                                                            | Males had 84.2% average LINE-1 methylation%, Females had 83.5% average LINE-1 methylation%                                                   |
| Andreotti et al., 2014 [45] | LINE-1    | Pyrosequencing     | 676 individuals aged 55–74 from the Prostate, Lung, Colorectal, Ovarian Cancer Screening Trial (PLCO) in the US | Significant differences ($p = 0.0004$) between male and female                                                                     | Males had 84.2% average LINE-1 methylation%, Females had 83.5% average LINE-1 methylation%                                                   |
| Cash et al., 2012 [46]  | LINE-1           | Pyrosequencing     | 528 individuals aged 25–74 from the Residents Registry of the Shanghai Municipal Government, China | Significant differences ($p = 0.0004$) between male and female                                                                     | Males had 82.09 average LINE-1 methylation%, Females had 81.53% average LINE-1 methylation%                                                   |
| Authors               | Methylation Type | Measurement method | Study participants                  | Findings                                                                 | Comments                                                                 |
|----------------------|------------------|--------------------|-------------------------------------|---------------------------------------------------------------------------|---------------------------------------------------------------------------|
| Chalitchagorn et al., 2004 [23] | LINE-1           | COBRA PCR          | 32 individuals ranging in age, Thailand | No differences                                                           |                                                                           |
| El-Maarri et al., 2011 [25] | LINE-1           | Pyrosequencing, SIRPH | 500 individuals aged 18–64, Bonn, Germany | Significant differences $(p = 0.01)$ between male and female               | Average gender difference 0.94%                                           |
| Hou et al., 2010 [27] | LINE-1           | Pyrosequencing     | 421 individuals aged 21–79 in Warsaw, Poland | No differences                                                           |                                                                           |
| Hsiung et al., 2007 [28] | LINE-1           | Cobra PCR          | 765 individuals aged 18–75, Greater Boston Metropolitan Area | Significant differences $(p = 0.002)$ between "male" and "female"         | Not given; adjusted for age, race, smoking, alcohol, HPV serology, dietary folate, MTHFR |
| Karami et al., 2015 [29] | LINE-1           | Pyrosequencing     | PLCO, 436 controls from individuals aged 55–74 in the US | PLCO, Significant differences $(p < 0.0001)$ between male and female   | Males had 77.15% average LINE-1 methylation%, females had 76.58% average LINE-1 methylation% |
| Liao et al., 2011 [30] | LINE-1           | Pyrosequencing     | 654 individuals aged 20–79 from the Central and Eastern European Renal Cancer Study (CEERCC) | Significant differences $(p = 0.0003)$ between male and female         | Males had 81.97% average LINE-1 methylation%, females had 81.4% average LINE-1 methylation% |
| Mirabello et al., 2010 [32] | LINE-1           | Pyrosequencing     | 314 individuals aged 12–75+ from the NCI Clinical Genetics Branch Familial TGTC Study in the US | Significant differences $(p = 0.002)$ between male and female         | Males had 79.6% average LINE-1 methylation%, females had 78.87% average LINE-1 methylation%. Adjusted for age |
| Pearce et al., 2012 [33] | LINE-1           | Pyrosequencing     | 228 individuals aged 49–51 from Newcastle, England | No differences                                                           |                                                                           |
| Perng et al., 2014 [34] | LINE-1           | Pyrosequencing     | 987 adults aged 45–84 from the Multi-Ethnic Study of Atherosclerosis (MESA), NY & LA | Significant differences $(p = 0.0001)$ between male and female         | Males had 80.94% average LINE-1 methylation%, Females had 80.54% average LINE-1 methylation% |
| Authors                  | Methylation Type | Measurement method | Study participants                                             | Findings                                                                 | Comments                                                                                     |
|-------------------------|------------------|--------------------|-----------------------------------------------------------------|--------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|
| Rusiecki et al., 2008 [42] | LINE-1           | Pyrosequencing     | 70 individuals aged 19–67 from Greenlandic Inuit, Greenland    | Significant differences ($p = 0.02$) between male and female             | Males had 79.05% average LINE-1 methylation%, Females had 77.73% average LINE-1 methylation% |
| Tajuddin et al., 2013 [47] | LINE-1           | Pyrosequencing     | 892 individuals aged 20–81 from the Spanish Bladder Cancer/EPICURO study, Spain | No differences                                                          | Significant differences ($p = 0.02$) between male and female before Bonferroni correction  |
| Wilhelm et al., 2010 [35] | LINE-1           | Pyrosequencing     | 465 individuals aged 25–74, from NH                            | Significant differences ($p = 0.04$) between male and female             | Not given                                                                                     |
| Zhang et al., 2011 [36]  | LINE-1           | Pyrosequencing     | 166 individuals aged 45–75 from the North Texas Healthy Heart Study | Significant differences ($p = 0.0001$) between male and female           | Males had 75% average LINE-1 methylation%, Females had 73.2% average LINE-1 methylation%   |
| Zhang et al., 2012 [37]  | LINE-1           | Pyrosequencing     | 165 individuals aged 18–78 from the COMIR (Commuting Mode and Inflammatory Response) study, NY | No differences                                                          |                                                                                            |
| Zhu et al., 2012 [38]    | LINE-1           | Pyrosequencing     | 1465 individuals total from a combination of 5 individual studies across MA; Warsaw, Poland; Milan, Italy; Brescia, Italy; Trissino, Italy | No differences                                                          |                                                                                            |
| El-Maarri et al., 2007 [48] | Alu              | SIRPH              | 192 individuals aged 18–43, Bonn, Germany                      | Significant differences ($p < 0.0003$) between male and female           | Slightly higher methylation in males                                                           |
| Kim et al., 2010 [40]    | Alu              | Pyrosequencing     | 86 individuals aged 42–69, South Korea                         | No differences                                                          | Adjusted for age                                                                            |
| Authors                  | Methylation Type | Measurement method | Study participants                                                                 | Findings                                                                 | Comments                                                                 |
|-------------------------|------------------|--------------------|--------------------------------------------------------------------------------------|--------------------------------------------------------------------------|--------------------------------------------------------------------------|
| Rusiecki et al., 2008 [42] | Alu              | Pyrosequencing     | 70 individuals aged 19–67 from Greenlandic Inuit, Greenland                           | Significant differences ($p = 0.0001$) between male and female           | Males had 25.35% average Alu methylation%, Females had 24.69% average Alu methylation% |
| Zhu et al., 2012 [38]   | Alu              | Pyrosequencing     | 1465 individuals total from a combination of 5 individual studies across MA; Warsaw, Poland; Milan, Italy; Brescia, Italy; Trissino, Italy | No differences                                                          |                                                                          |
| Fuke et al., 2004 [43]  | 5-mdC            | HPLC               | 76 individuals aged 4–94                                                             | Significant differences ($p < 0.0067$) between male and female            | Males had metC/(dC + metC) = 4.01 ± 0.069, females had metC/(dC + metC) = 3.975 ± 0.067 |
| Hsiung et al., 2007 [28] | LINE-1           | Cobra PCR          | 765 individuals aged 18–75, Greater Boston Metropolitan Area                         | Significant differences ($p = 0.03$) between “non-Caucasian” and “Caucasian” | Not provided; Adjusted for age, sex, smoking, alcohol, HPV serology, dietary folate, MTHFR |
| Perng et al., 2014 [34] | LINE-1           | Pyrosequencing     | 987 adults aged 45–84 from the Multi-Ethnic Study of Atherosclerosis (MESA), NY & LA | Significant differences ($p = 0.008$) found between “Caucasian Whites”, “African-American Blacks”, and Hispanics | Caucasian Whites had 80.5% average LINE-1 methylation%, African-American Blacks had 80.84% average LINE-1 methylation%, Hispanics had 80.75% average LINE-1 methylation% |
| Xu et al., 2012 [11]    | LINE-1           | Pyrosequencing     | 1101 women aged 20–98, from The Long Island Breast Cancer Study Project               | No differences                                                          |                                                                          |
| Zhang et al., 2011 [36] | LINE-1           | Pyrosequencing     | 161 individuals aged 45–75 from the North Texas Healthy Heart Study                   | Significant differences ($p = 0.001$) found between “non-Hispanic Whites”, “non-Hispanic Blacks”, and Hispanics | Non-Hispanic Whites had 75.3% average LINE-1 methylation%, non-Hispanic Blacks had 73.1% average LINE-1 methylation%, Hispanics had 74% average LINE-1 methylation% |
| Authors                    | Methylation Type | Measurement method | Study participants                                                                 | Findings       | Comments         |
|---------------------------|------------------|--------------------|------------------------------------------------------------------------------------|----------------|------------------|
| Zhang et al., 2012 [37]   | LINE-1           | Pyrosequencing     | 165 individuals aged 18–78 from the COMIR (Commuting Mode and Inflammatory Response) study, NY | No differences |                  |
| Choi et al., 2009 [1]     | 5-mdC            | LC/ESI-MS/MS       | 180 women aged 35–75                                                               | No differences |                  |
| Agodi et al., 2015 [21]   | LINE-1           | Pyrosequencing     | 177 women aged 13–50, Helsinki                                                    | No differences |                  |
| Hou et al., 2010 [27]     | LINE-1           | Pyrosequencing     | 421 individuals aged 21–79 in Warsaw, Poland                                        | No differences |                  |
| Karami et al., 2015 [29]  | LINE-1           | Pyrosequencing     | PLCO - 436 controls from individuals aged 55–74 in the US, ATBC - 575 controls from individuals aged 55–69 in Finland | PLCO - No differences ATBC - No differences |                  |
| Perng et al., 2014 [34]   | LINE-1           | Pyrosequencing     | 987 adults aged 45–84 from the Multi-Ethnic Study of Atherosclerosis (MESA), NY & LA | No differences |                  |
| Zhang et al., 2011 [36]   | LINE-1           | Pyrosequencing     | 161 adults aged 45–75 from the North Texas Healthy Heart Study                      | No differences |                  |
| Choi et al., 2009 [1]     | 5-mdC            | LC/ESI-MS/MS       | 180 women aged 35–75                                                               | No differences |                  |
| Authors                  | Methylation Type | Measurement method | Study participants                                                                 | Findings                                                                 | Comments                                                                 |
|-------------------------|------------------|--------------------|-------------------------------------------------------------------------------------|---------------------------------------------------------------------------|--------------------------------------------------------------------------|
| Duggan et al., 2014 [24] | LINE-1           | Pyrosequencing     | 300 overweight women aged 50–75 in the US                                            | No differences                                                            |                                                                          |
| Perng et al., 2014 [34] | LINE-1           | Pyrosequencing     | 987 adults aged 45–84 from the Multi-Ethnic Study of Atherosclerosis (MESA), NY & LA | No differences                                                            |                                                                          |
| White et al., 2013 [49] | LINE-1           | Pyrosequencing     | 647 non-Hispanic white women aged 35–74 from the NIH sister study, USA               | Significant differences ($p = 0.04$) between “0” and “3” physical activity duration level above the median of physical activity | Physical activity levels of women greater than or equal to the median of physical activity at three time points (ages 5–12, 13–19 and currently) had higher global methylation compared to women with activity levels below the median for all three time periods ($\beta = .33$, 95% CI: .01, 0.66) |
| Zhang et al., 2011 [50] | LINE-1           | Methylight         | 161 individuals aged 45–75 from the North Texas Healthy Heart Study                  | No differences                                                            |                                                                          |
| Zhang et al., 2012 [37] | LINE-1           | Pyrosequencing     | 165 individuals aged 18–78 from the COMIR (Commuting Mode and Inflammatory Response) study, NY | No differences                                                            |                                                                          |
| Agodi et al., 2015 [21] | LINE-1           | Pyrosequencing     | 177 women aged 13–50, Helsinki                                                      | No differences                                                            |                                                                          |
| Hou et al., 2010 [27]   | LINE-1           | Pyrosequencing     | 421 individuals aged 21–79 in Warsaw, Poland                                         | No differences                                                            |                                                                          |
| Hsiung et al., 2007 [28] | LINE-1           | COBRA PCR          | 765 individuals aged 18–75, Greater Boston Metropolitan Area                         | No differences                                                            | Adjusted for age, sex, race, smoking, HPV serology, dietary folate, MTHFR |
| Authors                  | Methylation Type | Measurement method | Study participants                                                                 | Findings                        | Comments                  |
|--------------------------|------------------|--------------------|-------------------------------------------------------------------------------------|--------------------------------|--------------------------|
| Karami et al., 2015 [29] | LINE-1           | Pyrosequencing     | PLCO - 436 controls from individuals aged 55–74 in the US, ATBC, 575 controls from individuals aged 55–69 in Finland | PLCO - No differences          | ATBC - No differences    |
| Mirabello et al., 2010 [32] | LINE-1           | Pyrosequencing     | 344 individuals aged 12–75+ from the NCI Clinical Genetics Branch Familial TGTC Study in the US | No differences                  |                          |
| Pearce et al., 2012 [33] | LINE-1           | Pyrosequencing     | 228 individuals aged 49–51 from Newcastle, England                                   | No differences                  |                          |
| Tajuddin et al., 2013 [47] | LINE-1           | Pyrosequencing     | 892 individuals aged 20–81 from the Spanish Bladder Cancer/EPICURO study, Spain       | No differences                  |                          |
| Perng et al., 2014 [34]  | LINE-1           | Pyrosequencing     | 987 adults aged 45–84 from the Multi-Ethnic Study of Atherosclerosis (MESA), NY & LA | No differences                  |                          |
| Xu et al., 2012 [11]     | LINE-1           | Pyrosequencing     | 1101 women aged 20–98, from The Long Island Breast Cancer Study Project                | No differences                  |                          |
| Zhang et al., 2011 [36]  | LINE-1           | Pyrosequencing     | 161 individuals aged 45–75 from the North Texas Healthy Heart Study                   | No differences                  |                          |
| Zhang et al., 2012 [37]  | LINE-1           | Pyrosequencing     | 165 individuals aged 18–78 from the COMIR (Commuting Mode and Inflammatory Response) study, NY | No differences                  |                          |
| Zhu et al., 2012 [38]    | LINE-1           | Pyrosequencing     | 1465 individuals total from a combination of 5 individual studies across MA; Warsaw, Poland; Milan, Italy; Brescia, Italy; Trissino, Italy | No differences                  |                          |
| Zhu et al., 2012 [38]    | Au               | Pyrosequencing     | 1465 individuals total from a combination of 5 individual studies across MA; Warsaw, Poland; Milan, Italy; Brescia, Italy; Trissino, Italy | No differences                  |                          |
| Authors               | Methylation Type | Measurement method | Study participants                                                                 | Findings                                                                                           | Comments                                                                                     |
|----------------------|------------------|--------------------|-------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|
| Kim et al., 2010 [40]| Alu              | Pyrosequencing     | 86 individuals aged 42–69, South Korea                                             | No differences                                                                                   | Adjusted for age                                                                              |
| Na et al., 2014 [41] | Alu              | Pyrosequencing     | 244 women aged 20–51, Korea                                                         | No differences                                                                                   |                                                                                               |
| Choi et al., 2009 [1] | 5-mdC            | LC/ESI-MS/MS       | 180 women aged 35–75                                                               | No differences                                                                                   |                                                                                               |
| Agodi et al., 2015 [21] | LINE-1          | Pyrosequencing     | 177 women aged 13–50, Helsinki                                                     | No differences                                                                                   |                                                                                               |
| Andreotti et al., 2014 [45] | LINE-1   | Pyrosequencing     | 676 individuals aged 55–74 from the Prostate, Lung, Colorectal, Ovarian Cancer Screening Trial (PLCO) in the US | No difference for females. Significant differences ($p = 0.008$) between “Never” and “Ever” smokers for males | “Never” smoked had 84% average LINE-1 methylation% and “Ever” smoked had 83.6% average LINE-1 methylation% for males |
| Gomes et al., 2012 [26] | LINE-1          | ELISA              | 126 individuals aged 60–88, Brazil                                                  | No differences                                                                                   |                                                                                               |
| Hou et al., 2010 [27] | LINE-1          | Pyrosequencing     | 421 individuals aged 21–79 in Warsaw, Poland                                        | No differences                                                                                   |                                                                                               |
| Hsiung et al., 2007 [28] | LINE-1          | COBRA PCR          | 765 individuals aged 18–75, Greater Boston Metropolitan Area                        | No differences                                                                                   |                                                                                               |
| Karami et al., 2015 [29] | LINE-1          | Pyrosequencing     | PLCO - 436 controls from individuals aged 55–74 in the US                           | PLCO - No differences for females. Significant difference ($p = 0.02$) between smokers and nonsmokers for males | PLCO, males who had never smoked have an average 77.35% LINE-1 methylation%, and males who had ever smoked have an average 77.02% LINE-1 methylation% |
| Liao et al., 2011 [30] | LINE-1          | Pyrosequencing     | 654 individuals aged 20–79 from the Central and Eastern European Renal Cancer Study (CEERCC) | No differences                                                                                  |                                                                                               |
| Authors                  | Methylation Type | Measurement method | Study participants                                                                 | Findings                  | Comments                                      |
|--------------------------|------------------|--------------------|-------------------------------------------------------------------------------------|---------------------------|-----------------------------------------------|
| Mirabello et al., 2010   | LINE-1           | Pyrosequencing     | 314 individuals aged 12–75+ from the NCI Clinical Genetics Branch Familial TGTC Study in the US | No differences            |                                               |
| Pearce et al., 2012      | LINE-1           | Pyrosequencing     | 228 individuals aged 49–51 from Newcastle, England                                    | No differences            |                                               |
| Perng et al., 2014       | LINE-1           | Pyrosequencing     | 957 adults aged 45–84 from the Multi-Ethnic Study of Atherosclerosis (MESA), NY & LA | No differences            |                                               |
| Tajuddin et al., 2013    | LINE-1           | Pyrosequencing     | 892 individuals aged 20–81 from the Spanish Bladder Cancer/EPICURO study, Spain       | No differences            | Adjusted for age, sex, region                 |
| Wilhelm et al., 2010     | LINE-1           | Pyrosequencing     | 465 individuals aged 25–74, from NH                                                  | No differences            |                                               |
| Xu et al., 2012          | LINE-1           | Pyrosequencing     | 101 women aged 20–98, from The Long Island Breast Cancer Study Project                | No differences            |                                               |
| Zhang et al., 2011       | LINE-1           | Pyrosequencing     | 161 individuals aged 45–75 from the North Texas Healthy Heart Study                  | No differences            |                                               |
| Zhu et al., 2012         | LINE-1           | Pyrosequencing     | 1465 individuals total from a combination of 5 individual studies across MA; Warsaw, Poland; Milan, Italy; Brescia, Italy; Trissino, Italy | No differences            |                                               |
| Kim et al., 2010         | Alu              | Pyrosequencing     | 86 individuals aged 42–69, South Korea                                               | No differences            | Adjusted for age                             |
| Na et al., 2014          | Alu              | Pyrosequencing     | 244 women aged 20–51, Korea                                                          | No differences            |                                               |
| Rusiecki et al., 2008    | Alu              | Pyrosequencing     | 70 individuals aged 19–67 from Greenlandic Inuit, Greenland                           | No differences            |                                               |
| Zhu et al., 2012         | Alu              | Pyrosequencing     | 1465 individuals total from a combination of 5 individual studies across MA; Warsaw, Poland; Milan, Italy; Brescia, Italy; Trissino, Italy | No differences            |                                               |
| Authors | Methylation Type | Measurement method | Study participants | Findings | Comments |
|---------|-----------------|--------------------|--------------------|----------|----------|
| Choietal., 2009 [1] | 5-mdC | LC/ESI-MS/MS | 180 women aged 35–75 | No differences | |
| Moore et al., 2008 [44] | 5-mdC | HPCE, HpaII digest, densitometry | 397 individuals aged 20–81 from the Spanish Bladder Cancer Study, Spain | No differences | |
| BMI | | | | | |
| Agodi et al., 2015 [21] | LINE-1 | Pyrosequencing | 177 women aged 13–50, Helsinki | No differences | |
| Duggan et al., 2014 [24] | LINE-1 | Pyrosequencing | 300 overweight women aged 50–75 in the US | No differences | |
| Gomes et al., 2012 [26] | LINE-1 | ELISA | 126 individuals aged 60–88, Brazil | No differences | |
| Karami et al., 2015 [29] | LINE-1 | Pyrosequencing | PLCO, 436 controls from individuals aged 55–74 in the US, ATBC, 575 controls from individuals aged 55–69 in Finland | PLCO, no differences. ATBC, significant differences between 16.7–<25, 25–30, and 30–62.1 BMI 16.7–<25 had 79.00% average LINE-1 methylation%, BMI 25–30 had 78.73% average LINE-1 methylation%, and BMI 30–62.1 had 78.39% average LINE-1 methylation% | |
| Liao et al., 2011 [30] | LINE-1 | Pyrosequencing | 654 individuals aged 20–79 from the Central and Eastern European Renal Cancer Study (CEERCC) | No differences | |
| Marques-Rocha, 2016 [31] | LINE-1 | MS-HRM | 156 individuals aged 19–27, Brazil | No differences | |
| Pearce et al., 2012 [33] | LINE-1 | Pyrosequencing | 228 individuals aged 49–51 from Newcastle, England | No differences | Adjusted for sex |
| Pernget al., 2014 [34] | LINE-1 | Pyrosequencing | 987 adults aged 45–84 from the Multi-Ethnic Study of Atherosclerosis (MESA), NY & LA | No differences | |
| Tajuddin et al., 2013 [47] | LINE-1 | Pyrosequencing | 892 individuals aged 20–81 from the Spanish Bladder Cancer/EPICURO study, Spain | No differences | |
| Zhang et al., 2011 [36] | LINE-1 | Pyrosequencing | 161 individuals aged 45–75 from the North Texas Healthy Heart Study | No differences | |
| Authors | Methylation Type | Measurement method | Study participants | Findings | Comments |
|---------|-----------------|--------------------|--------------------|----------|----------|
| Zhang et al., 2012 [37] | LINE-1 | Pyrosequencing | 165 individuals aged 18–78 from the COMIR (Commuting Mode and Inflammatory Response) study, NY | No differences | In unadjusted models, there was a statistically significant difference ($p = 0.03$) |
| Zhu et al., 2012 [38] | LINE-1 | Pyrosequencing | 1465 individuals total from a combination of 5 individual studies across MA; Warsaw, Poland; Milan, Italy; Brescia, Italy; Trissino, Italy | No differences | |
| Kim et al., 2010 [40] | Alu | Pyrosequencing | 86 individuals aged 42–69, South Korea | No differences | Adjusted for age |
| Na et al., 2014 [41] | Alu | Pyrosequencing | 244 women aged 20–51, Korea | Significant difference ($p < 0.001$) between normal weight, overweight, and obese groups | Normal weight had 26.28 Alu methylation%, overweight had 24.95 Alu methylation%, normal weight had 25.96 Alu methylation% |
| Zhu et al., 2012 [38] | Alu | Pyrosequencing | 1465 individuals total from a combination of 5 individual studies across MA; Warsaw, Poland; Milan, Italy; Brescia, Italy; Trissino, Italy | No differences | |
| Choi et al., 2009 [1] | 5-mdC | LC/ESI-MS/MS | 180 women aged 35–75 | No differences | Vegetables |
| Agodi et al., 2015 [21] | LINE-1 | Pyrosequencing | 177 women aged 13–50, Helsinki | No differences | |
| Cash et al., 2012 [46] | LINE-1 | Pyrosequencing | 528 individuals aged 25–74 from the Residents Registry of the Shanghai Municipal Government, China | Significant differences ($p = 0.002$) between “<4 times/week” and “≥4 times/week” intake of total cruciferous vegetables in men, not significant in women | Men with “<4 times/week” intake of total cruciferous vegetables had 81.31 average LINE-1 methylation% and men with “≥4 times/week” intake of total cruciferous vegetables had 82.2 average LINE-1 methylation% |
### Table 3: Continued.

| Authors                      | Methylation Type | Measurement method | Study participants                                                                 | Findings                                                                                                                               | Comments                                                                                           |
|------------------------------|------------------|--------------------|--------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------|
| Duggan et al., 2014 [24]     | LINE-1           | Pyrosequencing     | 300 overweight women aged 50–75 in the US                                             | No differences                                                                                                                      |                                                                                                     |
| Hou et al., 2010 [27]        | LINE-1           | Pyrosequencing     | 421 individuals aged 21–79 in Warsaw, Poland                                          | No differences                                                                                                                      |                                                                                                     |
| Karami et al., 2015 [29]     | LINE-1           | Pyrosequencing     | PLCO - 436 controls from individuals aged 55–74 in the US, ATBC, 575 controls from individuals aged 55–69 in Finland | PLCO, No differences. ATBC, significant differences ($p = 0.01$) between <690.9 grams of vegetables per day and ≥690.6 grams of vegetables per day | <690.9 grams of vegetables per day have an average 78.64% LINE-1 methylation% and ≥690.6 grams of vegetables per day have an average 78.90% LINE-1 methylation% |
| Liao et al., 2011 [30]       | LINE-1           | Pyrosequencing     | 654 individuals aged 20–79 from the Central and Eastern European Renal Cancer Study (CEERCC) | No differences                                                                                                                      |                                                                                                     |
| Martin-Núñez et al., 2014 [51]| LINE-1          | Pyrosequencing     | 155 individuals aged 40–65 from Spain                                                 | LINE-1 methylation increased in the control group ($p = 0.001$) but decreased in the Mediterranean diet intervention group ($p = 0.003$) | The control group had 66.8 average LINE-1 methylation% and the intervention group had 63.6 average LINE-1 methylation% after one year. Adjusted for age, gender, BMI at baseline |
| Tajuddin et al., 2013 [47]   | LINE-1           | Pyrosequencing     | 892 individuals aged 20–81 from the Spanish Bladder Cancer EPICURO study, Spain       | No differences                                                                                                                      | Adjusted for age, sex, region, smoking status                                                        |
| Zhang et al., 2012 [37]      | LINE-1           | Pyrosequencing     | 165 individuals aged 18–78 from the COMIR (Commuting Mode and Inflammatory Response) study, NY | No differences                                                                                                                      | Data given in tertiles of methylation; women with <201 grams/day fruit intake had lower average LINE-1 methylation% than women with >201 grams/day fruit intake |
| Agodi et al., 2015 [21]      | LINE-1           | Pyrosequencing     | 177 women aged 13–50, Helsinki                                                        | Significant differences ($p = 0.022$) between fruit intake groups of <201 grams/day and <201 grams/day                              | Data given in tertiles of methylation; women with <201 grams/day fruit intake had lower average LINE-1 methylation% than women with >201 grams/day fruit intake |
| Authors                  | Methylation Type | Measurement method | Study participants                                      | Findings                                                                 | Comments                                                                 |
|-------------------------|------------------|--------------------|--------------------------------------------------------|--------------------------------------------------------------------------|---------------------------------------------------------------------------|
| Hou et al., 2010 [27]    | LINE-1           | Pyrosequencing     | 421 individuals aged 21–79 in Warsaw, Poland          | No differences                                                           |                                                                           |
| Karami et al., 2015 [29] | LINE-1           | Pyrosequencing     | PLCO, 436 controls from individuals aged 55–74 in the US, ATBC, 575 controls from individuals aged 55–69 in Finland | PLCO, No differences. ATBC, No differences                               |                                                                           |
| Tajuddin et al., 2013    | LINE-1           | Pyrosequencing     | 892 individuals aged 20–81 from the Spanish Bladder Cancer/EPICURO study, Spain | No differences                                                          | Adjusted for age, sex, region, smoking status                             |
| Zhang et al., 2012 [37]  | LINE-1           | Pyrosequencing     | 165 individuals aged 18–78 from the COMIR (Commuting Mode and Inflammatory Response) study, NY | No differences                                                          |                                                                           |
| Agodi et al., 2015 [21]  | LINE-1           | Pyrosequencing     | 177 women aged 13–50, Helsinki                       | Significant differences ($p = 0.027$) between folate deficient and non-folate deficient groups | Data given in tertiles of methylation; women with folate deficiency had lower average LINE-1 methylation% than women without folate deficiency |
| Bae et al., 2014 [52]    | LINE-1           | LC-MS/MS           | 408 women aged 50–79 from the WHI-OS cohort, throughout the US | Significant differences ($p = 0.05$) among different levels of RBC folate | Women in “highest RBC folate group” had 5.12 baseline LINE-1 methylation% and women in “lowest RBC folate group” had 4.99 baseline LINE-1 methylation% |
| Gomes et al., 2012 [26]  | LINE-1           | ELISA              | 126 individuals aged 60–88, Brazil                   | No differences                                                           |                                                                           |
| Hou et al., 2010 [27]    | LINE-1           | Pyrosequencing     | 421 individuals aged 21–79 in Warsaw, Poland          | No differences                                                           |                                                                           |
| Hsiung et al., 2007 [28] | LINE-1           | COBRA PCR          | 765 individuals aged 18–75, Greater Boston Metropolitan Area | No differences                                                           | Adjusted for age, sex, race, smoking, alcohol, HPV serology, MTHFR       |
| Authors                    | Methylation Type | Measurement method           | Study participants                                                                 | Findings                                                                                     | Comments                                                                                   |
|----------------------------|------------------|------------------------------|-------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------|
| Karami et al., 2015 [29]   | LINE-1           | Pyrosequencing               | PLCO, 436 controls from individuals aged 55–74 in the US, ATBC, 575 controls from individuals aged 55–69 in Finland | PLCO, No differences. ATBC, No differences                                                   |                                                                                           |
| Perng et al., 2014 [34]    | LINE-1           | Pyrosequencing               | 987 adults aged 45–84 from the Multi-Ethnic Study of Atherosclerosis (MESA), NY & LA  | No differences                                                                               |                                                                                           |
| Tajuddin et al., 2013 [47] | LINE-1           | Pyrosequencing               | 892 individuals aged 20–81 from the Spanish Bladder Cancer/EPICURO study, Spain      | No differences                                                                               | Adjusted for age, sex, region                                                             |
| Xu et al., 2012 [11]       | LINE-1           | Pyrosequencing               | 1101 women aged 20–98, from The Long Island Breast Cancer Study Project               | No differences                                                                               |                                                                                           |
| Zhang et al., 2011 [36]    | LINE-1           | Pyrosequencing               | 161 individuals aged 45–75 from the North Texas Healthy Heart Study                   | No differences                                                                               |                                                                                           |
| Zhang et al., 2012 [37]    | LINE-1           | Pyrosequencing               | 165 individuals aged 18–78 from the COMIR (Commuting Mode and Inflammatory Response) study, NY | Significant differences ($p = 0.007$) among different levels of dietary folate from fortified foods | Dietary folate from fortified foods, μg/1,000 kJ spearman value 0.21                      |
| Moore et al., 2008 [44]    | 5-mdC            | HPCE, HpaII digest, densitometry | 397 individuals aged 20–81 from the Spanish Bladder Cancer Study, Spain               | No differences                                                                               |                                                                                           |
| Xu et al., 2012 [11]       | LINE-1           | Pyrosequencing               | 1101 women aged 20–98, from The Long Island Breast Cancer Study Project               | No differences                                                                               |                                                                                           |
| Choi et al., 2009 [1]      | 5-mdC            | LC/ESI-MS/MS                 | 180 women aged 35–75                                                                   | No differences                                                                               |                                                                                           |
| Authors                        | Methylation Type | Measurement method | Study participants                                                                 | Findings                                                                 | Comments                                                                 |
|-------------------------------|------------------|--------------------|-------------------------------------------------------------------------------------|--------------------------------------------------------------------------|--------------------------------------------------------------------------|
| **Fetal Birthweight**          |                  |                    |                                                                                      |                                                                          |                                                                          |
| Michelset al., 2011 [53]       | LINE-1           | Pyrosequencing     | 319 mother-child dyads from Brigham and Women's Hospital, Boston                     | Significant differences between low birthweight \( p = 0.007 \) and high birthweight \( p = 0.036 \) compared to normal birthweight infants | \"Low birthweight, <2500 g\" had a \(-0.82\) change in LINE-1 methylation% and \"High birthweight, 4000+ g\" had a \(-0.43\) change in LINE-1 methylation% |
| **Family history of breast cancer** |                  |                    |                                                                                      |                                                                          |                                                                          |
| Brennan et al., 2012 [9]       | LINE-1           | Pyrosequencing     | 769 individuals aged 23–83 from 3 cohorts, USA                                       | No differences                                                            |                                                                          |
| Delgado-Cruzata et al., 2014 [54] | LINE-1           | MethylLight        | 333 unaffected women who had a sister with breast cancer from the Breast Cancer Family Registry, NY | No differences                                                            |                                                                          |
| Wu et al., 2011 [55]           | LINE-1           | Pyrosequencing, MethylLight | 51 girls aged 6–17, USA                                                             | No differences                                                            |                                                                          |
| Xu et al., 2012 [11]           | LINE-1           | Pyrosequencing     | 1101 women aged 20–98, from The Long Island Breast Cancer Study Project               | No differences                                                            |                                                                          |
| Wu et al., 2011 [55]           | Alu              | MethylLight        | 51 girls aged 6–17, USA                                                             | Significant differences \( p < 0.05 \) between family history and no family history | Family history had 151.4 average Alu methylation% while no family history had 169.8 average Alu methylation% |
| Choi et al., 2009 [1]          | 5-mdC            | LC/ESI-MS/MS       | 180 women aged 35–75                                                                | No differences                                                            |                                                                          |
| Age at Menarche                |                  |                    |                                                                                      |                                                                          |                                                                          |
| Choi et al., 2009 [1]          | 5-mdC            | LC/ESI-MS/MS       | 180 women aged 35–75                                                                | No differences                                                            |                                                                          |
| Age at first birth             |                  |                    |                                                                                      |                                                                          |                                                                          |
| Choi et al., 2009 [1]          | 5-mdC            | LC/ESI-MS/MS       | 180 women aged 35–75                                                                | No differences                                                            |                                                                          |
| Parity                         |                  |                    |                                                                                      |                                                                          |                                                                          |
| Choi et al., 2009 [1]          | 5-mdC            | LC/ESI-MS/MS       | 180 women aged 35–75                                                                | No differences                                                            |                                                                          |
| Hormone Cycle                  |                  |                    |                                                                                      |                                                                          |                                                                          |
| El-Maarri et al., 2011 [25]    | LINE-1           | Pyrosequencing, SIRPH | 500 individuals aged 18–64, Bonn, Germany                                            | No differences                                                            |                                                                          |
| Sex Hormones                   |                  |                    |                                                                                      |                                                                          |                                                                          |
| Iwasaki et al., 2012 [56]      | LINE-1           | LUMA               | 185 women aged 55–74, Japan                                                         | No differences                                                            |                                                                          |
| Authors            | Methylation Type | Measurement method | Study participants                                    | Findings                  | Comments |
|--------------------|------------------|--------------------|------------------------------------------------------|---------------------------|----------|
| Ulrich et al., 2012 [57] | LINE-1           | Pyrosequencing     | 173 women aged 55–75 from the Physical Activity for Total Health Study | No differences           |          |
| Choi et al., 2009 [1]  | 5-mdC            | LC/ESI-MS/MS       | 180 women aged 35–75                                  | No differences           |          |
21 of the studies and a significant inverse relationship was reported for one study with smokers having lower DNA methylation.

3.3. Dietary Factors

3.3.1. BMI. A total of thirteen studies have examined the relationship between BMI and LINE-1 levels (Table 3). Twelve studies reported no relationship [21, 24, 26, 29–31, 33, 34, 36–38, 47] while one study found that a higher BMI was statistically significantly associated with a lower LINE-1 level [29]. Two studies found no significant association between BMI and Alu methylation [38, 40] while one study found that a higher BMI was significantly associated with a lower Alu methylation level [41]. One study found no relationship between BMI and 5-mdC levels [1]. In summary, of the 17 studies with data on BMI and estimates of global DNA methylation no relationship was reported for 15 of the studies, and significant inverse relationship was reported for two studies.

3.3.2. Vegetables. As shown in Table 3, eight studies conducted found no relationship between vegetable intake and LINE-1 levels [21, 24, 27, 29, 30, 37, 47]. Two other studies showed a significant association between lower vegetable intake and lower LINE-1 methylation [29, 46]. One study found a significant association between higher adherence to a Mediterranean diet and lower LINE-1 levels [51]. In summary, of the 11 studies with data on vegetables and estimates of global DNA methylation no relationship was reported for eight of the studies, and significant positive relationship was reported for three studies.

3.3.3. Fruit. Six studies investigated the relationship between fruit intake and global white blood cell DNA methylation levels (Table 3). Five studies found no significant association between levels of fruit intake and LINE-1 levels [27, 29, 37, 47]. One study found that, in women, there was a significant association between lower fruit intake and lower LINE-1 levels [21]. In summary, of the six studies with data on fruit and estimates of global DNA methylation no relationship was reported for five of the studies, and significant positive relationship was reported for one study in women.

3.3.4. Folate. As shown in Table 3, ten studies reported no significant relationship between dietary folate intake and LINE-1 levels [11, 26–29, 34, 36, 44, 47]. Two studies reported a statistically significant positive correlation between higher blood folate levels and higher LINE-1 levels [37, 52]. However, in one of the same studies, folate intake from natural foods and total dietary folate equivalents were not found to be associated with higher LINE-1 levels [37]. Another study reported that women with a folate deficiency had a statistically significantly lower LINE-1 level [21]. In summary, of the 13 studies with data on folate and global DNA methylation estimates no relationship was reported for ten of the studies and a significant positive relationship was reported for three of the studies.

3.4. Health History and Reproductive Factors

3.4.1. Menopause Status. As shown in Table 3, one study examined the relationship between menopausal status and LINE-1 methylation and did not find a significant association [11]. Another study did not find a significant association between menopausal status and 5-mdC levels [1].

3.4.2. Fetal Birthweight. A cross-sectional study investigated the relationship between fetal birthweight and LINE-1 levels and found a significant association between low (<2500 g) or high (4000+ g) birthweight and lower LINE-1 levels of the newborn [53] (Table 3).

3.4.3. Family History of Breast Cancer. Four studies reported no relationship between family history of breast cancer and LINE-1 levels [9, 11, 54, 55] (Table 3). Family history of breast cancer was unrelated to 5-mdC levels in another study [1]. However, one study did find a relationship between family history of breast cancer and lower Alu levels [55]. In summary, of the six studies with data on family history and estimates of global DNA methylation no relationship was reported for five of the studies, and significant inverse relationship was reported for one study.

3.4.4. Age at Menarche. One study found no association between the age at menarche and 5-mdC level [1] (Table 3).

3.4.5. Age at First Birth/Parity. As seen in Table 3, one study did not find a significant association between the age at first live birth or parity, and 5-mdC level [1].

3.4.6. Endogenous Hormones/Hormone Use. All four studies found no statistically significant association between LINE-1 levels and sex hormone levels [56, 57] or between LINE-1 levels and phase of the menstrual cycle [25] (Table 3). The only study that evaluated 5-mdC levels did not find a significant association between 5-mdC and hormone use [1].

4. Discussion

There was reasonably consistent evidence across studies that males have higher levels of global methylation in WBC DNA than females. There was little evidence across studies that age was associated with global methylation in WBC DNA but the populations studied were generally restricted to older adults. Age has been reported to be associated with WBC DNA LINE-1 methylation in a study that evaluated epigenetic changes throughout the lifetime of monozygotic twins [39]. None of the other demographic, lifestyle, dietary, or other risk factors were consistently associated with global WBC DNA methylation.

There are several factors that warrant consideration in interpreting the existing data on the associations between WBC DNA methylation and breast cancer risk factors. Nearly all the published studies used a composite of DNA from different subtypes of WBCs. As previously noted by others [18, 58], DNA methylation can vary by WBC subtype and
the distribution of WBC subtypes varies among individuals, which could possibly obscure associations. In addition, the type and method of assessing WBC DNA methylation differed across studies, which could potentially contribute to variation in results across studies. Another possible explanation for the general lack of association with breast cancer risk factors is that the assays used may not be optimal. However, the fact that global WBC DNA methylation levels appear to be slightly lower among women than men when measured by any of the three assays tends to suggest that laboratory measurement error is not the entire explanation. Finally, studies are generally cross-sectional in design [18], and for some of the risk factors examined the number of studies was quite limited. Overall, however, it seems unlikely that these considerations account for the consistently null findings observed.

4.1. Conclusion. In summary, with the exception of sex, there is very little evidence that the wide range of breast cancer risk factors we examined (demographic, lifestyle, dietary, and health conditions) were associated with global WBC DNA methylation markers including LINE-1, 5-mdC, and Alu. Although the possibility that global DNA methylation reflects a novel breast cancer risk factor cannot be ruled out on the basis of these findings, a plausible implication of the observed lack of association between global WBC DNA methylation and most known or suspected breast cancer risk factors is that the association between global WBC DNA methylation and breast cancer, if it exists, is due to a disease effect [16, 59].

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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