Association Between BMI and DNA Methylation in Blood or Normal Adult Breast Tissue: A Systematic Review

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Abstract. *Background/Aim:* Several studies have investigated the influence of obesity on DNA methylation (DNAm) to find biomarkers associated with the detection of chronic diseases, including breast cancer. The aim of the study was to systematically review studies examining the association of body mass index (BMI) and DNAm in blood or normal breast tissue. *Materials and Methods:* Three scientific literature databases (PubMed, Embase and Web of Science) were screened until May 2018. *Results:* Twenty-four studies were included along with ours in which we investigated this relation in the normal breast tissue of 40 breast cancer patients. *Conclusion:* BMI-associated CpG sites were highly variable with few identified in less than half of the studies. Nevertheless, a few genes potentially associated with BMI were highlighted in blood (CPT1A, ABCG1, SREBF1 and LGALS3BP) and in normal breast tissue (PTPRN2 and ABLIM2). The variability of the results could be explained by the tissue and cell-specificity of methylation and differences in methodology.

According to the World Health Organization (WHO), overweight and obesity are defined as “abnormal or excessive

fat accumulation that presents a risk to health” and are commonly measured by body mass index (BMI) calculated by dividing a person’s weight (in kilograms) by height (in metres squared). Thus, an adult with a BMI equal to or more than 25 kg/m² and lower than 30 kg/m² is considered overweight, and an adult with a BMI of 30 kg/m² or more is generally considered obese (1). In 2013, about 37% of the worldwide adult population were overweight or obese (2). These numbers and the fact that its prevalence has doubled in the past decades (3) sets obesity as a major public health concern. Indeed, overweightness and obesity are major risk factors for several chronic diseases such as diabetes, cardiovascular diseases and various cancers. Consequently, the overweight and obesity epidemic presents a significant challenge for the prevention of these diseases, including breast cancer.

Obesity is a well-known risk factor for breast cancer. Indeed, a well-established association has been recognized between overweight/obesity and an increased risk of postmenopausal hormone receptor-positive breast cancer (RR: 1.82; 95% confidence interval [CI], 1.55-2.14) (4). In contrast, being overweight/obese is associated with a lower risk of premenopausal hormone receptor-positive breast cancer (RR: 0.80; 95% CI, 0.70-0.92) (4) while normal body weight has a reported protective effect (5). Obesity is not only a breast cancer risk factor, but it is also a prognostic factor (6). In a meta-analysis of 82 studies on BMI and survival in women with breast cancer, BMI was significantly associated with breast cancer mortality regardless of menopausal status (RR: 1.35; 95% CI, 1.24-1.47 for obese women, 22 studies; and RR: 1.11, 95% CI, 1.06-1.17 for overweight women, 21 studies) (7). However, recent findings suggest that these associations may be limited to hormone receptor-positive breast cancer patients (8). To date, breast

This article is freely accessible online.

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Key Words: EWAS, normal breast tissue, blood, DNA methylation, obesity, body mass index, breast cancer, epigenetic biomarkers, review.

cancer is the most common cancer in women worldwide (9, 10), affecting over 1.5 million women each year. In 2012, more than 520,000 deaths were attributed to breast cancer (11). Several studies have been conducted with the aim of identifying causes of breast cancer to enhance prevention and treatment. Research on the relationship between breast cancer and obesity at genetic and epigenetic levels is a potential source of discovery.

Tumor progression is driven by a sequence of randomly occurring genetic mutations and epigenetic DNA alterations affecting the genes controlling cell proliferation, survival and other traits associated with a malignant cell phenotype (12). In contrast to classic genetic variations, epigenetic variations involve a few cellular reversible mechanisms that alter the information and interpretation of the genome without changing its nucleotide sequence (13). More importantly, the epigenome is tissue-specific and cell-specific. These characteristics contribute to the diverse phenotypes and expression patterns seen in different cell types (14). One of the most studied epigenetic mechanisms in humans is DNA methylation (DNAm) (15). DNAm is a mechanism for controlling gene expression (16), which occurs when a methyl group is added to the fifth carbon of cytosine (C), forming 5-methylcytosine (5mC). This mechanism is catalyzed by DNA methyltransferase (17), predominantly at cytosines within CG dinucleotides ('CpG' sites) in mammalian genomes. CpG rich regions in proximity of genes are called CpG islands and higher methylation in these regions is often associated with reduced expression of the nearby gene due to chromatin rearrangement, inhibition of transcription activators and/or recruitment of transcription repressors (18, 19). DNAm is mainly associated with gene silencing when this occurs at gene promoters and enhancers, and with active gene expression when established within gene bodies (20). For instance, in cancer, alterations in DNAm include global hypomethylation of the genome accompanied by CpG island hypermethylation (15) that inactivates tumor suppressor genes. DNAm modifications are assumed to provide a link between environmental exposure and clinical phenotypes and are therefore suspected of contributing to the unexplained heritability of cancers (21). Regarding environmental exposure, obesity is highly heritable, but genetic variants seem to explain only part of the variation in heritability (22). DNAm alterations may explain part of the missing variability. Identification of obesity-related DNAm changes in adults may provide new insights into the mechanisms linking obesity to breast cancer and may provide new biomarkers for early prevention or research treatment.

Although DNAm is tissue- and cell-specific, studies generally assess DNAm in the blood instead of primary targeted tissues because these are often difficult to obtain. Blood then may serve as a surrogate tissue for breast tissue

when measuring DNA methylation. The aim of this study was to conduct a systematic review to identify all available studies on BMI and DNAm in blood or normal breast tissue using an epigenome-wide approach to summarize and discuss the current state of knowledge. This review also includes new data on the association between BMI and DNAm in the normal breast tissue of women diagnosed with an estrogen receptor-positive, non-metastatic breast cancer.

Materials and Methods

A systematic review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) protocol.

Search methods for identification of studies. We systematically searched three scientific literature databases (PubMed, Embase and Web of Science) until May 7th, 2018, for studies in humans of the association between BMI and DNAm in blood or normal adult breast tissue. Combinations of the following terms were used "methylation" and "genome-wide association study" or "epigenome-wide association study" and "overweight" or "obesity" or "body mass index", and duplicates were removed. Studies had to be fully published, available as full-text and written in English.

Screening of unique studies identified. We reviewed titles and abstracts of the studies identified by the three databases. Records, where only the abstract was available, were excluded.

Inclusion criteria. We included studies that met the following criteria (1) association analysis between BMI and DNAm was assessed, (2) at an epigenome-wide scale, (3) with DNAm measured in blood or normal breast tissue (4) in adults.

Exclusion criteria. We excluded studies according to the following criteria 1) abstract/full-text not available, 2) not in English, 3) not an analysis of the association between methylation and BMI, 4) not genome-wide, 5) not in humans, 6) not in adults, 7) preselection of common sites for the analysis and 8) correlation analysis of common DNA methylation between blood and adipose tissue.

Selection of studies. References identified by the search strategy were reviewed by one author (D. Dragic) in a two-step process. First, titles and abstracts were screened to exclude clearly non-eligible studies. Then, full-text articles were assessed for eligibility based on selection criteria. Whenever required, a second review author (C. Diorio or SL Chang) was consulted. We also included new results from our study in this review.

Data extraction. We extracted information about the study population (sample size, sex, disease status, mean age, mean BMI), DNA source (blood or normal breast tissue), technique used to measure DNAm, statistical analysis (parametric or not, robust or not), variables used for adjustment, assessment of BMI (by a trained person or self-reported), BMI treatment in the analysis (categorical or continuous), correction used to account for multiple tests and number of BMI-associated CpG sites and genes. Data were extracted several times over three months to ensure their reliability.

Figure 1. PRISMA flow diagram representing the selection process of the articles, which met our inclusion criteria (Epigenome-wide association analysis of body mass index and DNA methylation in blood or normal adult breast tissue).

Data synthesis. Considering that high heterogeneity between studies was expected, quantitative synthesis of data was not considered appropriate. Instead, we adopted a qualitative systematic review approach to investigate the relationship between BMI and DNAm in blood or normal breast tissue.

Regarding our study, 40 women were selected from a cohort of 757 diagnosed with a first invasive, non-metastatic, estrogen receptor-positive breast cancer between 2000 and 2007 and followed at the Centre des Maladies du Sein (CMS) in Quebec City, Quebec. Normal breast tissue located at more than one centimetre of the tumor was obtained, and gene methylation was assessed using the Illumina HumanMethylation450 BeadChip array (Illumina Inc., San Diego, CA, USA). Statistical analysis was performed with R statistical software (Version 3.4.3). To detect global methylation differences between normal weight (BMI <25 kg/m²) and overweight/obese (BMI ≥25 kg/m²) women, we first compared median M-values with Wilcoxon-Mann-Whitney test and mean M-values with Student's *t*-test. Robust linear regression was then used to detect differentially methylated positions with methylation levels (M-value) as the dependent variable and BMI as the independent variable. Box-Cox transformation (23) was applied to BMI values, which were not normally distributed (Shapiro-Wilk test *p*-

value=3.9×10⁻⁴), using the MASS package. The model was adjusted for age (*a priori*).

Results

Results of the search. We screened titles and abstracts of 908 unique references. Thirty-three studies were selected for full-text review. Of these, 23 studies met our inclusion criteria, as well as the present study. Thus, in total, 24 studies were included in this systematic review (Figure 1).

Description of studies. Characteristics of included studies are summarized in Table I. The 24 studies involved 32 to 5,387 participants, and 23 were published between 2010 and 2018. Twenty studies included breast cancer-free individuals. Of these, two included only women (13, 25), 16 included both men and women (24, 26-40), one was a meta-analysis of three cohorts (KORA, LOLIPOP, EPICOR) (41) and another a meta-analysis of two cohorts (FHS, LBC) (42). Three studies had mixed breast cancer patients and breast cancer-free

Table I. Summary of characteristics of EWASs.

First author (year) (Reference No)	Country	Discovery sample			BMI	Measurement	Statistical analysis	LDA ¹	Adjustment co-variables	Correction for multiple tests	Number of CpG (genes)
		Size (n)	Mean age (year)	Mean BMI (kg/m ²)							
DNA methylation measured in blood (n=21)											
Xu (2018) (24)	USA	378/ 132*	27.9 32.2	24.5 34.7	Measured Cat	450K	Linear regression	Yes ²	Age, sex, race, smoking status, years of education, first 30PC	FDR	20 (15)
Campanella (2018) (43)	Europe	1,941 [#]	53.8 [#]	25.7 [#]	Measured Reported Cont	450K	Meta-analysis of four cohorts	No	Batch and array position, sex, age at blood draw, case-control status	Bonferroni	26 (21)
Wilson (2017) (44)	USA	573	54.7	27.0	Measured Cat	27K	Robust linear regression	Yes ²	PC (plate, bisulfite conversion intensities and age), breast cancer status	FDR	2 (2)
		871	55.2	27.1		450K				FDR	4 (4)
		181	50.4	26.7		FDR				23 (17)	
Wahl (2017) (41)	Europe	5,387 [#]	54.5 [#]	27.7 [#]	Measured Reported Cont	450K	Meta-analysis by inverse variance of three cohorts	Yes ²	Age, sex, smoking status, physical activity index, alcohol consumption, 20PC	Bonferroni	278 (214)
Sayols- Baixeras (2017) (26)	Spain	3,156 [#]	65.6 [#]	28.1 [#]	Measured Cont	450K	Fixed-effects meta-analyses of two cohorts	Yes ²	Age, sex, smoking exposure and surrogate variables	Bonferroni	92 (71)
Mendelson (2017) (42)	USA, Scotland	3,743 [#]	69.2 [#]	27.9 [#]	Measured Cont	450K	Meta-analysis of two cohorts	Yes ^{3,4}	Age, sex	Bonferroni	135 (105)
Meeks (2017) (27)	Ghanaians individuals	547	50.5	26.7	Measured Cont	450K	Linear regression	Yes ²	Age, sex, recruitment site, technical effects (batch and array position), first PC	FDR	18 (18)
Geurts (2017) (28)	Australia	5,361 [#]	60.5 [#]	27.0 [#]	Measured Cont	450K	Fixed-effects meta-analysis of two cohorts	Yes ²	Age, sex, country of birth, smoking status, sample type. Study, plate, and chip as random effects	Bonferroni	310 (256)
Crujeiras (2017) (29)	Spain	10/ 45*	27.4 37.4	23.0 36.2	Measured Cat	450K	Non-parametric Wilcoxon rank sum tests	No	-	FDR	4,815 (3,828)
Al Muftah (2016) (30)	Qatar, UK	123	37.9°	28.7°	NA Cont	450K	Linear mixed model	Yes ²	Age, sex, type II diabetes status, smoking status	Bonferroni	0
Shah (2015) (31)	Europe	1,366	72.6	27.3	Measured Cont	450K	Linear regression	Yes ³	Age, sex, technical variables (plate, array, array position)	Bonferroni	9 (8) 5 (5)
		752	45.5	25.4							
Ollikainen (2015) (32)	Finland	30 30 twin	27 27	19.7-40.6 24.2-48.6	Measured Cat	450K	Linear regression	No	Smoking status	FDR	0
Mansego (2015) (33)	Spain	73	45	34.6	Measured Cat	450K	Linear regression	Yes ²	Gender, smoking, metabolic syndrome, research group that made each study and batch effect	FDR	0

Table I. Continued

Table I. *Continued*

First author (year) (Reference No)	Country	Discovery sample			BMI	Measurement	Statistical analysis	LDA ¹	Adjustment co-variables	Correction for multiple tests	Number of CpG (genes)
		Size (n)	Mean age (year)	Mean BMI (kg/m ²)							
Huang (2015) (34)	USA	16/ 16*	44.4 47.9	21.9 34.5	Reported Cat	450K	Linear regression	Yes ²	-	FDR	0
Demerath (2015) (35)	USA	2,097	56.2	30.1	Measured Cont	450K	Linear mixed- effect regression	Yes ²	Chip specified as a random effect and sex, age, study center, total WBC, education, household income, current cigarette smoking, current alcohol consumption, leisure time physical activity and 10 PCs as fixed effect	Bonferroni	76 (60)
Aslibekyan (2015) (36)	USA	991	49.0	28.0	Measured Cont	450K	Linear mixed model	Yes ⁵	Age, sex, study site, T-cell purity, smoking, family structure and first four CD4 ⁺ T-cell purity PCs	Bonferroni	8 (4)
Agha (2015) (37)	USA	106 [#]	47	31.4 [#]	Measured Cont	450K	Linear regression	Yes ²	Sex, race, current smoking	-	0
Dick (2014) (38)	Europe	459 [#]	55.2 [#]	27.2 [#]	NA Cont	450K	Linear regression	No	Age, sex, smoking status, methylation array batch, centre	FDR	5 (5)
Almen (2014) (13)	Latvia	22/ 24*	55.0 57.0	22.0 35.0	Reported Cat	27K	Robust linear model	No	-	FDR	1 (1)
Carless (2013) (39)	USA	183	42.2	32.4	NA Cont	Golden Gate	Polygenic regression models	No	Sex, age, age ² and their interactions	Bonferroni	0
Feinberg (2010) (40)	Iceland	V6: 48 V7: 64	74.1 82.8	26.6 26.0	NA Cont	CHARM	For each stable VMR: linear regression model	No	-	FDR ^A	4 VMR
DNA methylation measured in normal breast tissue (n=3)											
Dragic (2020)	Canada	40	51.0	24.2	Reported Cont	450K	Robust linear regression	-	Age	FDR	6 (6)
Johnson (2017) (25)	USA	100	37.2	27.6	Reported Cont	450K	Multivariable linear model	-	Differences in cellular mixtures	FDR ^B	0
Hair (2015) (45)	USA	81	19-84	22NW 26OW 33OB	Reported Cont	450K	Robust linear regression	-	Age, race, alcohol use	FDR	2,573 (1,987)

Discovery sample column: *n not obese/n obese; [#]Data from meta-analysis are combined; [°]Mean between women's data and men's data; NW stands for normal weight, OW for overweight, OB for obese and V for visit. LDA column: ¹Leukocyte distribution adjustment; ²The influence of leukocyte composition was adjusted using the algorithm described by Houseman *et al.* (80); ³The influence of leukocyte composition was adjusted by subtype cell count; ⁴The influence of leukocyte composition was adjusted using SVA function; ⁵CD4⁺ T-cell purity was estimated using the methods described by Abbas *et al.*, and Houseman *et al.* Adjustment co-variables column: PC: principal component, WBC: white blood cells. BMI column: Information on the BMI variable: if it was measured by a trained person, if it was reported by the participant or if it was not available (NA); and if it was used as a categorical (cat) or continuous (cont) variable. Correction for multiple tests column: FDR for false discovery rate and fixed at $q < 0.05$; ^A $q < 0.1$; ^B $q < 0.01$. Identified number of CpG (genes) column: VMR: variably methylated region.

participants: one was a meta-analysis of four studies (EPIC-Italy, EnviroGenoMarkers, NOWAC, EPIC-Netherlands) that included both men and women (43), another was derived from the Sister Study cohort which included only healthy women at enrollment with a sister with breast cancer (44), and finally, one that included only women (45). Our study included only female patients with breast cancer.

The mean age of included participants varied. Two studies reported mean age under 30 years (24, 32), eight included participants with a mean age of 30-50 years (25, 29, 30, 33, 34, 36, 37, 39), nine others included participants with a mean age of 50-60 years [(13, 27, 35, 38, 41, 43-45) and the present study], and the last five had participants with a mean age above 60 years (26, 28, 31, 40, 42). The forty study participants from our study ranged in age from 33 to 69 years, with a mean age of 51.0 (standard deviation [SD] ± 8.2) years.

Out of the 24 studies, five classified participants as non-obese (mean BMI between 21.9 and 24.5 kg/m²) and obese (mean BMI between 34.5 and 36.2 kg/m²) (13, 24, 29, 34, 44), one only involved overweight/obese participants with mean BMI of 34.6 kg/m² (33), one had BMI-discordant twin pairs with a leaner twin (BMI between 19.7 and 40.6 kg/m²) and a heavier twin (BMI between 24.2 and 48.6 kg/m²) (32) and the remaining 17 had participants with mean BMI that varied between 25.4 and 32.4 kg/m². In our study, we classified participants as either having a normal weight (NW: BMI <25 kg/m²; n=27) or being overweight/obese (OWOB: BMI $\geq$ 25 kg/m²; n=13). Characteristics are presented in Table II. No significant differences were observed between the two groups, except for BMI. BMI was 21.8 $\pm$ 1.9 kg/m² (mean $\pm$ SD) in NW women and 29.3 $\pm$ 4.7 kg/m² in OWOB women.

In total, 21 of the 24 epigenome-wide studies used the HumanMethylation 450K BeadChip (Illumina) [(24-38, 41-45) and the present study]. The other three used the Illumina 27K (13) (450K predecessor), the Illumina GoldenGate Assay for Methylation (39) and the CHARM technique (40). All three were the oldest studies identified in the review.

Statistical analysis to detect BMI-related differentially methylated loci varied across studies. Thirteen studies used non-robust linear regression (24, 25, 27, 30-38, 40), four used robust linear regression [(13, 44, 45) and the present study], one used non-parametric tests (29) and one used polygenic regression models (39). Five studies conducted meta-analysis (26, 28, 41-43), thus combining several cohorts (sample size ranging between 106 and 5,387 individuals). Given that methylation is influenced by several exposures, models were adjusted for multiple variables, including age, sex, ethnicity, smoking status, disease status, physical activity index and alcohol consumption. Most studies adjusted for age [(16 studies (24, 26-28, 30, 31, 35, 36, 38, 39, 41-45) and the present study]. Eight studies also adjusted for technical variables (plate, array, array position) to correct for batch effects (27, 28, 31, 33, 35, 38, 43, 44).

Table II. Characteristics of participants in study groups.

	BMI <25 kg/m ² n=27	BMI $\geq$ 25 kg/m ² n=13
Age (years)	50.0 $\pm$ 7.5	53.0 $\pm$ 9.5
BMI (kg/m ²)	21.8 $\pm$ 1.9	29.3 $\pm$ 4.7
Smokers (ever)	11 (41%)	8 (62%)
Menopausal status		
Premenopausal	12 (44%)	4 (31%)
Postmenopausal	15 (56%)	9 (69%)
Parity (Yes)	16 (59%)	10 (77%)
Age at first pregnancy (years)	25.4 $\pm$ 5.1	27.3 $\pm$ 5.3
Breastfeeding (Yes)	9 (56%)	4 (40%)
Family history of breast cancer (Yes)	21 (78%)	11 (85%)
Histological type		
Ductal, invasive	26 (96%)	12 (92%)
Lobular, invasive	1 (4%)	1 (8%)
Tumor size (mm)	20.9 $\pm$ 8.0	23.5 $\pm$ 13.7
Stage		
I	11 (41%)	5 (38.5%)
II	13 (48%)	7 (53.8%)
III	3 (11%)	1 (7.7%)
Tumor grade		
1	6 (22.2%)	5 (38.5%)
2	12 (44.4%)	5 (38.5%)
3	7 (26%)	2 (15%)
Indeterminate	2 (7.4%)	1 (8%)
PR status		
Positive	18 (67%)	12 (92%)
Negative	9 (33%)	1 (8%)
HER2 status		
Not evaluated	4 (15%)	2 (15%)
Positive	2 (7%)	0
Negative	21 (78%)	11 (85%)
Radiotherapy (Yes)	23 (85%)	12 (92%)
Adjuvant chemotherapy (Yes)	21 (78%)	9 (69%)
Adjuvant hormone therapy (Yes)	27 (100%)	13 (100%)

Data are mean $\pm$ sd, n (%)

Concerning DNA samples, 21 studies measured DNAm in blood, three assessed DNAm in normal breast tissue [(25, 45) and the present study]. In these three studies, two used tissue samples majorly composed of epithelial cells [(25) and the present study], and one involved tissue specimens sampled $\geq$ 4 cm from breast tumor margins but lacked cell composition information [(45) and the present study]. Since blood contains several cell types, 14 of the 21 blood studies accounted for the proportion of each cell type (24, 26-28, 30, 31, 33-37, 41, 42, 44).

Up to now, only one of the three breast tissue studies assessed the association between global methylation and obesity, and results are presented in Table III. No significant differences were found between the two groups: median M-values of 0.439 (OWOB) and 0.462 (NW) (Wilcoxon-Mann-Whitney test: $p=0.424$) and mean M-values of 0.056 (OWOB) and 0.056 (NW) (Student's t -test: $p=0.959$).

Table III. (A) Median and (B) mean M-values by CpG island localisation.

A

Localisation	Nb of CpG loci	Median M-values NW	Median M-values OWOB	Wilcoxon <i>p</i> -value
Total	397,315	0.462	0.439	0.424
Island	133,333	-2.289	-2.295	0.862
Shelves	35,816	1.768	1.787	0.820
Shores	93,343	0.104	0.073	0.076
OpenSea	134,823	1.616	1.635	0.758

Nb: number; NW : Normal weight; OWOB: overweight/obese.

B

Localisation	Nb of CpG loci	Mean M-values NW	Mean M-values OWOB	T-test
Total	397,315	0.056	0.056	0.959
Island	133,333	-1.585	-1.585	0.730
Shelves	35,816	1.545	1.551	0.867
Shores	93,343	0.021	0.013	0.233
OpenSea	134,823	1.304	1.308	0.873

Table IV. BMI-associated CpG sites passing the false discovery rate ($q < 0.05$).

CpG sites	Chromosome	Gene	Gene region	CpG island region	β coefficient*	FDR
cg03724010	1	C1orf70	Body	N_Shore	88.51	0.018
cg07695909	2	NCKAP5	Body	OpenSea	84.77	0.049
cg04718733	4	ABLIM2	Body	OpenSea	67.88	0.049
cg02439266	7	HOXA3	5'UTR; Body	Island	-76.43	0.049
cg18894200	7	PTPRN2	Body	Island	-93.40	0.049
cg14585892	8	LY6D	TSS200	OpenSea	68.72	0.049

* β coefficient is the methylation (m-value) change associated with one unit increase in Box-Cox transformed-BMI.

However, borderline significant hypomethylation was observed in the CpG Island shores in OWOB compared to NW women (median M-values of 0.073 (OWOB) and 0.104 (NW); $p=0.076$).

The number of BMI-associated CpG sites identified differed in each of the three studies carried out in the normal breast tissue, including one with none (25), one with 2,573 sites (45), and ours with 6 CpG sites identified. Table IV lists the six CpG sites significantly associated with BMI at FDR q -value < 0.05 after adjustment for age. These sites were located at different loci on different genes and chromosomes (Chr): *C1orf70* (cg03724010) on Chr1, *NCKAP5* (cg07695909) on Chr2, *ABLIM2* (cg04718733) on Chr4 and *LY6D* (cg14585892) on Chr8, except for *HOXA3* (cg02439266) and *PTPRN2* (cg18894200) both positioned on Chr7. DNAm was positively associated with BMI at CpG sites cg03724010, cg07695909, cg04718733 and cg14585892, and negatively associated with BMI at cg02439266 and cg18894200.

No identical CpG sites were observed between the three studies, but BMI-associated CpG sites were found at different locations in *PTPRN2* (cg26337914, cg18894200) and *ABLIM2* (cg00861207, cg04718733) in two of them [(45) and the present study]. *PTPRN2* loci cg26337914 and cg18894200 were both hypomethylated with increased BMI.

The number of BMI-associated CpG sites identified in the 21 blood studies ranged from 0 to 4,815. Of these, only two evaluated the association between global methylation and obesity but did not highlight differences between non-obese and obese participants. In total, 5,537 unique BMI-associated CpG sites were identified in 14 studies that assessed the number of differentially methylated positions (13, 24, 26-29, 31, 35, 36, 38, 41-44). Six studies showed no association between BMI and DNAm after multiple testing corrections (30, 32-34, 37, 39) and one analysed variably methylated regions (VMR) (40). Among the 5,537 BMI-associated CpG sites identified, 177 were found in more than one study. The

cg00574958 locus, located in the *CPT1A* gene on Chr11, was the most frequent position identified and was consistently hypomethylated with increased BMI (24, 27, 28, 35, 36, 41-43). On the other hand, *ABCG1* locus cg06500161 on Chr21 (28, 31, 35, 41-44), *SREBF1* locus cg11024682 (28, 31, 35, 41-43) and *LGALS3BP* locus cg04927537 (27, 28, 35, 41-43), both located on Chr17, were consistently hypermethylated with increased BMI. There were also several BMI-associated CpG sites found at other gene locations. Two CpG sites, cg00574958 (24, 27, 28, 35, 36, 41-43) and cg17058475 (28, 41, 42) in the *CPT1A* gene were identified in several studies and were hypomethylated across studies with increased BMI. Four CpG sites in *ABCG1* were also identified in several studies, cg06500161 (28, 31, 35, 41-44), cg27243685 (24, 35, 41-43), cg01881899 (41, 42) and cg10192877 (31, 41, 42). All these sites were hypermethylated with increased BMI. Six CpG sites in the *LGALS3BP* gene were identified in several studies including cg04927537 (27, 28, 35, 41-43), cg25178683 (27, 28, 35, 41, 42), cg11202345 (28, 41, 42), cg14870271 (41, 42, 44), cg17836612 (41, 42) and cg27470213 (29, 42). All were hypermethylated with increased BMI, except cg27470213, which was hypermethylated in one study (29) and hypomethylated in another (42).

Discussion

We systematically reviewed the literature for all studies assessing the association between BMI and DNAm quantified either in the blood or in normal breast tissue. To our knowledge, this is the first systematic review to report BMI-related DNAm changes occurring in various genes. Twenty-four articles were included in this review, mostly conducted in blood (n=21), with only three using breast tissue, including our study. Among the 24 studies, only three assessed the link of global methylation with obesity and no association was found [(13, 29) and the present study]. In the three studies involving breast tissue, BMI-associated DNAm changes notably implicated two genes, *PTPRN2* and *ABLIM2*. Although CpG sites were not identical in all studies, DNAm changes at other loci in *PTPRN2* and *ABLIM2* were observed in two [(45) and the present study]. Recently, *PTPRN2* has been identified as a possible obesity susceptibility gene (46), and its tumor expression was reported as a novel candidate biomarker and therapeutic target in estrogen receptor-positive breast cancer (47). Concerning the *ABLIM2* gene, little is known besides its potential role in lung cancer metastasis (48) and regulation by estradiol (49). These findings suggest that BMI is associated with DNAm in normal breast tissue in estrogen receptor-positive breast cancer patients. More specifically, we found that higher BMI may affect DNAm of *PTPRN2* gene. Although none of the 5,537 unique CpG sites identified

in blood (13, 24, 26-29, 31, 35, 36, 38, 41-44) overlapped with those found in breast tissue, one study identified three BMI-associated CpG sites in the *PTPRN2* gene (29). However, these results should be interpreted separately as findings in breast tissue are different from those in blood. Nevertheless, the implication of DNAm of the *PTPRN2* gene in obesity deserves more attention.

Genes (n=105) containing one of the 177 BMI-associated CpG sites that were reported in several blood studies are implicated in cellular growth and proliferation, cellular development and cell death and survival. Of these, *CPT1A* (cg00574958), *ABCG1* (cg06500161), *SREBF1* (cg11024682) and *LGALS3BP* (cg04927537) had one CpG site frequently identified in several studies. Other CpG sites located in *CPT1A* (cg17058475), *ABCG1* (cg27243685, cg01881899 and cg10192877) and *LGALS3BP* (cg25178683, cg11202345, cg14870271, cg17836612 and cg27470213) were also identified in some of the studies. In the literature, the expression of *CPT1A*, *ABCG1*, *SREBF1* and *LGALS3BP* has been associated with obesity (50-54) and linked to various cancers (55-62), including breast cancer (63-69) adding plausibility to the findings. Indeed, *CPT1A* was recently found to be up-regulated in co-cultured adipocytes isolated from human breast adipose tissue with hormone receptor-positive or -negative breast cancer cells (70). Altogether, these findings support the notion that BMI is associated with the expression of *CPT1A*, *ABCG1*, *SREBF1* and *LGALS3BP* in normal tissue among breast cancer patients.

The main strength of this review includes the use of an exhaustive search strategy following a rigorous methodology to identify all available studies. Because the vast majority of studies used the Illumina HumanMethylation450 BeadChipArray and BMI was primarily measured and not reported, it was easier to compare data since studies had a similar methodology. It is also important to highlight that most studies in blood accounted for the proportion of each cell type, thus allowing the comparison of the results. The originality of this review is the inclusion of our study data, added to identify obesity-related DNAm changes among breast cancer patients. Our study used normal breast tissues to quantify DNA methylation. More precisely, our tissues were rich in epithelial cell content thereby decreasing the risk of bias due to cell type variability. Our six CpG sites found associated with BMI are biologically plausible. These six sites were located in *C1orf70*, *NCKAP5*, *ABLIM2*, *HOXA3*, *PTPRN2* and *LYD6*. In the literature, DNAm of *PTPRN2* and *HOXA3* gene expression have both been associated with obesity (71, 72). Furthermore, DNAm of *PTPRN2*, *HOXA3*, *C1orf70* and expression of *PTPRN2*, *ABLIM2*, *NCKAP5* and *LYD6* have been linked to various cancers (48, 73-78) including breast cancer (47, 79).

Limitations include the small number of studies quantifying DNAm in normal breast tissue and their small

sample sizes. Most studies measured DNAm in blood. This is mainly because blood is easier to obtain than other tissues. However, results found in blood and breast tissues cannot be compared because they contain different cell types, each having a characteristic methylation profile, which can confound DNAm associations with the outcome. Limitations for studies in this systematic review include the differences in the statistical analysis used and adjustment variables. Concerning our study, subjects were all diagnosed with an estrogen receptor-positive breast cancer, potentially limiting the generalizability of our results. Nevertheless, since obesity was found to be associated with estrogen receptor-positive breast cancer risk and mortality, our study was conducted as a first step in identifying obesity-related DNAm changes that could potentially be used as biomarkers for early prevention or treatment of breast cancer.

Conclusion

Out of the three breast tissue studies from the systematic review, two BMI-associated CpG sites in genes *PTPRN2* and *ABLIM2* were identified. Among these, *PTPRN2* has been previously associated with obesity and reported as a potential therapeutic target in breast cancer. Further investigations of the effect of obesity on breast tissue epithelial cells are needed to understand their relation with cancer-associated pathways. In blood, our systematic review of the association between BMI and DNAm highlighted a few genes potentially associated with BMI (*CPT1A*, *ABCG1*, *SREBF1* and *LGALS3BP*). These genes were also associated with breast cancer in other studies. Taken together, further validation in independent large cohorts will be needed to confirm that these identified BMI-associated genes are potential biomarkers for early prevention and treatment of breast cancer.

Authors' Contributions

Conceived and designed the experiments: CD. Performed the experiments: KEI AM. Analyzed the data: DD CD SLC. Wrote the paper: DD CD SLC. Manuscript review: FD KEI AM.

Conflicts of Interest

The Authors have no conflicts of interest to declare regarding this study.

Acknowledgements

The Authors thank the participants for their generosity and for providing samples. The Authors also thank E. Issa for DNA extraction from the normal breast tissue. This work was supported by grants from the Canadian Cancer Society (Grant # 702501) and the Fondation du cancer du sein du Québec and the Banque de tissus et données of the Réseau de recherche sur le cancer of the

Fond de recherche du Québec – Santé (FRQS), associated with the Canadian Tumor Repository Network (CTRNet). KEI holds a Vanier Canada Graduate Scholarship from the Canadian Institutes of Health Research. CD holds a Senior Investigator Award from the FRQS.

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Received February 6, 2020

Revised February 21, 2020

Accepted February 25, 2020