University Library

MINERVA ACCESS

A gateway to Melbourne's research publications

Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:
Li, S;Dugué, PA;Baglietto, L;Severi, G;Wong, EM;Nguyen, TL;Stone, J;English, DR;Southey, MC;Giles, GG;Hopper, JL;Milne, RL

Title:
Genome-wide association study of peripheral blood DNA methylation and conventional mammographic density measures

Date:
2019-10-01

Citation:
Li, S., Dugué, P. A., Baglietto, L., Severi, G., Wong, E. M., Nguyen, T. L., Stone, J., English, D. R., Southey, M. C., Giles, G. G., Hopper, J. L. & Milne, R. L. (2019). Genome-wide association study of peripheral blood DNA methylation and conventional mammographic density measures. International Journal of Cancer, 145 (7), pp.1768-1773. https://doi.org/10.1002/ijc.32171.

Persistent Link:
https://hdl.handle.net/11343/285437

Check for updates

Li Shuai (Orcid ID: 0000-0002-8696-8594)
Stone Jennifer (Orcid ID: 0000-0001-5077-0124)

Genome-wide association study of peripheral blood DNA methylation and conventional mammographic density measures

Shuai Li[1,2*], Pierre-Antoine Dugué[1,3,4*], Laura Baglietto[5], Gianluca Severi[1,3,6], Ee Ming Wong[4,7], Tuong L. Nguyen[1], Jennifer Stone[8], Dallas R. English[1,3], Melissa C. Southey[4,7], Graham G. Giles[1,3], John L. Hopper[1,3†‡], and Roger L. Milne[1,3,4†]

1. Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Australia
2. Centre for Cancer Genetic Epidemiology, Department of Public Health and Primary Care, University of Cambridge, UK
3. Cancer Epidemiology and Intelligence Division, Cancer Council Victoria, Australia
4. Precision Medicine, School of Clinical Sciences at Monash Health, Monash University, Australia
5. Department of Clinical and Experimental Medicine, University of Pisa, Italy
6. Centre de Recherche en Épidémiologie et Santé des Populations (INSERM U1018), Université Paris-Saclay, Université Paris-Sud, Université Versailles Saint-Quentin-en-Yvelines, Institut Gustave Roussy, Villejuif, France
7. Genetic Epidemiology Laboratory, Department of Clinical Pathology, The University of Melbourne, Australia
8. Centre for Genetic Origins of Health and Disease, Curtin University and the University of Western Australia, Australia

[*]These authors contributed equally to this work

[†]These authors contributed equally to this work

[‡]Corresponding author

Address: Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, 207 Bouverie Street, Carlton, Victoria 3053, Australia

Email: j.hopper@unimelb.edu.au

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/ijc.32171

This article is protected by copyright. All rights reserved.

Author Manuscript

Telephone: +61 3 8344 0697

Author Manuscript

This article is protected by copyright. All rights reserved.

Key words

DNA methylation, epigenetics, mammographic density, breast cancer

List of abbreviations

AMDTSS: Australia Mammographic Density Twins and Sisters Study. BMI: Body mass index. GWAS: Genome-wide association study. HM450: HumanMethylation450 BeadChip. MCCS: Melbourne Collaborative Cohort Study. OPERA: Odds per adjusted standard deviation.

Article category

Cancer Genetics and Epigenetics

Novelty and impact

This study is the first genome-wide association analysis of DNA methylation and mammographic density measures that predict breast cancer risk. For over 1,000 middle-aged Australian women, blood DNA methylation was measured using the HumanMethylation450 BeadChip assay, and mammographic density (dense area, non-dense area, and percentage dense area) defined by the conventional brightness threshold was measured using the CUMULUS software. This study did not find evidence of an association between blood DNA methylation and the conventional mammographic density measures.

This article is protected by copyright. All rights reserved.

Author Manuscript

Author Manuscript

Abstract

Age- and body mass index (BMI)-adjusted mammographic density is one the strongest breast cancer risk factors. DNA methylation is a molecular mechanism that could underlie inter-individual variation in mammographic density. We aimed to investigate the association between breast cancer risk-predicting mammographic density measures and blood DNA methylation. For 436 women from the Australian Mammographic Density Twins and Sisters Study and 591 women from the Melbourne Collaborative Cohort Study, mammographic density (dense area, non-dense area and percentage dense area) defined by the conventional brightness threshold was measured using the CUMULUS software, and peripheral blood DNA methylation was measured using the HumanMethylation450 (HM450) BeadChip assay. Associations between DNA methylation at >400,000 sites and mammographic density measures adjusted for age and BMI were assessed within each cohort and pooled using fixed-effect meta-analysis. Associations with methylation at genetic loci known to be associated with mammographic density were also examined. We found no genome-wide significant ($P<10^{-7}$) association for any mammographic density measure from the meta-analysis, or from the cohort-specific analyses. None of the 299 methylation sites located at genetic loci associated with mammographic density was associated with any mammographic density measure after adjusting for multiple testing (all $P>0.05/299 = 1.7 \times 10^{-4}$). In summary, our study did not detect associations between blood DNA methylation, as measured by the HM450 assay, and conventional mammographic density measures that predict breast cancer risk.

This article is protected by copyright. All rights reserved.

Author Manuscript

Introduction

Mammographic density has conventionally been defined as the white or bright region on a mammographic image of the breast. Mammographic density declines with increasing age and body mass index (BMI). After adjustment for age and BMI, mammographic density has been found to be one of the strongest risk factors for breast cancer in terms of differentiating cases from controls: age- and BMI-adjusted mammographic density has an odds per adjusted standard deviation (OPERA) of approximately 1.4, which is comparable to other strong breast cancer risk factors such as polygenic risk scores[1]. Women with more than 75% dense breast tissue have a four- to five-fold increased breast cancer risk compared with women of the same age and BMI with less than 10% dense breast tissue[2].

Variation across the population in mammographic density has both environmental and genetic determinants. Age, BMI, and other breast cancer risk factors explain about 30% of the variation in mammographic density, and over 60% of the residual variation appears to be accounted for by additive genetic factors[3]. Genome-wide association studies (GWAS) have identified variants at specific genetic loci associated with mammographic density[4, 5], and about 15% of the genetic variants known to be associated with breast cancer risk are also associated with mammographic density measures[6], although they explain only a small fraction of the latter. Despite these known determinants, the molecular mechanisms underlying variation in mammographic density are not well understood, nor is how mammographic density translates into breast cancer risk at the biological level[7].

DNA methylation, a process whereby typically a methyl group is added to a cytosine-guanine dinucleotide, plays an important role in modulating gene expression without changing the underlying DNA sequence. There is increasing evidence that blood DNA methylation is associated with variation in traits or disease risks, and also considerable interest in determining if there is any relationship between blood DNA methylation and breast cancer risk[8]. We tested the hypothesis that blood DNA methylation underlies inter-individual variation in breast cancer risk-predicting mammographic density measures using data for middle-aged women from two Australian cohorts.

Material and methods

Sample

The study sample included 436 women from the Australian Mammographic Density Twins and Sisters Study (AMDTSS) and 591 women from the Melbourne Collaborative Cohort

This article is protected by copyright. All rights reserved.

Study (MCCS). Included participants from the two cohorts had, on average, similar characteristics (Table 1).

The AMDTSS is a twin family cohort study of mammographic density[9]. Between 2004 and 2009, twins aged 40-70 years who participated in the Australian Twin Study of Mammographic Density between 1995 and 1999 were asked to participate further, and their non-twin sisters were also invited to participate. Participants completed questionnaires through telephone-administered interviews, and gave blood samples and permission to access their mammograms. A total of 479 women from 130 families oversampled for having one or more participating sisters were selected for DNA methylation research[10]. Among them, 436 women also had mammographic density data available and were included in this analysis. All women were of European descent.

The MCCS is a prospective cohort of 41,513 adults (24,469 women) aged between 27 and 76 years (99.3% aged 40-69) when recruited between 1990 and 1994[11]. Participants completed questionnaires in face-to-face interviews, had clinical measures taken by trained staff and gave a blood sample, the latter collected as dried blood spots on Guthrie cards, as peripheral blood mononuclear cells or as buffy coats. All MCCS participants were of European descent, including 25% born in Italy or Greece. Blood DNA methylation was measured for 6,404 participants in one of six nested-case control studies for breast cancer, colorectal cancer, mature B cell neoplasms, urothelial cell carcinoma, kidney cancer, or lung cancer[12, 13]. In the breast cancer study, only women who included as controls were selected, to avoid the possibility of collider bias[14]. A total of 591 women who had mammographic density and DNA methylation data available were included in this analysis.

DNA methylation

DNA was extracted from peripheral blood samples and methylation was measured using the Illumina Infinium HumanMethylation450 (HM450) BeadChip and following the same protocol for both cohorts [10, 13]. DNA was extracted from dried blood spots stored on Guthrie cards using a previously reported method[15], and from peripheral blood mononuclear cells and buffy coat specimens using QIAamp mini spin columns (Qiagen, Hilden, Germany). Bisulfite conversion was performed using EZ DNA Methylation-Gold single-tube kit (Zymo Research, Irvine, CA) according to the manufacturer's instructions. A total of 200ng of bisulfite-converted DNA was whole-genome amplified and hybridised onto HM450 BeadChips. DNA methylation was assessed according to manufacturer's instructions. Raw intensity data was processed using the Bioconductor *minfi* package. Illumina's reference factor-based normalization methods (*preprocessIllumina*) and subset-quantile within array normalization for type I and II probe bias correction[16] were used for data normalisation. In the AMDTSS, the empirical Bayes batch-effects removal method *ComBat*[17] was applied. Probes with

This article is protected by copyright. All rights reserved.

Author Manuscript

missing value (detection *P*-value>0.01) in one or more samples were excluded. In the MCCS, samples were excluded if >5% probes had a detection *P*-value higher than 0.01, which were regarded as missing values, while probes were excluded from further analysis if more than 20% of samples had missing values. After quality control, 439,085 autosomal methylation sites were common to both datasets.

Mammographic density measures

Both cohorts measured mammographic density using the same protocol[9, 18]. In the AMDTSS, mammograms were retrieved from BreastScreen Services, from private clinics, and from women who kept their films at home. In the MCCS, mammograms were retrieved from BreastScreen Victoria via record linkage. For a woman with several mammograms available, the mammogram taken closest to the date at blood draw was selected. Mammographic films were digitized by the Australian Mammographic Density Research Facility at the University of Melbourne. The digitized images were then masked so that extraneous features on the mammogram other than the breast were excluded, and randomized into reading sets of approximately 100 images. The total breast area and the dense area defined as the amount of the white or bright regions were measured by independent readers using a computer-assisted thresholding technique CUMULUS 3.0 (Imaging Research Program, Sunnybrook Health Sciences Centre, University of Toronto, Toronto, Canada). The non-dense area was calculated as the total breast area minus the dense area, and the percentage dense area was calculated as the dense area divided by the total breast area.

Genome-wide association study

We used linear mixed-effects models to assess the association between the M-value (logit transformation of Beta-value, which quantifies the percentage of methylated cytosines) of each methylation site as the dependent variable, and each mammographic density risk measure as an independent variable. Because mammographic density measures were right-skewed, they were power-transformed to obtain approximately Gaussian distributions (dense area, power 0.25; non-dense area, power 0.4; percentage dense area, power 0.3). Models were adjusted by fitting fixed effects for age at blood draw (continuous), the difference between age at mammogram and age at blood draw (continuous), BMI (continuous), smoking status (never/ever in the AMDTSS and never/former/current in the MCCS), menopausal status (pre-/post-menopausal), use of hormone replacement treatment (never/ever), number of live births (continuous), all collected at blood draw and cell type proportions (CD4T cells, CD8T cells, natural killer cells, B cells, monocytes, granulocytes) estimated from DNA methylation data using the Houseman algorithm[19]. For the AMDTSS, the model was additionally adjusted for family and zygosity as random effects. For the MCCS, the model was additionally adjusted for sample type (dried blood spots/mononuclear cells/buffy coats) and country of birth

This article is protected by copyright. All rights reserved.

Author Manuscript

(Australia/UK/Italy/Greece) as fixed effects, and for study, plate and chip as random effects[20]. For the AMDTSS, the Bioconductor package *bacon* was used to adjust for the observed inflation of test statistics. Results from the two cohorts were pooled using fixed-effect meta-analysis[21]. A *P*-value of 10^{-7} was used to account for multiple testing.

Associations for methylation sites at genetic loci associated with mammographic density

We annotated the analysed methylation sites to genetic loci associated with mammographic density identified by the largest GWAS to date[4], using the column 'UCSC_RefGene_Name' of the annotation file provided by Illumina. A total of 299 methylation sites were annotated: 4 sites to the *AREG* locus, 76 sites to the *ESR1* locus, 15 sites to the *IGF1* locus, 48 sites to the *LSP1/TNNT3* locus, 60 sites to the *PRDM6* locus, 40 sites to the *SGSM3/MKL1* locus, 17 sites to the *TMEM184B* locus and 39 sites to the *ZNF365* locus. Associations between these 299 sites and mammographic density measures were examined using results from the meta-analysis. The observed number of sites with $P<0.05$ was compared with the expected number using the binomial test.

Results

None of the studied mammographic density measures was associated with DNA methylation at any site (all $P>3.4 \times 10^{-7}$ from meta-analysis; Figure 1). No evidence of inflation was found from the meta-analysis: inflation factors were 0.99, 0.97 and 0.96 from the analyses of dense area, non-dense area and percentage dense area, respectively. No genome-wide significant association was found from either of the cohort-specific analyses (all $P>1.5 \times 10^{-7}$; Figure 2). From the meta-analyses of dense area, non-dense area and percentage dense area, a total of 30, 50 and 30 sites, respectively, had $P<10^{-4}$ (Supplementary Table 1). Of these sites, seven were overlapping for dense area and percentage dense area, one was overlapping for non-dense area and percentage dense area, and none was overlapping for dense area and non-dense area. None of these sites was located at known genetic loci associated with mammographic density[4] or breast cancer[22].

Of the 299 methylation sites located at the eight genetic loci associated with mammographic density, none was associated with any of the studied mammographic density measures after Bonferroni adjustment for multiple testing (all $P>0.05/299 = 1.7 \times 10^{-4}$). From the analyses of dense area, non-dense area and percentage dense area, a total of 22, 21 and 21 sites, respectively, had a nominally significant association at $P<0.05$ (Supplementary Table 2), but this was not significantly greater than expected by chance (all $P>0.08$).

Discussion

This article is protected by copyright. All rights reserved.

Author Manuscript

Author Manuscript

Our genome-wide analysis of data for middle-aged Australian women did not detect associations between blood DNA methylation and conventional mammographic density measures that predict breast cancer risk. No association was found either at any of the loci for which genetic variants were reported to be associated with mammographic density.

Several factors could explain our null results. First, our study included over 1,000 participants, but this might not have been sufficient to detect existed but moderate or weak associations; for the most significant site (P=3.4 × 10^{-7} for non-dense area), the percentage difference in DNA methylation between participants in the top compared with bottom quartile of non-dense area was 1.36% in the AMDTSS and 0.24% in the MCCS, and our study had a power of 41.0% to detect this difference. Second, DNA methylation was measured with the widely used HM450 assay, which covers the promoter regions of 99% RefSeq genes but only a fraction of the genome and might therefore not include sites associated with mammographic density. Third, there could be substantial measurement error in methylation measures obtained from the HM450 assay, which would have weakened the statistical power of our analysis[13, 23]. Fourth, we used the conventional site-by-site analytic approach and a stringent threshold for statistical significance, which might not detect gene- or region-level methylation associated with mammographic density. Fifth, we measured DNA methylation in peripheral blood, not breast tissue, because it is easily accessible and potentially useful for public and clinical health translation[24]. While methylation marks are usually quite stable across tissues, to our knowledge this has not been carefully studied for breast tissue compared with peripheral blood, and not regarding biological relevance to mammographic density. Finally, peripheral blood DNA methylation might not be related to mammographic density at all, or at most trivially, which is indirectly supported by the fact that inconsistent or null results were observed in studies of the relationship between blood DNA methylation and breast cancer risk[8].

In this study, mammographic density was defined by the conventional brightness threshold using the CUMULUS software and has been referred to as *Cumulus*. New mammographic density measures defined by higher pixel brightness thresholds, *Cirrocumulus* and *Altocumulus*, have been found to predict breast cancer risk better than the *Cumulus* measures, and multivariate analyses suggest that the *Cumulus* measures are only surrogates for the causal aspects of a mammographic image that predict risk[25]. A new breast cancer risk-predicting measure called *Cirrus* has also been developed using machine learning[26]. The *Cirrocumulus*, *Altocumulus* and *Cirrus* measures might be more aetiologically relevant to breast cancer risk. Further research is therefore warranted to investigate associations between DNA methylation and these new mammographic density measures.

Our study has two main strengths. First, to the best of our knowledge, it is the first to investigate the association between DNA methylation and mammographic density. Second,

This article is protected by copyright. All rights reserved.

we pooled data from relatively homogenous samples of middle-aged Australian women, for whom DNA methylation and mammographic density were measured using the same protocols.

In summary, no association between blood DNA methylation measured by the HM450 assay and conventional mammographic density measures that predict breast cancer risk was detected by our analysis of middle-aged Australian women.

Author Manuscript

This article is protected by copyright. All rights reserved.

Figure legends

Figure 1. Q-Q plot of the genome-wide association analysis results from the meta-analysis of the two cohorts

Figure 2. Q-Q plots of the genome-wide association analysis results from the AMDTSS and MCCS

Author Manuscript

This article is protected by copyright. All rights reserved.

Author Manuscript

Ethics approval and consent to participate

The AMDTSS and MCCS were approved by the Human Research Ethics Committees of the University of Melbourne and Cancer Council Victoria, respectively. All participants provided informed consent.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing financial interests.

Funding

The AMDTSS was supported by the National Health and Medical Research Council (NHMRC) (grant numbers 1050561 and 1079102), Cancer Australia and National Breast Cancer Foundation (grant number 509307). The MCCS cohort recruitment was funded by VicHealth and Cancer Council Victoria. The MCCS was further augmented by Australian National Health and Medical Research Council grants number 209057, 396414 and 1074383 and by infrastructure provided by Cancer Council Victoria. Additional support was received from the NHMRC project grant numbers 1011618, 1026892, 1027505, 1050198, and 1043616, and the Victorian Breast Cancer Research Consortium (PI Southey).

Authors' contributions

Study design: SL, PAD, LB, DRE, MCS, GGG, JLH and RLM. Data collection and preparation: LB, GS, EMW, TLN, JS, DRE, MCS, GGG, JLH and RLM. Data analysis: SL and PAD. Data interpretation: SL, PAD, GS, GGG, MCS, JLH and RLM. Manuscript drafting - SL, PAD, JLH and RLM. All authors contributed to the preparation of and approved the final version of the manuscript.

Acknowledgements

We would like to thank all women participating in the two cohorts. The AMDTSS was facilitated through access to Twins Research Australia, a national resource supported by a Centre of Research Excellence Grant (ID: 1079102), from the NHMRC. The AMDTSS data analysis was facilitated by Spartan, the High Performance Computer and Cloud hybrid system of the University of Melbourne. SL and TLN are Cancer Council Victoria Postdoctoral Research Fellows. MCS is a NHMRC Senior Research Fellow (APP1061177). JLH is a NHMRC Senior Principal Research Fellow.

This article is protected by copyright. All rights reserved.

Author Manuscript

References

1. Hopper JL. Odds per adjusted standard deviation: comparing strengths of associations for risk factors measured on different scales and across diseases and populations. *Am J Epidemiol* 2015;**182**: 863-7.

2. Boyd NF, Guo H, Martin LJ, Sun L, Stone J, Fishell E, Jong RA, Hislop G, Chiarelli A, Minkin S, Yaffe MJ. Mammographic density and the risk and detection of breast cancer. *N Engl J Med* 2007;**356**: 227-36.

3. Stone J, Dite GS, Gunasekara A, English DR, McCredie MR, Giles GG, Cawson JN, Hegele RA, Chiarelli AM, Yaffe MJ, Boyd NF, Hopper JL. The heritability of mammographically dense and nondense breast tissue. *Cancer Epidemiol Biomarkers Prev* 2006;**15**: 612-7.

4. Lindstrom S, Thompson DJ, Paterson AD, Li J, Gierach GL, Scott C, Stone J, Douglas JA, dos-Santos-Silva I, Fernandez-Navarro P, Verghase J, Smith P, et al. Genome-wide association study identifies multiple loci associated with both mammographic density and breast cancer risk. *Nat Commun* 2014;**5**: 5303.

5. Lindstrom S, Vachon CM, Li J, Varghese J, Thompson D, Warren R, Brown J, Leyland J, Audley T, Wareham NJ, Loos RJ, Paterson AD, et al. Common variants in ZNF365 are associated with both mammographic density and breast cancer risk. *Nat Genet* 2011;**43**: 185-7.

6. Stone J, Thompson DJ, Dos Santos Silva I, Scott C, Tamimi RM, Lindstrom S, Kraft P, Hazra A, Li J, Eriksson L, Czene K, Hall P, et al. Novel Associations between Common Breast Cancer Susceptibility Variants and Risk-Predicting Mammographic Density Measures. *Cancer Res* 2015;**75**: 2457-67.

7. Sherratt MJ, McConnell JC, Streuli CH. Raised mammographic density: causative mechanisms and biological consequences. *Breast Cancer Res* 2016;**18**: 45.

8. Sturgeon SR, Pilsner JR, Arcaro KF, Ikuma K, Wu H, Kim SM, Chopra-Tandon N, Karpf AR, Ziegler RG, Schairer C, Balasubramanian R, Reckhow DA. White blood cell DNA methylation and risk of breast cancer in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO). *Breast Cancer Res* 2017;**19**: 94.

9. Odefrey F, Stone J, Gurrin LC, Byrnes GB, Apicella C, Dite GS, Cawson JN, Giles GG, Treloar SA, English DR, Hopper JL, Southey MC, et al. Common genetic variants associated with breast cancer and mammographic density measures that predict disease. *Cancer Res* 2010;**70**: 1449-58.

10. Li S, Wong EM, Joo JE, Jung CH, Chung J, Apicella C, Stone J, Dite GS, Giles GG, Southey MC, Hopper JL. Genetic and Environmental Causes of Variation in the Difference Between Biological Age Based on DNA Methylation and Chronological Age for Middle-Aged Women. *Twin Res Hum Genet* 2015;**18**: 720-6.

This article is protected by copyright. All rights reserved.

Author Manuscript

11. Milne RL, Fletcher AS, MacInnis RJ, Hodge AM, Hopkins AH, Bassett JK, Bruinsma FJ, Lynch BM, Dugue PA, Jayasekara H, Brinkman MT, Popowski LV, et al. Cohort Profile: The Melbourne Collaborative Cohort Study (Health 2020). *Int J Epidemiol* 2017;**46**: 1757-i.

12. Dugue PA, Bassett JK, Joo JE, Jung CH, Ming Wong E, Moreno-Betancur M, Schmidt D, Makalic E, Li S, Severi G, Hodge AM, Buchanan DD, et al. DNA methylation-based biological aging and cancer risk and survival: Pooled analysis of seven prospective studies. *Int J Cancer* 2018;**142**: 1611-9.

13. Dugue PA, English DR, MacInnis RJ, Jung CH, Bassett JK, FitzGerald LM, Wong EM, Joo JE, Hopper JL, Southey MC, Giles GG, Milne RL. Reliability of DNA methylation measures from dried blood spots and mononuclear cells using the HumanMethylation450k BeadArray. *Sci Rep* 2016;**6**: 30317.

14. Cole SR, Platt RW, Schisterman EF, Chu H, Westreich D, Richardson D, Poole C. Illustrating bias due to conditioning on a collider. *Int J Epidemiol* 2010;**39**: 417-20.

15. Joo JE, Wong EM, Baglietto L, Jung CH, Tsimiklis H, Park DJ, Wong NC, English DR, Hopper JL, Severi G, Giles GG, Southey MC. The use of DNA from archival dried blood spots with the Infinium HumanMethylation450 array. *BMC biotechnol* 2013;**13**: 23.

16. Maksimovic J, Gordon L, Oshlack A. SWAN: Subset-quantile within array normalization for illumina infinium HumanMethylation450 BeadChips. *Genome Biol* 2012;**13**: R44.

17. Johnson WE, Li C, Rabinovic A. Adjusting batch effects in microarray expression data using empirical Bayes methods. *Biostatistics* 2007;**8**: 118-27.

18. Baglietto L, Krishnan K, Stone J, Apicella C, Southey MC, English DR, Hopper JL, Giles GG. Associations of mammographic dense and nondense areas and body mass index with risk of breast cancer. *Am J Epidemiol* 2014;**179**: 475-83.

19. Jaffe AE, Irizarry RA. Accounting for cellular heterogeneity is critical in epigenome-wide association studies. *Genome Biol* 2014;**15**: R31.

20. Geurts YM, Dugue PA, Joo JE, Makalic E, Jung CH, Guan W, Nguyen S, Grove ML, Wong EM, Hodge AM, Bassett JK, FitzGerald LM, et al. Novel associations between blood DNA methylation and body mass index in middle-aged and older adults. *Int J Obes (Lond)* 2018;**42**: 887-96.

21. Viechtbauer W. Conducting Meta-Analyses in R with the metafor Package. *Journal of Statistical Software* 2010;**36**: 1-48.

22. Michailidou K, Lindstrom S, Dennis J, Beesley J, Hui S, Kar S, Lemacon A, Soucy P, Glubb D, Rostamianfar A, Bolla MK, Wang Q, et al. Association analysis identifies 65 new breast cancer risk loci. *Nature* 2017;**551**: 92-4.

23. Li S, Wong EM, Nguyen TL, Joo JE, Stone J, Dite GS, Giles GG, Saffery R, Southey MC, Hopper JL. Causes of blood methylomic variation for middle-aged women measured by the HumanMethylation450 array. *Epigenetics* 2017;**12**: 973-81.

This article is protected by copyright. All rights reserved.

24. Li S, Dugué P-A, Milne RL, Hopper JL. Epigenetic Prospects in Epidemiology and Public Health. In: Tollefsbol TO. *Epigenetics in Human Disease*ed., vol. 6: Academic Press, 2018: 995-1017.

25. Nguyen TL, Aung YK, Evans CF, Yoon-Ho C, Jenkins MA, Sung J, Hopper JL, Song YM. Mammographic density defined by higher than conventional brightness threshold better predicts breast cancer risk for full-field digital mammograms. *Breast Cancer Res* 2015;**17**: 142.

26. Schmidt DF, Makalic E, Goudey B, Dite GS, Stone J, Nguyen TL, Dowty JG, Baglietto L, Southey MC, Maskarinec G. Cirrus: An Automated Mammography-Based Measure of Breast Cancer Risk Based on Textural Features. *JNCI Cancer Spectrum* 2018;**2**: pky057.

Author Manuscript

This article is protected by copyright. All rights reserved.

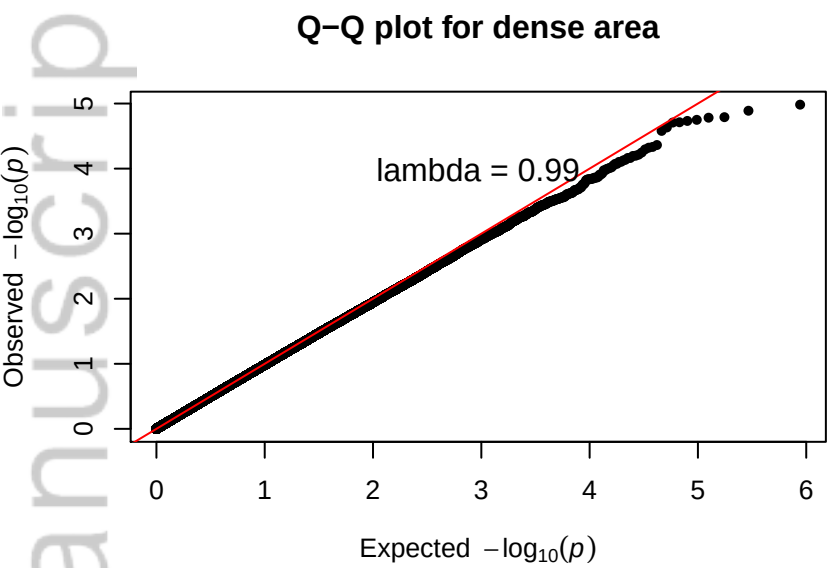
Table 1. Characteristics of samples from the two cohorts

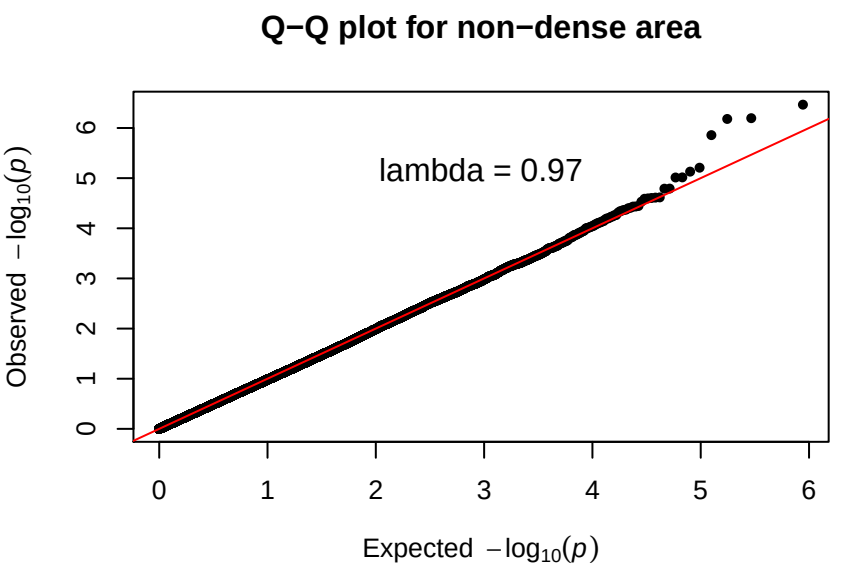
Sample characteristics	AMDTSS (N=436)	MCCS (N=591)
Age at mammogram (years), mean ± SD	55.6 ± 8.1	59.5 ± 7.3
Age at blood draw (years), mean ± SD	56.7 ± 7.9	57.2 ± 7.9
Body mass index (kg/m^2), mean ± SD	26.8 ± 5.8	26.6 ± 4.5
Post-menopausal, N (%)	305 (70)	435 (74)
Hormone replacement therapy use, N (%)	53 (12)	119 (20)
Number of live births, mean ± SD	2.6 ± 1.5	2.4 ± 1.5
Ever smoker, N (%)	166 (38)	198 (34)
Mammographic density measures		
Dense area (cm^2), median (IQR)	25.6 (10.1-45.7)	11.3 (4.2-20.7)
Non-dense area (cm^2), median (IQR)	99.1 (65.8-143.3)	116.4 (82.7-159.2)
Percentage dense area (%), median (IQR)	21.5 (7.0-39.6)	9.5 (3.1-19.2)

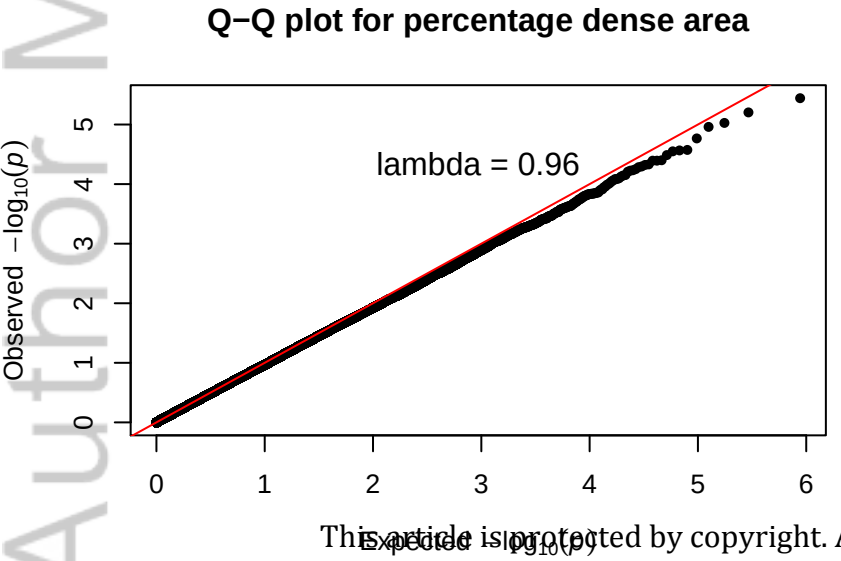
N, sample size; SD: standard deviation; IQR: inter-quartile range

Author Manuscript

This article is protected by copyright. All rights reserved.





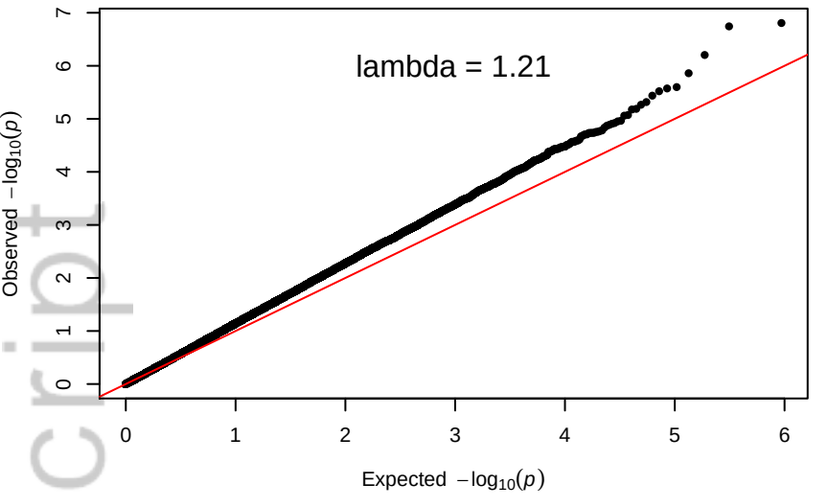


This article is protected by copyright. All rights reserved.

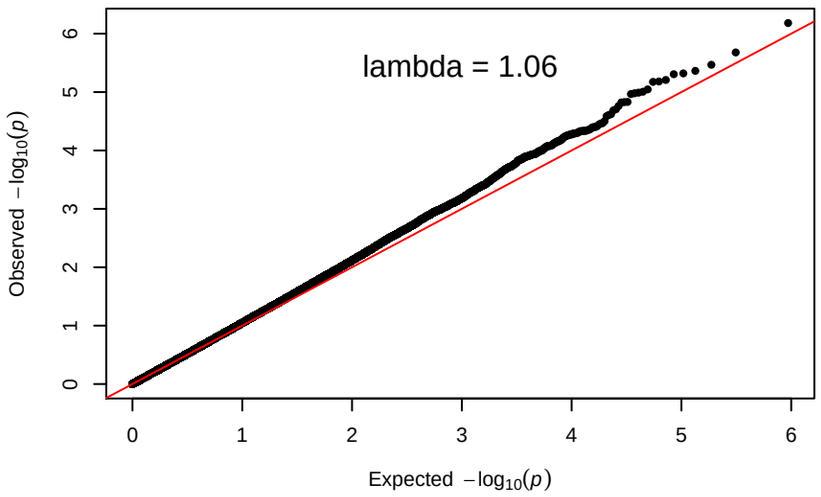
Author Manuscript

Author Manuscript

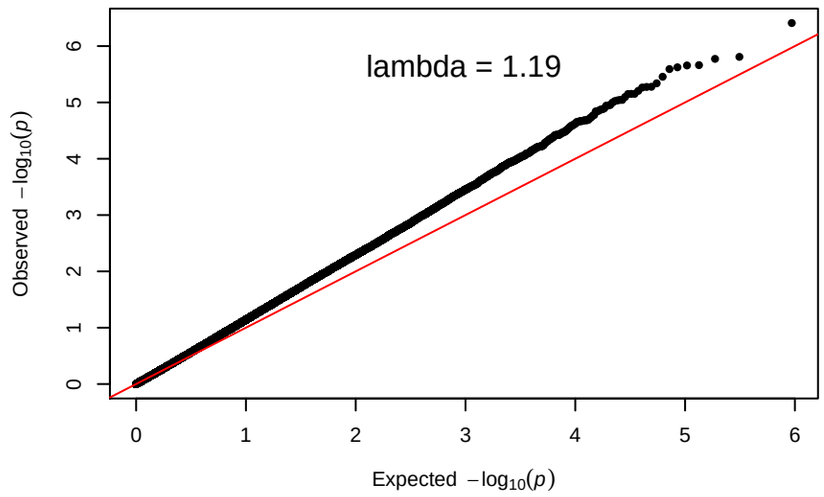
AMDTSS: DA before Bacon correction



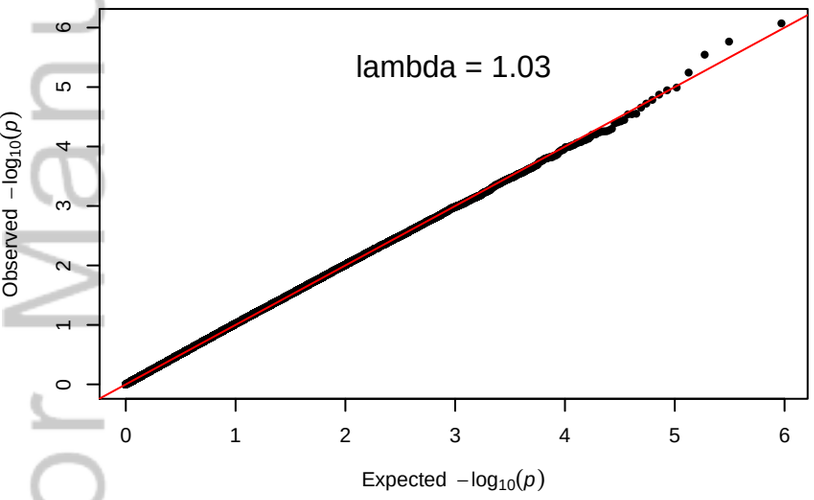
AMDTSS: NDA before Bacon correction



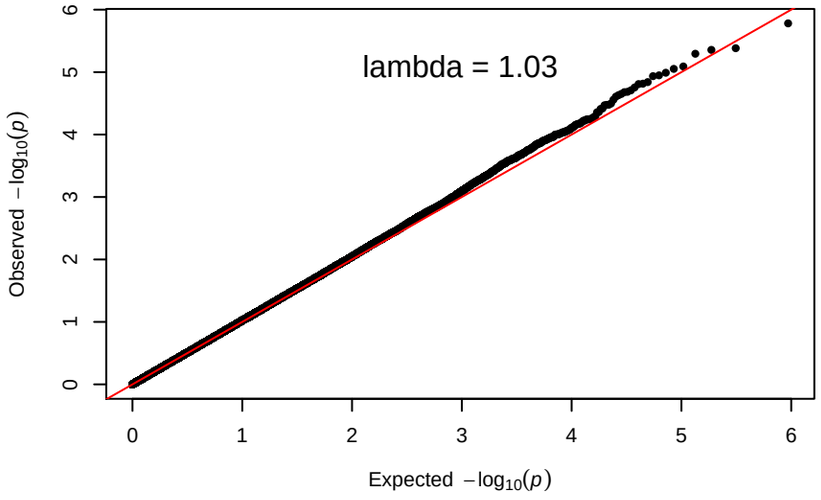
AMDTSS: PDA before Bacon correction



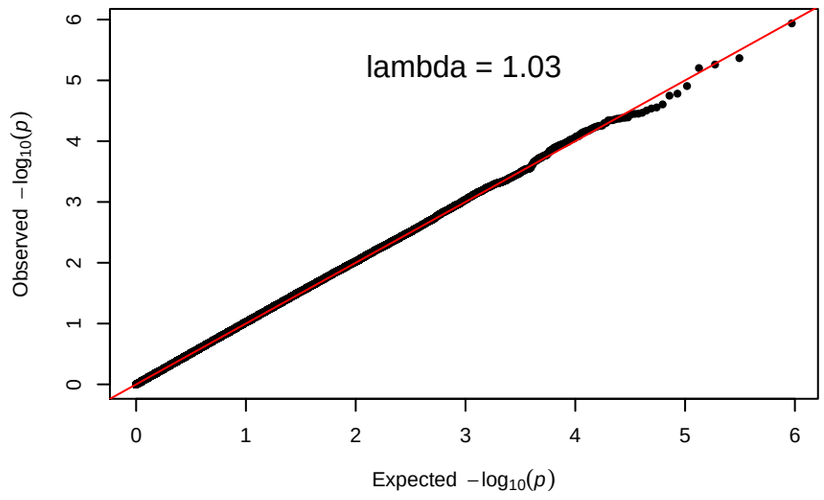
AMDTSS: DA after Bacon correction



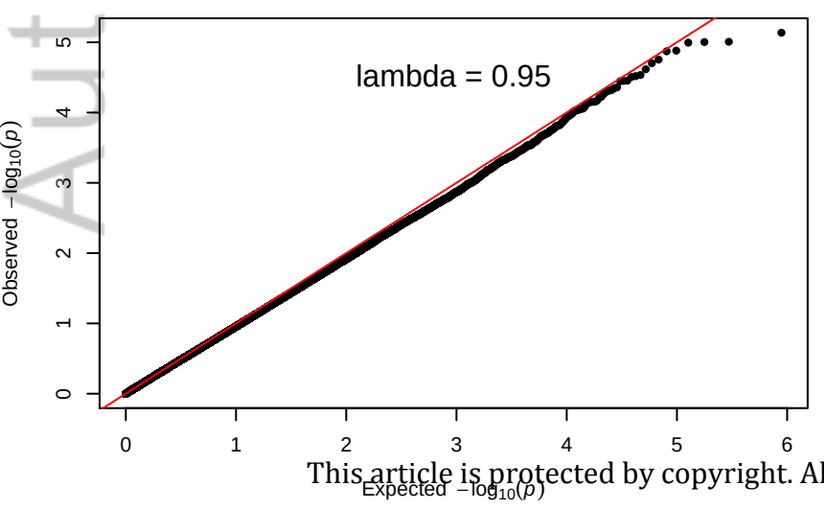
AMDTSS: NDA after Bacon correction



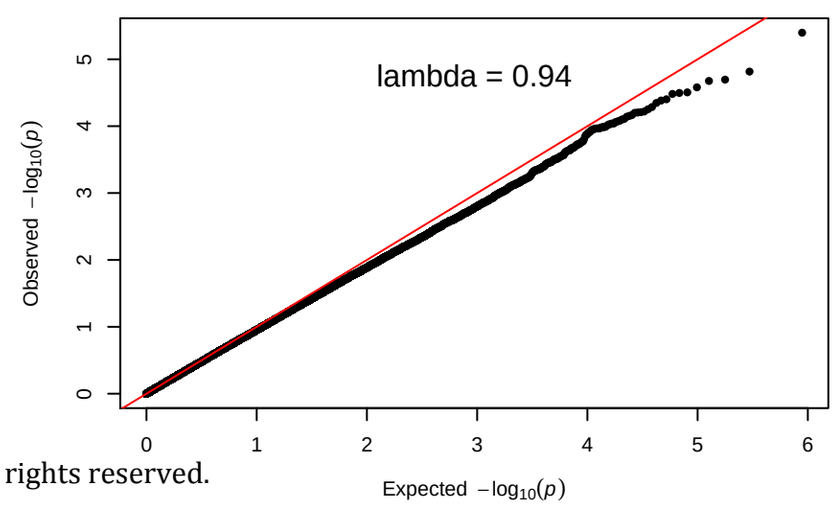
AMDTSS: PDA after Bacon correction



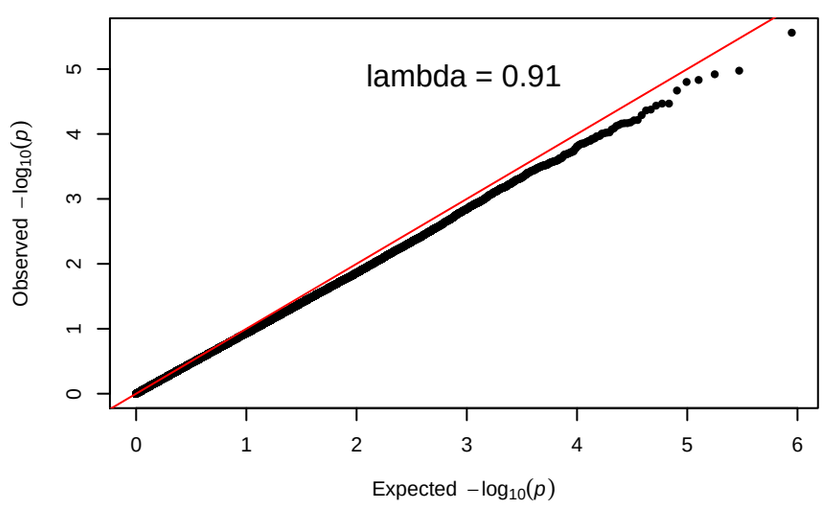
MCCS: DA



MCCS: NDA



MCCS: PDA



This article is protected by copyright. All rights reserved.

Novelty & Impact Statement: IJC-18-3498.R1

While breast density is a strong predictor of breast cancer risk, little is known about the molecular mechanisms that determine breast density and their influence on cancer risk. Here, in women ages 40 to 70 in two Australian study cohorts, including one twin cohort, genome-wide association analysis was used to investigate relationships between DNA methylation and mammographic density measures that predict breast cancer. DNA methylation assessed from peripheral blood and conventional mammographic density measures were not significantly associated. Moreover, no associations were detected between breast density measures and DNA methylation at genetic loci known to be associated with mammographic density.

Author Manuscript

This article is protected by copyright. All rights reserved.

Table 1. Characteristics of samples from the two cohorts

Sample characteristics	AMDTSS (N=436)	MCCS (N=591)
Age at mammogram (years), mean ± SD	55.6 ± 8.1	59.5 ± 7.3
Age at blood draw (years), mean ± SD	56.7 ± 7.9	57.2 ± 7.9
Body mass index (kg/m^2), mean ± SD	26.8 ± 5.8	26.6 ± 4.5
Post-menopausal, N (%)	305 (70)	435 (74)
Hormone replacement therapy use, N (%)	53 (12)	119 (20)
Number of live births, mean ± SD	2.6 ± 1.5	2.4 ± 1.5
Ever smoker, N (%)	166 (38)	198 (34)
Mammographic density measures		
Dense area (cm^2), median (IQR)	25.6 (10.1-45.7)	11.3 (4.2-20.7)
Non-dense area (cm^2), median (IQR)	99.1 (65.8-143.3)	116.4 (82.7-159.2)
Percentage dense area (%), median (IQR)	21.5 (7.0-39.6)	9.5 (3.1-19.2)

N, sample size; SD: standard deviation; IQR: inter-quartile range

Author Manuscript

This article is protected by copyright. All rights reserved.