Published in final edited form as:

Exp Mol Pathol. 2018 December ; 105(3): 404–410. doi:10.1016/j.yexmp.2018.11.006.

Genome-Wide DNA Methylation Assessment of ‘*BRCA1*-like’ Early-Onset Breast Cancer: data from the Australian Breast Cancer Family Registry.

Cameron M. Scott^{a,b,1}, Ee Ming Wong^{a,c,1}, JiHoon Eric Joo^{a,d,1}, Pierre-Antoine Dugué^{e,f}, Chol-Hee Jung^g, Neil O’Callaghan^a, James Dowty^e, Graham G. Giles^{e,f}, John L. Hopper^e, and Melissa C. Southey^{a,c,#,1}

^aGenetic Epidemiology Laboratory, Department of Pathology, The University of Melbourne, Victoria 3010, Australia

^bOlivia Newton-John Cancer Research Institute & School of Cancer Medicine, La Trobe University, Heidelberg, VIC 3084, Australia

^cPrecision Medicine, School of Clinical Sciences at Monash Health, Monash University, Clayton, 3168, Australia

^dColorectal Oncogenomics Group, Department of Clinical Pathology, University of Melbourne Centre for Cancer Research, The University of Melbourne

^eCentre for Epidemiology and Biostatistics, The University of Melbourne, Victoria 3010, Australia

^fCancer Epidemiology and Intelligence Division, Cancer Council Victoria, Victoria 3004, Australia

^gMelbourne Bioinformatics, The University of Melbourne, Parkville, VIC, Australia

Abstract

Breast cancers arising in women carrying a germline mutation in *BRCA1* are typically high-grade, early-onset and have distinct morphological features (*BRCA1*-like). However, the majority of early-onset breast cancers of this morphological type are not associated with germline *BRCA1* mutations or constitutional *BRCA1* promoter methylation. We aimed to assess DNA methylation across the genome for associations with the “*BRCA1*-like” morphology. Genome-wide methylation in blood-derived DNA was measured using the Infinium HumanMethylation450K BeadChip assay for women under the age of 40 years participating in the Australian Breast Cancer

Author for Correspondence Professor Melissa C. Southey, Precision Medicine, School of Clinical Sciences at Monash Health, Monash University, Clayton 3168, Australia, Ph: 61 3 8344 4895 msouthey@unimelb.edu.au.

¹Present address

Competing Interests

The authors have no Conflict of Interest to declare.

Ethics declaration

The blood and tumour samples were collected as part of the ABCFS. Written informed consent was obtained from each ABCFS participant. This study was approved by the Human Research Ethics Committee of the University of Melbourne (Project 0608818) and meets the principles of the Declaration of Helsinki.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Family Study (ABCFS) diagnosed with: i) *BRCA1*-like breast cancer (n=30); and ii) breast cancer without *BRCA1*-like morphological features (non *BRCA1*-like; n=30), and age-matched unaffected women (controls; n=30). Corresponding tumour-derived DNA from 43 of the affected women was also assessed. Methylation of blood-derived DNA was found to be elevated across 17 consecutive marks in the *BRCA1* promoter region and decreased at several other genomic regions (including *TWIST2* and *CTBPI*) for 7 women (23%) diagnosed with *BRCA1*-like breast cancer compared with women in the other groups. Corresponding tumour-derived DNA available from 5 of these 7 women had elevated methylation within the *BRCA1* and *SPHK2* promoter region and decreased methylation within the *ADAP1*, *IGF2BP3* and *SPATA13* promoter region when compared with the other breast tumours. These methylation marks could be biomarkers of risk for *BRCA1*-like breast cancer, and could be responsible in part for their distinctive morphological features and biology. As such, they may assist with prevention and targeted therapies for this cancer subtype.

Keywords

Genome-Wide; Methylation; HM450K; *BRCA1*; Early-Onset Breast Cancer

INTRODUCTION

Breast cancers arising in women who carry a germline mutation in *BRCA1* are often high grade and early-onset, with poor prognosis and limited treatment options (Anders et al., 2010). These breast cancers also have distinct morphological features that include a high mitotic index, high nuclear grade, little or no tubule formation, pushing margins, necrosis, lymphocytic infiltrate and trabecular, circumscribed and syncytial growth patterns (Armes et al., 1998b; Eisinger et al., 1996; Johannsson et al., 1997; Lakhani et al., 1998). Breast cancers with a large number of these features are commonly referred to as “*BRCA1*-like”, irrespective of their germline *BRCA1* mutation status.

Approximately 25% of all women diagnosed with *BRCA1*-like breast cancer before the age of 40 years carry an identifiable *BRCA1* germline mutation, suggesting the majority are due to other mechanisms that result in the same histological pattern (Southey et al., 2011). We previously detected blood-derived DNA methylation at the *BRCA1* promoter region in approximately 30% of young women diagnosed with *BRCA1*-like breast cancers (who were not known to carry a *BRCA1* germline mutation). These methylation marks were associated with a 3.5-fold (95% CI, 1.4–10.5) increased risk of early-onset breast cancer (Wong et al., 2011). A similar association was reported by Iwamoto et al (2011) in the Japanese population (OR 1.73 95% CI, 1.01–2.96) although the association was not specific to any age group or histological type (Iwamoto et al., 2011). *BRCA1* promoter methylation has been well-described in tumour-derived DNA from *BRCA1*-like breast cancers, including the tumours from women with elevated blood-derived DNA methylation at this region (Dobrovic and Simpfendorfer, 1997; Snell et al., 2008a; Wong et al., 2011).

Until recently, research in this area has been limited by low-resolution molecular techniques with the capacity to measure only a single or a small number of methylation marks. It was,

therefore, not known if the *BRCA1* promoter methylation observed in these *BRCA1*-like breast cancers (both in blood and tumour-derived DNA) was specific to the *BRCA1* promoter, part of an extended methylation signature, or even a genome-wide phenomenon. Here, we hypothesize that aberrant DNA methylation marks, not restricted to the *BRCA1* promoter region, are associated with the *BRCA1*-like breast cancers.

To test this, we measured genome-wide methylation in blood-derived and tumour-derived DNA samples from women with early-onset breast cancer (selected by tumour morphology), and age-matched unaffected women (controls) from the ABCFS using the Illumina HM450K Beadchip assay.

MATERIALS AND METHODS

Study samples

Blood and tumour-derived DNA samples were obtained from women participating in the ABCFS, a population-based case-control family study of breast cancer diagnosed before the age of 40 years (Dite et al., 2003; Hopper et al., 1999; John et al., 2004). Blood samples from affected women were collected on average 8 months after cancer diagnosis (Dite et al., 2003). These women had been previously screened for mutations in *BRCA1*, as well as other breast cancer susceptibility genes *ATM*, *CHEK2*, *PALB2*, *TP53* and *BRCA2* (Andrulis et al., 2002; Apicella et al., 2007; Dite et al., 2003; Goldgar et al., 2011; Hopper et al., 1999; Le Calvez-Kelm et al., 2011; Leong et al., 2000; Mouchawar et al., 2010; Neuhausen et al., 2009; Smith et al., 2007; Southey et al., 2010; Southey et al., 1999). All women selected for this study were known not to carry mutations in *BRCA1*, *BRCA2*, *TP53*, *ATM*, *CHEK2* or *PALB2*. DNA methylation at the *BRCA1* promoter had also been assessed for a subset of these women using a locus-specific MethyLight Real-time PCR assay (Trinh et al., 2001; Wong et al., 2011).

The morphological features of the tumours arising in these women had been reviewed and scored for features such as mitotic index, nuclear grade, tubule formation, pushing margins, necrosis, lymphocytic infiltrate and trabecular, circumscribed and syncytial growth patterns (Armes et al., 1998a; Wong et al., 2011). *BRCA1*-like breast cancer was defined as having five or more features associated with carrying a *BRCA1* germline mutation, and non *BRCA1*-like breast cancer was defined as having three or fewer of these features (Wong et al., 2011). We classified the subjects into three groups of: i) women diagnosed with *BRCA1*-like breast cancer (*BRCA1*-like; n=30); ii) women diagnosed with non *BRCA1*-like breast cancer (non *BRCA1*-like; n=30); and iii) age-matched women who were unaffected by breast cancer at the time of recruitment into the ABCFS (controls; n=30).

DNA extraction from archival blood spots

Blood-derived DNA was extracted from archival dried blood spots on Whatman filter paper (GE Healthcare, United Kingdom) as previously described (Joo et al., 2013). DNA was extracted from twenty-eight 3.2 mm diameter punches using the QIAamp 96 DNA Blood Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions, except the DNA was eluted three times in 50 µl nuclease-free water to obtain a final volume of 150 µl.

DNA extraction from formalin-fixed paraffin-embedded (FFPE) tumour sections

A haematoxylin and eosin (H&E) stained slide, marked up by a pathologist, was used as a reference slide for each case. Tumour-enriched tissue was macrodissected from at least two corresponding Methyl Green-stained sections using both a scalpel (Swann-Morton, Sheffield, England) and a 21-gauge syringe needle (Terumo, Tokyo, Japan), as previously described (Wong et al., 2015a). DNA was extracted using the QIAamp DNA FFPE Tissue Kit as per the manufacturer's instructions (Qiagen, Hilden, Germany), except the tissue was incubated for 48 hours with 20 µl of Proteinase K (20 mg/ml) replenished at 0 and 24 hours. The tumour-derived DNA was eluted twice in 15 µl elution buffer to obtain a final volume of 30 µl and stored long-term at 4°C.

Quality assessment of tumour-derived DNA

The quality of tumour-derived DNA was assessed using the Infinium HD QC assay (Illumina, CA, United States) according to manufacturer's instructions. All samples (diluted 1 ng/ul) were assayed in triplicate and run on the LightCycler® 480 System (Roche, Basel, Switzerland). Samples that amplified within 4 cycles of the positive control were progressed through the experimental workflow (Wong et al., 2015b).

Sodium bisulfite modification of blood and tumour-derived DNA

Five hundred nanograms of blood-derived DNA and a minimum of 20 ng of tumour-derived DNA was sodium bisulfite modified using the EZ DNA Methylation-Gold™ Kit (Zymo Research, CA, United States), according to the manufacturer's protocol. Blood-derived DNA was eluted in 10 ul Elution Buffer and stored long-term at -20°C. Tumour-derived DNA was eluted in 8 ul Elution Buffer and stored long-term at -20°C prior to restoration using the Infinium HD FFPE DNA Restore Kit (Illumina, CA, United States).

Infinium HumanMethylation450K BeadChip (HM450K)

The success of the sodium bisulfite modification and restoration steps was assessed using an in-house developed bisulfite-specific quantitative-PCR, as described previously (Wong et al., 2015b). DNA samples that amplified at least 4 cycles earlier than the negative control were progressed to the HM450K assay.

The HM450K assay was performed as per the manufacturer's protocol (Illumina, CA, United States). Blood-derived (n=90) and tumour-derived (n=46) DNA were assayed separately, with an additional six technical replicates of blood-derived DNA samples and two technical replicates of the tumour-derived DNA samples run simultaneously during the respective assays. To control for batch effects, all samples were assayed simultaneously and samples from the three study sample groups were equally distributed between beadchips (12 DNA samples per chip). The extension and staining steps were performed using the Freedom EVO® automated liquid handler (Tecan, Männedorf, Switzerland). The beadchips were scanned using the iScan® Microarray Scanner (Illumina, CA, United States).

Quality control and statistical analysis

Raw methylation data were imported into the R statistical environment and processed using the Bioconductor package *minfi* (version 3.2) (Aryee et al., 2014). The data were filtered and normalized according to the Illumina protocol, where the raw fluorescence data were normalized using control probes. Additional normalization using Subset-quantile Within Array Normalization (SWAN) was performed to correct for technical discrepancies between Type I and Type II probes (Maksimovic et al., 2015). Probes with detection p-values > 0.05 were considered as failed and excluded from subsequent analyses. M-values and β -values were calculated using *minfi*.

Differentially Methylated Probes (DMPs) were identified by a regression analysis using the empirical Bayes methods using *limma* R package (Smyth, 2004). Statistical significance was estimated by false discovery rate (FDR) adjusted p-value cut-off of 0.01, calculated using moderated t-statistics (Smyth, 2004). Unsupervised clustering and Multi-Dimensional Scaling (MDS) plots were generated using the *lumi* R package (Du et al., 2008).

Estimation of blood cell type composition

Cell type composition (proportion of CD8⁺ T cells, CD4⁺ T cells, natural killer (NK) cells, B cells, monocytes and granulocytes) was estimated using the Houseman algorithm for blood-derived DNA samples (implemented in the function '*estimateCellCounts*' of the package *minfi*) (Houseman et al., 2012; Jaffe and Irizarry, 2014). The granulocyte/lymphocyte ratio was calculated as the estimated granulocyte proportion divided by the sum of estimated CD8⁺ T cell, CD4⁺ T cell, NK cell and B cell proportions. To investigate whether differences in methylation levels could be explained by differences in blood cell type composition, t-tests were performed for each cell type and the granulocyte/lymphocyte ratio.

RESULTS

DNA methylation of blood-derived DNA

Overall, all samples had average detection p-values (calculated across all 485,512 probes) of < 0.01. No sample had > 3000 failed probes ($p > 0.01$) (Bibikova et al., 2011). In total, 477,380 probes remained after removing probes with detection p-values > 0.05 (Du et al., 2010). The six technical replicates had r^2 values of < 0.999. Multidimensional scaling was performed based on their principal component across all remaining probes (477,380) and demonstrated consistency (no outliers) for all samples irrespective of group (Supplementary Figure S1).

Regression analysis did not identify statistically significant DMPs with FDR adjusted p-values < 0.01 for any of the comparisons between the three groups but some heterogeneity was observed in the methylation patterns within each group and this was especially strong for the *BRCA1*-like group.

DNA methylation at the *BRCA1* promoter region

The observed heterogeneity of the *BRCA1*-like group was further considered by specific analysis of the *BRCA1* promoter region. There was a total of 22 HM450K probes encompassing *BRCA1* exon 1a, *NBR2* exon 1 and their bi-directional promoter (Chr 17: 41276607–41277848 (hg19)) (Figure 1). A subset of samples (n=7) within the *BRCA1*-like group had higher levels of methylation at the *BRCA1* promoter region. The average β methylation across the 22 promoter-associated probes for these seven samples was 25.6% compared with 15.5% for the non *BRCA1*-like samples. The remaining 23 *BRCA1*-like samples had an average beta methylation across the promoter probes of 15.9%, which was similar to the non *BRCA1*-like group.

Based on this observation in blood derived DNA, the 30 women of the *BRCA1*-like group were further divided into two subgroups: 1) a non-*BRCA1*-methylated *BRCA1*-like group (NM-*BRCA1*-like; n=23) sharing a similar methylation pattern with both the women with non *BRCA1*-like breast cancer and the controls, and 2) a *BRCA1*-methylation-associated *BRCA1*-like group (M-*BRCA1*-like; n=7) (Figure 1).

DNA methylation of M-*BRCA1*-like subgroup

Further analysis was performed to compare M-*BRCA1*-like with the other 83 blood-derived DNA samples (from all three groups of women). There were extensive methylation differences associated with the M-*BRCA1*-like samples; 477 and 5,369 probes were identified to be differentially methylated (FDR adjusted p-value < 0.01) in this group compared with NM-*BRCA1*-like and all other groups, respectively. There were 472 probes common to the two comparisons. Seventeen *BRCA1* promoter-associated probes were found to be amongst the most differentially methylated. When ranked by their statistical significance (i.e. FDR adjusted p-value), six of the 17 *BRCA1* promoter-associated probes were amongst the ten most DMPs (Supplementary Table S1). Probes overlapping *TWIST2*, an intergenic region (chromosome 7: 96,483,662), *C4orf51*, and *CTBP1* were also included in the ten most DMPs. In contrast to the six probes in the *BRCA1* promoter region, the other four probes had lower levels of methylation in the M-*BRCA1*-like samples.

Blood cell type composition between M-*BRCA1*-like and NM-*BRCA1*-like samples

We did not find significant cell type composition differences between blood samples from women in the M-*BRCA1*-like and NM-*BRCA1*-like subgroups for any individual cell type proportion or the granulocyte/lymphocyte ratio (p > 0.1) (Table 1).

DNA methylation of tumour-derived DNA

DNA derived from FFPE breast tumour tissue was available for 46 of the 60 affected women. Three samples performed poorly on the HM450K with average detection p-values > 0.01 and were excluded. In total, we obtained genome-wide methylation data from 21 *BRCA1*-like breast tumours (5 M-*BRCA1*-like and 16 NM-*BRCA1*-like breast tumours) and 22 non *BRCA1*-like breast tumours. There were 407,610 common probes across the 43 samples after normalization and data processing. MDS based on all detected probes showed weak clustering between the *BRCA1*-like and the non *BRCA1*-like groups (data not shown).

Of these 407,610 probes, we detected 862 (0.21%) statistically significant DMPs between the *BRCA1*-like and non *BRCA1*-like breast tumour samples (Supplementary Table S2). The *BRCA1* promoter region was one of the most differentially methylated regions. Other genes with multiple DMPs in the promoter region include *SPHK2*, *SPATA13* and *IGF2BP3* (Figure 2A-C).

As a group, the *BRCA1*-like tumours (n=21), had increased methylation levels compared with the non *BRCA1*-like breast tumours at the *BRCA1* promoter ($\beta = \sim 15\%$). When the *BRCA1*-like tumours were divided into sub-groups based on their respective blood-derived DNA methylation patterns, the 5 M-*BRCA1*-like tumours showed a further increase in methylation compared with the non *BRCA1*-like group ($\beta_{\text{M-}BRCA1\text{-like}}: \sim 23\%$; $\beta_{\text{NM-}BRCA1\text{-like}}: \sim 10\%$) (Figure 3).

We identified 49 DMPs between the M-*BRCA1*-like and NM-*BRCA1*-like subgroups. The majority of the M-*BRCA1*-like-associated DMPs displayed lower levels of methylation compared with the NM-*BRCA1*-like samples (Supplementary Table S3). In most instances the DMPs were identified in isolation and their significance is difficult to determine. However, multiple DMPs were identified at the *ADAPI*, *POLG* and *CYP2A7* gene regions. Consistent and distinct lower levels of DNA methylation at *ADAPI* were identified in the M-*BRCA1*-like samples across the entire gene (although only two probes had a FDR adjusted p-value of < 0.01) (Figure 2D).

DISCUSSION

This genome-wide assessment of methylation in blood and tumour-derived DNA from women diagnosed with breast cancer before age 40 years further characterized a remarkable subtype of breast cancer that is associated with distinct histological features commonly observed in *BRCA1*-mutation carriers. We found elevated methylation across a region of the *BRCA1* promoter in blood-derived DNA (known to be associated with a 3.5-fold (95% CI, 1.4–10.5) increased risk of having early-onset breast cancer (Snell et al., 2008b; Wong et al., 2011). In addition to the *BRCA1* promoter, a number of other differentially methylated marks were characteristic of this breast cancer subtype in both the blood and tumour-derived DNA. This raises the possibility of a common underlying predisposition and tumourigenic pathway presenting as a distinct genome-wide methylation profile and histology. This is the first report to extend the characterisation of this subtype beyond a single site assessment within the *BRCA1* promoter and to describe its genomewide features.

Analysis of the HM450K data measured from blood-derived DNA

Our analysis identified 477 DMPs specific to the M-*BRCA1*-like group and 17 of these were across the promoter region of *BRCA1* (Figure 1). Six of these *BRCA1* promoter region probes were ranked in the ten most significant DMPs. *TWIST2* and *CTBP1* also contained single probes within the ten most significant DMPs. These two genes have both been found to be deregulated in breast cancer with oncogenic potential (Deng et al., 2012; Fang et al., 2011). Overexpression of *TWIST2* in breast cancer aids in the regulation of epithelial to mesenchymal transition (EMT), in part by down-regulating *CDH1* expression (Fang et al., 2011). The majority of cancers develop from epithelial cells which, as they progress to

malignancy, undergo EMT and lose the expression of epithelial specific genes (Yang et al., 2004). *CTBPI*, which has been shown via immunohistochemistry to be expressed in 92% of invasive ductal breast cancers is also thought to play a role in the down-regulation of *CDH1* as well as *BRCA1* (Deng et al., 2012). Interestingly, blood-derived DNA from women with the M-*BRCA1*-like subtype had reduced levels of methylation at probes in the *TWIST2* and *CTBPI* gene bodies that has been associated with reduced expression levels of the corresponding gene products (Ball et al., 2009; Hellman and Chess, 2007). Differences in blood cell composition are unlikely to have played a major role in the methylation profile observed in the M-*BRCA1*-like subgroup.

Analysis of the HM450K data measured from tumour-derived DNA

The comparison between *BRCA1*-like and non *BRCA1*-like tumours revealed 862 statistically significant DMPs. We speculate that the large differences in DNA methylation between the *BRCA1*-like and non *BRCA1*-like samples were driven largely by the distinct methylation pattern in the M-*BRCA1*-like samples.

The M-*BRCA1*-like samples had higher methylation levels than the non *BRCA1*-like samples at the *SPHK2* promoter region ($\beta = \sim 39\%$). *SPHK2* has been shown to suppress cell growth and proliferation, and enhance apoptosis within breast cancer cells (Igarashi et al., 2003; Liu et al., 2003). A decrease in *SPHK2* expression might lead to an increase in tumour cell growth and proliferation, consistent with the associated tumour histological features. In contrast, *SPATA13* showed a consistent trend of decreased methylation within the M-*BRCA1*-like tumour samples. Previous research has shown *SPATA13* to be a key regulatory gene for cell migration and adhesion in adenomatous polyposis coli (Kawasaki et al., 2007). There was a distinct decrease in methylation within the M-*BRCA1*-like samples across all ten *IGF2BP3* promoter-associated probes on the HM450 array. Similar to *TWIST2* and *CTBPI* in the germline analysis, *IGF2BP3* overexpression has been shown to be associated with EMT, cell proliferation and migration in breast cancer (Su et al., 2014). With the majority of the *BRCA1*-like tumours being triple negative (*ER-/PR-/HER2-*), these findings are keeping with previous studies where *IGF2BP3* has been found to be preferentially expressed within triple-negative breast cancers (Walter et al., 2009).

ADAP1 also displayed consistently lower levels of methylation across the gene in M-*BRCA1*-like samples. *ADAP1* has been previously shown to play a role in conserving interactions at adheren junctions and epithelial vesicle traffic within epithelial-like tumour cells, thereby preventing EMT (Kohn et al., 2014). Lower expression levels of *ADAP1* may therefore contribute to tumour cell invasion and metastasis. This is a novel finding within this subset of breast cancers.

This first report of genome-wide methylation assessment of *BRCA1*-like breast cancer is small and further work in expert-histologically annotated studies is required to validate these findings

CONCLUSION

In the absence of a germline mutation, our prior work found that the constitutional methylation of *BRCA1* is associated with risk of *BRCA1*-like breast cancer (similar to germline *BRCA1* mutations). This report provides additional information demonstrating that the methylation status of several other genomic regions is characteristic of this breast cancer subtype and could play a role in M-*BRCA1*-like breast cancer tumourigenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We extend our thanks to the many women and their families who generously participated in the Australian Breast Cancer Family Study and consented to allow us access to their pathology material.

Grant Support

The Australia site of Breast Cancer Family Registry was supported by the National Health and Medical Research Council of Australia, the New South Wales Cancer Council, the Victorian Health Promotion Foundation (Australia) and grant UM1 CA164920 from the USA National Cancer Institute. The content of this manuscript does not necessarily reflect the views or policies of the National Cancer Institute or any of the collaborating centers in the Breast Cancer Family Registry (BCFR), nor does mention of trade names, commercial products, or organizations imply endorsement by the USA Government or the BCFR. JLH is a Senior Principle Research Fellow of the NHMRC and MCS is a Senior Research Fellow. CMS is a recipient of a Genetic Epidemiology Laboratory Honours Scholarship, Department of Pathology, The University of Melbourne.

ABBREVIATIONS

ABCFS	Australian Breast Cancer Family Study
CGI	CpG Island
DMP	Differentially Methylation Probe
FFPE	Formalin-fixed Paraffin Embedded
TSS	Transcriptional Start Site
HM450K	Illumina HumanMethylation450K BeadChip assay
SWAN	Subset-quantile WithinArray Normalization
FDR	False Discovery Rate
MDS	Multidimensional Scaling

References

- Anders CK, et al., 2010 Poly(ADP-Ribose) polymerase inhibition: “targeted” therapy for triplenegative breast cancer. Clin Cancer Res. 16, 4702–10. [PubMed: 20858840]
- Andrulis IL, et al., 2002 Comparison of DNA- and RNA-based methods for detection of truncating BRCA1 mutations. Hum Mutat. 20, 65–73. [PubMed: 12112659]

- Apicella C, et al., 2007 Validation study of the LAMBDA model for predicting the BRCA1 or BRCA2 mutation carrier status of North American Ashkenazi Jewish women. *Clin Genet.* 72, 87–97. [PubMed: 17661812]
- Armes JE, et al., 1998a The histologic phenotypes of breast carcinoma occurring before age 40 years in women with and without BRCA1 or BRCA2 germline mutations: a population-based study. *Cancer.* 83, 2335–45. [PubMed: 9840533]
- Armes JE, et al., 1998b The histologic phenotypes of breast carcinoma occurring before age 40 years in women with and without BRCA1 or BRCA2 germline mutations - A population-based study. *Cancer.* 83, 2335–2345. [PubMed: 9840533]
- Aryee MJ, et al., 2014 Minfi: a flexible and comprehensive Bioconductor package for the analysis of Infinium DNA methylation microarrays. *Bioinformatics.* 30, 1363–9. [PubMed: 24478339]
- Ball MP, et al., 2009 Targeted and genome-scale strategies reveal gene-body methylation signatures in human cells. *Nat Biotechnol.* 27, 361–8. [PubMed: 19329998]
- Bibikova M, et al., 2011 High density DNA methylation array with single CpG site resolution. *Genomics.* 98, 288–95. [PubMed: 21839163]
- Deng Y, et al., 2012 Transcriptional down-regulation of Brca1 and E-cadherin by CtBP1 in breast cancer. *Mol Carcinog.* 51, 500–7. [PubMed: 21681822]
- Dite GS, et al., 2003 Familial risks, early-onset breast cancer, and BRCA1 and BRCA2 germline mutations. *J Natl Cancer Inst.* 95, 448–57. [PubMed: 12644538]
- Dobrovic A, Simpfendorfer D, 1997 Methylation of the BRCA1 gene in sporadic breast cancer. *Cancer Res.* 57, 3347–50. [PubMed: 9269993]
- Du P, et al., 2008 lumi: a pipeline for processing Illumina microarray. *Bioinformatics.* 24, 1547–8. [PubMed: 18467348]
- Du P, et al., 2010 Comparison of Beta-value and M-value methods for quantifying methylation levels by microarray analysis. *BMC bioinformatics.* 11, 587. [PubMed: 21118553]
- Eisinger F, et al., 1996 Germ line mutation at BRCA1 affects the histoprognostic grade in hereditary breast cancer. *Cancer Research.* 56, 471–474. [PubMed: 8564955]
- Fang X, et al., 2011 Twist2 contributes to breast cancer progression by promoting an epithelial-mesenchymal transition and cancer stem-like cell self-renewal. *Oncogene.* 30, 4707–20. [PubMed: 21602879]
- Goldgar DE, et al., 2011 Rare variants in the ATM gene and risk of breast cancer. *Breast Cancer Res.* 13, R73. [PubMed: 21787400]
- Hellman A, Chess A, 2007 Gene body-specific methylation on the active X chromosome. *Science.* 315, 1141–3. [PubMed: 17322062]
- Hopper JL, et al., 1999 Population-based estimate of the average age-specific cumulative risk of breast cancer for a defined set of protein-truncating mutations in BRCA1 and BRCA2. Australian Breast Cancer Family Study. *Cancer Epidemiol Biomarkers Prev.* 8, 741–7. [PubMed: 10498392]
- Houseman EA, et al., 2012 DNA methylation arrays as surrogate measures of cell mixture distribution. *BMC bioinformatics.* 13, 86. [PubMed: 22568884]
- Igarashi N, et al., 2003 Sphingosine kinase 2 is a nuclear protein and inhibits DNA synthesis. *Journal of Biological Chemistry.* 278, 46832–46839. [PubMed: 12954646]
- Iwamoto T, et al., 2011 BRCA1 promoter methylation in peripheral blood cells is associated with increased risk of breast cancer with BRCA1 promoter methylation. *Breast cancer research and treatment.* 129, 69–77. [PubMed: 20882403]
- Jaffe AE, Irizarry RA, 2014 Accounting for cellular heterogeneity is critical in epigenome-wide association studies. *Genome biology.* 15, R31. [PubMed: 24495553]
- Johannsson OT, et al., 1997 Tumour biological features of BRCA1-induced breast and ovarian cancer. *Eur J Cancer.* 33, 362–71. [PubMed: 9155518]
- John EM, et al., 2004 The Breast Cancer Family Registry: an infrastructure for cooperative multinational, interdisciplinary and translational studies of the genetic epidemiology of breast cancer. *Breast Cancer Res.* 6, R375–89. [PubMed: 15217505]
- Joo JE, et al., 2013 The use of DNA from archival dried blood spots with the Infinium HumanMethylation450 array. *Bmc Biotechnology.* 13.

- Kawasaki Y, et al., 2007 Identification and characterization of Asef2, a guanine-nucleotide exchange factor specific for Rac1 and Cdc42. *Oncogene*. 26, 7620–267. [PubMed: 17599059]
- Kohn KW, et al., 2014 Gene expression correlations in human cancer cell lines define molecular interaction networks for epithelial phenotype. *PLoS One*. 9, e99269. [PubMed: 24940735]
- Lakhani SR, et al., 1998 Multifactorial analysis of differences between sporadic breast cancers and cancers involving BRCA1 and BRCA2 mutations. *Journal of the National Cancer Institute*. 90, 1138–1145. [PubMed: 9701363]
- Le Calvez-Kelm F, et al., 2011 Rare, evolutionarily unlikely missense substitutions in CHEK2 contribute to breast cancer susceptibility: results from a breast cancer family registry case-control mutation-screening study. *Breast Cancer Res*. 13, R6. [PubMed: 21244692]
- Leong T, et al., 2000 Mutation analysis of BRCA1 and BRCA2 cancer predisposition genes in radiation hypersensitive cancer patients. *Int J Radiat Oncol Biol Phys*. 48, 959–65. [PubMed: 11072151]
- Liu H, et al., 2003 Sphingosine kinase type 2 is a putative BH3-only protein that induces apoptosis. *Journal of Biological Chemistry*. 278, 40330–40336. [PubMed: 12835323]
- Maksimovic J, et al., 2015 Removing unwanted variation in a differential methylation analysis of Illumina HumanMethylation450 array data. *Nucleic Acids Res*.
- Mouchawar J, et al., 2010 Population-based estimate of the contribution of TP53 mutations to subgroups of early-onset breast cancer: Australian Breast Cancer Family Study. *Cancer Res*. 70, 4795–800. [PubMed: 20501846]
- Neuhausen SL, et al., 2009 BRCA1 and BRCA2 mutation carriers in the Breast Cancer Family Registry: an open resource for collaborative research. *Breast Cancer Res Treat*. 116, 379–86. [PubMed: 18704680]
- Smith LD, et al., 2007 BRCA1 promoter deletions in young women with breast cancer and a strong family history: a population-based study. *Eur J Cancer*. 43, 823–7. [PubMed: 17317153]
- Smyth GK, 2004 Linear models and empirical bayes methods for assessing differential expression in microarray experiments. *Stat Appl Genet Mol Biol*. 3, Article3.
- Snell C, et al., 2008a BRCA1 promoter methylation in peripheral blood DNA of mutation negative familial breast cancer patients with a BRCA1 tumour phenotype. *Breast Cancer Res*. 10, R12. [PubMed: 18269736]
- Snell C, et al., 2008b BRCA1 promoter methylation in peripheral blood DNA of mutation negative familial breast cancer patients with a BRCA1 tumour phenotype. *Breast Cancer Res*. 10, R12. [PubMed: 18269736]
- Southey MC, et al., 2011 Morphological predictors of BRCA1 germline mutations in young women with breast cancer. *Br J Cancer*. 104, 903–9. [PubMed: 21343941]
- Southey MC, et al., 2010 A PALB2 mutation associated with high risk of breast cancer. *Breast Cancer Res*. 12, R109. [PubMed: 21182766]
- Southey MC, et al., 1999 BRCA1 mutations and other sequence variants in a population-based sample of Australian women with breast cancer. *Br J Cancer*. 79, 34–9. [PubMed: 10408690]
- Su P, et al., 2014 IMP3 expression is associated with epithelial-mesenchymal transition in breast cancer. *International Journal of Clinical and Experimental Pathology*. 7, 3008–3017. [PubMed: 25031719]
- Trinh BN, et al., 2001 DNA methylation analysis by MethyLight technology. *Methods*. 25, 456–62. [PubMed: 11846615]
- Walter O, et al., 2009 IMP3 is a novel biomarker for triple negative invasive mammary carcinoma associated with a more aggressive phenotype. *Human Pathology*. 40, 1528–1533. [PubMed: 19695680]
- Wong EM, et al., 2015a Tools for translational epigenetic studies involving formalin-fixed paraffin-embedded human tissue: applying the Infinium HumanMethylation450 Beadchip assay to large population-based studies. *BMC Res Notes*. 8, 543. [PubMed: 26438025]
- Wong EM, et al., 2015b Tools for translational epigenetic studies involving formalin-fixed paraffin-embedded human tissue: applying the Infinium HumanMethylation450 Beadchip assay to large population-based studies. *BMC research notes*. 8, 543. [PubMed: 26438025]

- Wong EM, et al., 2011 Constitutional methylation of the BRCA1 promoter is specifically associated with BRCA1 mutation-associated pathology in early-onset breast cancer. *Cancer Prev Res (Phila)*. 4, 23–33. [PubMed: 20978112]
- Yang J, et al., 2004 Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. *Cell*. 117, 927–39. [PubMed: 15210113]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Highlights

- Some young women have widespread blood derived DNA methylation at the *BRCA1* promoter
- These women also have reduced blood derived DNA methylation at *TWIST2* and *CTBP1*
- Corresponding tumour DNA from these women have elevated methylation at *BRCA1* These tumours also have other DMR when compared to other breast cancer molecular subtypes

Figure 1. Blood-derived DNA methylation at the *BRCA1* promoter region.

The mean beta values for each sample group at each CpG (as indicated) across the *BRCA1* promoter region. Error bars are plotted Standard Error of Mean. [CGI (CpG Island), TSS (Transcriptional Start Site)]

Figure 2. Beta methylation values at DMPs identified between tumour-derived DNA. Groups: *BRCA1*-like and non-*BRCA1*-like, as well as M-*BRCA1*-like and NM-*BRCA1*-like, beta methylation values are plotted at each probe across (A) *SPHK2* (B) *SPATA13* (C) *IGF2BP3* (D) *ADAP1*. A schematic of CGIs and exons is included. Errors bars denoted Standard Error of Mean. [CGI (CpG Island), TSS (Transcriptional Start Site)]

Figure 3. Methylation at the *BRCA1* promoter in tumour-derived DNA.

Beta values for breast tumours from the *BRCA1*-like, non *BRCA1*-like, M-*BRCA1*-like and NM-*BRCA1*-like groups at probes across the *BRCA1* promoter region. Error bars are plotted Standard Error of Mean. [CGI (CpG Island), TSS (Transcriptional Start Site)]

Table 1:

Estimated blood cell type compositions for M-*BRCA1*-like and NM-*BRCA1*-like samples.

Study group	Cell type	Median	Mean	SD ^a	t-test
1; NM- <i>BRCA1</i> -like (N=23)	CD8 ⁺ T	0.12	0.12	0.03	P=0.54
1A; M- <i>BRCA1</i> -like (N=7)		0.13	0.14	0.06	
1; NM- <i>BRCA1</i> -like (N=23)	CD4 ⁺ T	0.11	0.11	0.05	P=0.13
1A; M- <i>BRCA1</i> -like (N=7)		0.12	0.14	0.03	
1; NM- <i>BRCA1</i> -like (N=23)	NK	0.03	0.04	0.03	P=0.79
1A; M- <i>BRCA1</i> -like (N=7)		0.04	0.03	0.01	
1; NM- <i>BRCA1</i> -like (N=23)	B cell	0.07	0.08	0.03	P=0.59
1A; M- <i>BRCA1</i> -like (N=7)		0.07	0.08	0.03	
1; NM- <i>BRCA1</i> -like (N=23)	Mono	0.07	0.08	0.04	P=0.21
1A; M- <i>BRCA1</i> -like (N=7)		0.08	0.10	0.03	
1; NM- <i>BRCA1</i> -like (N=23)	Gran	0.59	0.61	0.09	P=0.28
1A; M- <i>BRCA1</i> -like (N=7)		0.62	0.56	0.11	
1; NM-<i>BRCA1</i>-like (N=23)	Gran / Lymphocytes^b	1.83	1.96	0.81	P=0.16
1A; M-<i>BRCA1</i>-like (N=7)		1.86	1.55	0.57	

^aSD: sample standard deviation

^bLymphocyte proportion calculated as CD8T + CD4T + NK + B cell proportions