Non-BRCA1/BRCA2 *High-Risk Familial Breast Cancers are Not Associated with a High Prevalence* of BRCAness

Lars Andersen (laandersen@health.sdu.dk)
University of Southern Denmark https://orcid.org/0000-0002-6148-1341
Martin Larsen
University of Southern Denmark
Helen Davies
University of Cambirdge
Andrea Degasperi
MRC Cancer Unit https://orcid.org/0000-0001-6879-0596
Henriette Nielsen
European Sperm Bank
Lone Kroeldrup
Odense University Hospital
Anne-Marie Gerdes
Copenhagen University Hospital Rigshospitalet
Anne-Vibeke Lænkholm
Slagelse Hospital
Torben Kruse
University of Southern Denmark
Serena Nik-Zainal
University of Cambridge
Mads Thomassen
Odense University Hospital

Brief Communication

Keywords: Breast cancer, hereditary breast cancer, non-BRCA1/BRCA2, whole genome sequencing, mutational signatures, HRDetect, BRCAness

Posted Date: August 26th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-827829/v1

Abstract

Familial breast cancer is in most cases unexplained due to the lack of identifiable pathogenic variants in the BRCA1 and BRCA2 genes. Using genome sequencing, we first noted for non-BRCA1/BRCA2 tumours, only a small proportion (3/23) demonstrated features of BRCAness, with high HRDetect scores and concomitant somatic BRCA1 mutation or promoter hypermethylation to explain their BRCAness. Second, a small proportion (4/23) showed no features of BRCAness but had mutationally active tumours. Third, the remaining tumours lacked features of BRCAness and were mutationally quiescent. Only few families could be explained by pathogenic germline variants in other genes or polygenic risk score.

Main Text

Approximately 5–10% of all breast cancer cases are familial (1–3), however, less than 17–28% are attributed to inherited mutations in the *BRCA1* and *BRCA2* susceptibility genes (4–6). This challenges clinical genetic counselling of families with a strong history of breast cancer without identified germline mutations in *BRCA1* and *BRCA2* (herewith referred to as non-*BRCA1/BRCA2* high-risk families). Recent studies using whole-genome sequencing (WGS) have resulted in the comprehensive landscape of somatic mutations revealing the mutational processes that have left specific mutational signatures in the tumours. These signatures may be predictive for treatment response. HRDetect is a robust prediction model incorporating mutational signatures, HRD-index and deletion of microhomology (7). HRDetect has been shown to be predictive among non-*BRCA1/BRCA2* patients for response to platinum-based chemotherapy (8). A recent clinical trial demonstrated that breast cancer patients with germline mutations in BRCA1 or BRCA2 benefit from Poly(adenosine diphosphate–ribose) polymerase (PARP) inhibitor treatment (9). Non-*BRCA1/BRCA2* patients with a high HRDetect score may potentially also benefit from this treatment.

In this study, we performed whole-genome sequencing of flash frozen primary breast samples and matched normal blood samples from 23 breast cancer patients from high-risk breast and ovarian cancer families screened negative for mutations in *BRCA1* and *BRCA2* together with seven patients carrying a pathogenic *BRCA1* or *BRCA2* variant (Supplementary Figure S1, Supplementary Table S1-S2). We identified somatic substitutions, insertions and deletions (indels) and rearrangements in our cohort (Fig. 1e-g, Supplementary Figure S2-3, Supplementary Table S3) and fitted the catalogues of somatic mutations to the previously identified substitution and rearrangement signatures in breast cancer (Fig. 1c, Supplementary Figure S4) using a mathematical model (10). Unsupervised hierarchical clustering was applied to stratify the tumours based on the somatic mutational signatures (Fig. 1a and 1c). Furthermore, we applied the HRDetect model to identify *BRCA1/BRCA2*-deficient tumours (BRCAness) driving tumorigenesis by defective homologous recombination (7).

We observed that HRDetect scores resulted in a very strong separation of tumours with all *BRCA1* and *BRCA2* positive tumours having a HRDetect score of > 0.99 (Fig. 1d). Furthermore, clustering supported that *BRCA1/BRCA2*-mutated tumours are clearly separated from non-*BRCA1/BRCA2* tumours. However,

three non-*BRCA1/BRCA2* tumours had a HRDetect score of > 0.99 and were thus identified as having *BRCA1/BRCA2*-deficient tumours and clustered together with *BRCA1/BRCA2* tumours (cluster D in Fig. 1, Supplementary Table S4). All three tumours had *BRCA1*-like characteristics i.e. were triple negative (negative estrogen/progesterone receptor (ER/PR) and HER2 receptor status normal), basal-like and had *BRCA1* loss of heterozygosity (LOH). Promoter hypermethylation of *BRCA1* could explain BRCA-deficiency in two of these tumours and in the third a somatic *BRCA1* missense variant of unknown significance (VUS), might in principle explain the BRCA-deficiency in this tumour. However, the variant is located in exon 11 that although containing more than half of the coding region of *BRCA1*, does not contain reported pathogenic germline missense mutations. Low variant allele frequency and a high copy-number level in the *BRCA1* region questions if the variant is causal for the high HRDetect score.

The clustering also revealed a group of tumours with high mutational burden and high level of the APOBEC associated substitution signature (SBS) 13 but low HRDetect score (cluster C in Fig. 1). These tumours more frequently harboured *BRCA1/BRCA2* LOH, *TP53* mutations and had negative ER/PR and positive HER2 status. Finally, the clustering revealed two clusters of non-*BRCA1/BRCA2* tumours with distinct molecular profiles not related to known molecular subtypes (cluster A and B in Fig. 1). These tumours all had very low mutational burden, low HRDetect scores (0.03 or less) and had higher frequency of somatic *PIK3CA* mutations and lower frequency of *TP53* mutations compared to tumours with high mutational burden (Fig. 1, Supplementary Figure S2, Supplementary Table S4-S5). Cluster A was mainly described by SBS 1 and 5, lack of rearrangement signature (RS) 2, were mainly luminal A or B, and commonly had *CDH1* mutations. Cluster B was primarily defined by SBS 1 and 5, RS 2, and were mainly luminal B. Cluster B clustered close to cluster C containing tumours with high mutational burden, as these tumours also had high proportions of RS 2 (Fig. 1).

We also integrated the results from our previously published RNA classifier to classify basal-like tumours as *BRCA1*-like or non-*BRCA1*-like, and LumB-subtype tumours as *BRCA2*-like or non-*BRCA2* like. (11, 12). The RNA classifier identified four basal-subtype tumours as *BRCA1*-like, and one LumB-subtype tumour as *BRCA2*-like (Fig. 1). However, compared to HRDetect the RNA classifier had lower accuracy for *BRCA1/BRCA2*-deficient tumours as it failed to classify one *BRCA1* positive tumour as *BRCA1*-like. The RNA classifier also did not classify the tumour, harbouring the somatic *BRCA1* VUS, as *BRCA1*-like. The RNA classifier also identified a rather low fraction of BRCAness among non-*BRCA1/BRCA2* cases (22%) though higher than HRDetect (13%). Further studies are required to investigate if BRCAness at the RNA level exists due to other mechanisms than mutational patterns.

Germline variants could only explain few cases of familial aggregation. We identified rare germline variants in the *FANCD2*, *RAD51D*, *TP53*, *SLX4*, *MSH6* and *CHEK2* genes of which *TP53* and *CHEK2* are likely to contribute to the familial aggregation (Fig. 2, Supplementary Table S6). Furthermore, we applied the polygenic risk score (PRS) (13) incorporated in BOADICEA (14) to obtain the estimated lifetime risk and the combined risk with family history. The patient carrying the pathogenic *CHEK2* mutation had a high PRS score resulting in an estimated lifetime risk of 57%, where PRS contributed with 22% (patient 39, Fig. 2). This was further supported by bilateral breast cancer of the patient and a family history with

multiple breast cancer cases. The remaining patients showed little or negative effect contributed by the PRS (Fig. 2, Supplementary Figure S1, Supplementary Table S1).

The finding of low fraction of non-*BRCA1/BRCA2* familial breast cancers with high HRDetect score is striking. One might expect non-*BRCA1/BRCA2* high-risk familial breast tumours to have high HRDetect score similar to tumours from families with mutations in these high-penetrant genes. Especially, considering that our families are selected based on a combination of multiple breast cancer cases, early onset of breast cancer and ovarian cancer in the families very similar to families with a *BRCA1/BRCA2* phenotype.

To the best of our knowledge, only one other study by Nones et al. (15) has applied WGS to characterise familial breast cancer. Their study included 30 familial non-*BRCA1/BRCA2* breast tumours. They identified 4 (13%) non-*BRCA1/BRCA2* tumours with high *BRCA1/BRCA2* HRDetect score that could not be explained by promotor methylation or somatic mutations. This is a slightly higher fraction than in our material where only one such tumour (4%) was identified and even that harboured a somatic VUS in *BRCA1*. Nones et al. also identified a cluster of silent tumours with RS 2 like we did (cluster B in Fig. 1). However, they also found a large cluster of 13/30 (43%) tumours with high contribution of RS 4 affecting known driver genes. We only identified 3/23 (13%) tumours having high proportions of RS 4. The differences between the two studies might to some extend be explained by the approaches used to identify mutational signatures. Nones et al. extracted novel signatures and correlated these to the known COSMIC signatures, whereas we opted to fit the catalogue of somatic mutations directly to the known breast cancer signatures as this is more suitable for small sample sizes (10, 16, 17). Their study population of non-*BRCA1/BRCA2* tumours had very similar sample size to our study. However, both studies are statistically underpowered to draw significant conclusions. Nevertheless, their findings of limited BRCAness among cases with suspicion of hereditary breast cancer is very similar to ours. The combined results from the two studies strongly indicates low frequency of BRCAness among non-*BRCA1/BRCA2* familial breast cancer patients.

Further larger studies are demanded to validate these findings and to identify the missing genetic cause. Critically, whatever these unknown genetic factors are, they clearly drive the increased risk of carcinogenesis through other pathophysiological mechanisms than through mutagenesis. The identified substructures among the non-*BRCA1/BRCA2* tumors may point to common genetic mechanisms within the subgroups. Our results may also be relevant for future treatment decision. A potential benefit of platinum-based chemotherapy has been reported for non-*BRCA1/BRCA2* patients with high HRDetect score compared to those with low HRDetect score (8). PARP inhibitor treatment, which is routinely used for *BRCA1/BRCA2*-deficient ovarian cancer patients (18, 19), has recently been shown to also benefit *BRCA1/BRCA2*-deficient breast cancer patients (9).

Declarations

Ethics approval and consent to participate

The study was approved by the Danish Ethical Committee System (case no. S-20150167). All patients were informed in writing and following oral consultation. All patients have provided written consent before inclusion in the study.

Consent for publication

Not applicable

Availability of data and materials

The datasets generated and/or analysed during the current study are not publicly available since publication of individual genome sequencing data is not approved by the Danish Data Protection Agency for this study.

Competing interests

The authors declare that they have no competing interests

Funding

This work was supported by funding from Danish Cancer Society (Journal number R146-A9247-16-S2), Dansk Kræftforskningfond (Journal number 153/1), The Odense University Hospital Fund for Free Research, Faculty of Health Sciences at University of Southern Denmark, Karen Elise Jensen Foundation, Dagmar Marshalls Foundation, Snedkermester Sophus Jacobsens og hustru Astrid Jacobsens Foundation, Arvid Nilssons Foundation and Overlægerådets Research Foundation.

Authors' contributions

LvBA, MJL, TAK and MT designed the study and performed the main data interpretation. HRN, LK and AMG performed patient information. AVL collected and characterized tumour samples. LvBA performed the data analysis with guidance and help from HD, AD and SNZ. LvBA wrote the manuscript and the other authors read, commented on and approved the final manuscript.

Acknowledgements

We wish to thank the patients for participating in the project.

Materials And Methods

Patient material

In this study, 23 non-*BRCA1/BRCA2* patients from families with a strong history of breast cancer, previously included in a study predicting BRCAness (12), were selected where matched tumour and blood samples were available. Inclusion criteria to enter the study were 1) a pedigree indicating monogenic inheritance of breast cancer predisposition, or 2) presence of ovarian cancer in pedigrees with breast

cancer cases, or 3) a very young age at diagnosis of breast cancer (<30 years). Furthermore, four *BRCA1* and three *BRCA2* patients carrying a pathogenic BRCA1/BRCA2 variant with unknown family history were selected as controls for BRCAness classification. All tumour tissues were freshly frozen primary breast tumours collected between 1982 and 2008 in Odense and had been stored in the tumour biobanks of Department of Pathology, Odense University Hospital and Danish Breast Cancer Cooperative Group (DBCG). Data for Immunohistochemistry (IHC) of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) status was received from DBCG. The ER, PR and HER2 hormone receptor status not identified by the pathological review were estimated from gene expression levels of *ESR1, PGR* and *ERBB2*. The PAM50 subtypes were also classified for all samples from the gene expression (Supplementary Table S1).

Family risk from BOADICEA breast cancer estimation model

The Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm (BOADICEA) was used to validate the increased risk of breast cancer in the patients based on their family history (14). Five patients did not show increased risk of breast cancer according to BOADICEA but were still included due to either early-onset breast cancer, bilateral breast cancer, multiple breast or ovarian cancers in the family, or a combination of those (Supplementary Figure S1, Supplementary Table S1).

Whole-genome sequencing (WGS)

Sample preparation was performed using Illumina TruSeq Nano protocol with 550 bp insert length to strengthen detection of structural variants. Samples were sequenced on Illumina Novaseq 6000 with paired-end 2x150bp. The average sequencing coverage was 50.2X for tumour samples and 38.5X for normal samples (Supplementary Table S2).

Gene expression

Gene expression analysis Gene expression analysis was performed using a customized version of Agilent SurePrint G3 Human GE 8x60K Microarray and raw data were pre-processed as previously described (11). Microarray data have been deposited to the Gene Expression Omnibus (GSE49481).

Alignment of WGS data

The paired-end reads resulting from the sequencing was aligned to the human reference genome (GRCh37) using BWA-MEM v0.7.17. The specific version used can be found in the cgpmap-3.0.4 docker image (https://dockstore.org/containers/quay.io/wtsicgp/dockstore-cgpmap:3.0.4).

Processing of WGS data

The whole-genome sequencing data was processed using the same bioinformatic pipeline as in Nik-Zainal et al. (20).

CaVEMan (Cancer Variants Through Expectation Maximization: http://cancerit.github.io/CaVEMan/) was used for calling somatic and germline single nucleotide variants (SNVs). A lightly modified version of Pindel 2.0 (http://cancerit.github.io/cgpPindel/) was used for calling somatic and germline insertions and deletions (indels).

BRASS (BReakpoint AnalySiS: https://github.com/cancerit/BRASS) was used to detect rearrangements and other structural variants.

The Battenberg algorithm (https://github.com/cancerit/cgpBattenberg) was used for the detection of copy number variation in matched tumour-normal samples.

The specific versions of the tools used are found in the cgpwgs-2.1.0 docker image (https://dockstore.org/containers/quay.io/wtsicgp/dockstore-cgpwgs:2.1.0).

Filtering variants

Germline variants

Germline variants were filtered using a candidate gene list of 170 pathogenic and likely pathogenic germline variants associated with hereditary cancer (21). Then filters were applied keeping only frameshift, splice-site, and nonsynonymous variants with strong bioinformatic prediction and with frequency <0.01 according to gnomAD and ExAC (22). The variants were evaluated using the variant databases ClinVar and HGMD, and six missense variant predictors implemented in VarSeq. Loss-of-function (protein truncating) and splice variants, variants with strong bioinformatic prediction, and variants in genes associated with breast cancer risk with an odds ratio above two (23) were selected for further investigation.

Somatic variants

Somatic variants were filtered using the default settings of the tools in the bioinformatic pipeline. Somatic driver mutations were identified by filtering the list of somatic variants for the driver genes previously identified in 560 breast cancers using identical criteria for reporting a driver event as in (20).

Polygenic risk score

We applied the polygenic risk score with 313 SNPs (PRS_{313}) developed for breast cancer risk prediction (13) incorporated in the latest version of BOADICEA (14) to predict the risk of getting breast cancer for each individual in our cohort under the assumption that they did not already develop breast cancer.

Mutational signatures

We applied a mathematical model (10) implemented in the Signature Tools Lib R package (24) (https://github.com/Nik-Zainal-Group/signature.tools.lib) to fit substitution and rearrangement signatures imprinted in the breast cancer genomes i.e. first a catalogue of substitutions and rearrangements was

created for each sample and then fitted using bootstrap for robustness to the twelve substitution and six rearrangement signatures previously identified (20).

Stratification of tumours using unsupervised hierarchical clustering

Unsupervised hierarchical clustering with Euclidean distance and Ward's linkage criterion (ward.D2 in the statistical programming language R) was used to stratify the breast cancer tumours. We incorporated both substitution and rearrangement signatures in the clustering. To make the signatures comparable, we needed to normalise the signatures to correct for the fact that cancer genomes often carry more substitution than rearrangement signatures thereby giving higher weight to the rearrangement signatures in the clustering. Proportions of signatures were normalised by dividing all substitution and rearrangement signatures by the highest proportion identified in their respective mutation categories.

BRCAness: HRDetect and our RNA classifier

The HRDetect model for detection of *BRCA1/BRCA2*-deficient tumours (7) was applied to the patient cohort. The HRDetect model incorporates information from substitution and rearrangement signatures, HRD score and deletion of microhomology and computes the probability of each tumour being *BRCA1/BRCA2*-deficient. We used the HRDetect model implemented in the Signature Tools Lib R package (24).

We included the BRCAness classification from our in-house developed RNA classifier published in an earlier study (12). The RNA classifier has been developed to classify basal and LumB subtype tumours i.e. basal-like tumours can be classified as either *BRCA1*-like or non-*BRCA1*-like, and LumB-subtype tumours can be classified as either *BRCA2*-like or non-*BRCA1/BRCA2* like. Other subtypes are not yet supported. Molecule subtypes were identified using PAM50 as previously described (11).

Detection of promotor methylation

Detection of promotor methylation of the breast cancer predisposition genes *BRCA1* and *BRCA2* in the patients was done in an earlier study using MLPA (12).

Whole-genome profiles and heatmap figures

Breast cancer whole-genome profiles were created using the Signature Tools Lib R package (24) and are presented in Supplementary Figure S3. Heatmaps and stacked figures (Figures 1-3 and Supplementary Figure S1) were created using the ComplexHeatmap R package (25).

Additional information about variant interpretation

We identified very few rare germline variants in known breast cancer candidate genes. In one family, a well-known pathogenic mutation in *CHEK2 (26-28)* was found as well as high PRS score resulting in a predicted life-time risk of 57%. In another family, a missense TP53 germline variant, previously shown to be deleterious in functional assay (29), accompanied by a somatic second hit in *TP53* is very likely to

explain the extreme early onset breast cancer at the age of 29 years. The effect of loss of function mutations identified in the candidate genes *FANCD2, RAD51D, SLX4, MSH6* is less clear.

These variants included loss of function variants in *CHEK2*, *FANCD2*, *RAD51D* and *SLX4*. In addition to the deleterious variant in *CHEK2* we identified in another patient an in-frame *CHEK2* deletion of unknown significance, c.246_260delCCAAGAACCTGAGGA previously shown to have intermediate functional impact (27). In another family, two affected members both carried a *MSH6* missense variant of unknown significance (VUS) c.1813A>G, p.Thr605Ala, predicted deleterious by 5 of 6 bioinformatic predictors. No MMR signatures were identified indicating that the variant might not be pathogenic.

FANCD2 and *SLX4* are well-established Fanconi Anemia genes similar to several other breast cancer genes. Nevertheless, mutations in these genes are expected to have low penetrance for breast cancer (23, 30-34). In combination with other genetic risk factors e.g. a high PRS this might explain the strong familial phenotype. However, the contribution from PRS estimated from BOADICEA was minor. Nevertheless, the included families had pedigrees indicative of a strong pattern of inheritance, and therefore other yet unknown genetic risk factors are likely to play a role in these families.

Our study also indicates that tumours with pathogenic mutations in *TP53* and *CHEK2*, which are associated with DNA-damage signalling and detection of double-stranded breaks, did not classify as *BRCA1/BRCA2*-deficient tumours according to both prediction models tested. This confirms findings from earlier studies (7, 35).

The only tumour with high HRDetect score and no clear *BRCA1/BRCA2* inactivating mechanism (germline variant or methylation) had a somatic VUS in BRCA1. The variant is located on exon 11 that although containing more than half of the coding region of BRCA1, does not contain reported pathogenic germline missense mutations. Low allele frequency and a high copy-number level in the *BRCA1* region questions if the variant is causal for the high HRDetect score. Our finding of low BRCAness measured by HRDetect among non-*BRCA1/BRCA2* familial cancer indicates a low false positive rate for classification of VUS in this clinically relevant patient group. Although HRDetect have a strong potential for classification of VUS our case illustrates that caution should be taken for this approach.

References

1. Hall MJ, Reid JE, Burbidge LA, Pruss D, Deffenbaugh AM, Frye C, et al. BRCA1 and BRCA2 mutations in women of different ethnicities undergoing testing for hereditary breast-ovarian cancer. Cancer. 2009;115(10):2222-33.
2. Honrado E, Benítez J, Palacios J. The molecular pathology of hereditary breast cancer: genetic testing and therapeutic implications. Modern Pathology. 2005;18(10):1305-20.
3. Larsen MJ, Thomassen M, Gerdes AM, Kruse TA. Hereditary breast cancer: clinical, pathological and molecular characteristics. Breast cancer : basic and clinical research. 2014;8:145-55.

4. Prevalence and penetrance of BRCA1 and BRCA2 mutations in a population-based series of breast cancer cases. Anglian Breast Cancer Study Group. British journal of cancer. 2000;83(10):1301-8.
5. Gerdes AM, Cruger DG, Thomassen M, Kruse TA. Evaluation of two different models to predict BRCA1 and BRCA2 mutations in a cohort of Danish hereditary breast and/or ovarian cancer families. Clinical genetics. 2006;69(2):171-8.
6. Melchor L, Benitez J. The complex genetic landscape of familial breast cancer. Hum Genet. 2013;132(8):845-63.
7. Davies H, Glodzik D, Morganella S, Yates LR, Staaf J, Zou X, et al. HRDetect is a predictor of BRCA1 and BRCA2 deficiency based on mutational signatures. Nat Med. 2017;23(4):517-25.
8. Zhao EY, Shen Y, Pleasance E, Kasaian K, Leelakumari S, Jones M, et al. Homologous Recombination Deficiency and Platinum-Based Therapy Outcomes in Advanced Breast Cancer. Clin Cancer Res. 2017;23(24):7521-30.
9. Tutt ANJ, Garber JE, Kaufman B, Viale G, Fumagalli D, Rastogi P, et al. Adjuvant Olaparib for Patients with BRCA1- or BRCA2-Mutated Breast Cancer. N Engl J Med. 2021;384(25):2394-405.
10. Huang P-J, Chiu L-Y, Lee C-C, Yeh Y-M, Huang K-Y, Chiu C-H, et al. mSignatureDB: a database for deciphering mutational signatures in human cancers. Nucleic Acids Research. 2017;46(D1):D964-D70.
11. Larsen MJ, Kruse TA, Tan Q, Laenkholm AV, Bak M, Lykkesfeldt AE, et al. Classifications within molecular subtypes enables identification of BRCA1/BRCA2 mutation carriers by RNA tumor profiling. PLoS One. 2013;8(5):e64268.
12. Larsen MJ, Thomassen M, Tan Q, Laenkholm AV, Bak M, Sorensen KP, et al. RNA profiling reveals familial aggregation of molecular subtypes in non-BRCA1/2 breast cancer families. BMC Med Genomics. 2014;7:9.
13. Mavaddat N, Michailidou K, Dennis J, Lush M, Fachal L, Lee A, et al. Polygenic Risk Scores for Prediction of Breast Cancer and Breast Cancer Subtypes. The American Journal of Human Genetics. 2019;104(1):21-34.
14. Lee A, Mavaddat N, Wilcox AN, Cunningham AP, Carver T, Hartley S, et al. BOADICEA: a comprehensive breast cancer risk prediction model incorporating genetic and nongenetic risk factors. Genet Med. 2019.
15. Nones K, Johnson J, Newell F, Patch AM, Thorne H, Kazakoff SH, et al. Whole-genome sequencing reveals clinically relevant insights into the aetiology of familial breast cancers. Ann Oncol. 2019.
16. Alexandrov LB, Nik-Zainal S, Wedge DC, Campbell PJ, Stratton MR. Deciphering signatures of mutational processes operative in human cancer. Cell Rep. 2013;3(1):246-59.
17. Rosenthal R, McGranahan N, Herrero J, Taylor BS, Swanton C. DeconstructSigs: delineating mutational processes in single tumors distinguishes DNA repair deficiencies and patterns of carcinoma evolution. Genome Biol. 2016;17:31.
18. Mittica G, Ghisoni E, Giannone G, Genta S, Aglietta M, Sapino A, et al. PARP Inhibitors in Ovarian Cancer. Recent Pat Anticancer Drug Discov. 2018;13(4):392-410.

19. Yap TA, Plummer R, Azad NS, Helleday T. The DNA Damaging Revolution: PARP Inhibitors and Beyond. Am Soc Clin Oncol Educ Book. 2019;39:185-95.
20. Nik-Zainal S, Davies H, Staaf J, Ramakrishna M, Glodzik D, Zou X, et al. Landscape of somatic mutations in 560 breast cancer whole-genome sequences. Nature. 2016;534(7605):47-54.
21. Bertelsen B, Tuxen IV, Yde CW, Gabrielaite M, Torp MH, Kinalis S, et al. High frequency of pathogenic germline variants within homologous recombination repair in patients with advanced cancer. npj Genomic Medicine. 2019;4(1):13.
22. Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, et al. Analysis of protein-coding genetic variation in 60,706 humans. Nature. 2016;536:285.
23. Lu HM, Li S, Black MH, Lee S, Hoiness R, Wu S, et al. Association of Breast and Ovarian Cancers With Predisposition Genes Identified by Large-Scale Sequencing. JAMA Oncol. 2019;5(1):51-7.
24. Degasperi A, Amarante TD, Czarnecki J, Shooter S, Zou X, Glodzik D, et al. A practical framework and online tool for mutational signature analyses show inter-tissue variation and driver dependencies. Nat Cancer. 2020;1(2):249-63.
25. Gu Z, Eils R, Schlesner M. Complex heatmaps reveal patterns and correlations in multidimensional genomic data. Bioinformatics. 2016;32(18):2847-9.
26. Vahteristo P, Bartkova J, Eerola H, Syrjäkoski K, Ojala S, Kilpivaara O, et al. A CHEK2 Genetic Variant Contributing to a Substantial Fraction of Familial Breast Cancer. The American Journal of Human Genetics. 2002;71(2):432-8.
27. Bell DW, Kim SH, Godwin AK, Schiripo TA, Harris PL, Haserlat SM, et al. Genetic and functional analysis of CHEK2 (CHK2) variants in multiethnic cohorts. Int J Cancer. 2007;121(12):2661-7.
28. Schmidt MK, Hogervorst F, van Hien R, Cornelissen S, Broeks A, Adank MA, et al. Age- and Tumor Subtype-Specific Breast Cancer Risk Estimates for CHEK2*1100delC Carriers. J Clin Oncol. 2016;34(23):2750-60.
29. Giacomelli AO, Yang X, Lintner RE, McFarland JM, Duby M, Kim J, et al. Mutational processes shape the landscape of TP53 mutations in human cancer. Nat Genet. 2018;50(10):1381-7.
30. Wasielewski M, Riaz M, Vermeulen J, van den Ouweland A, Labrijn-Marks I, Olmer R, et al. Association of rare MSH6 variants with familial breast cancer. Breast Cancer Res Treat. 2010;123(2):315-20.
31. Sun J, Meng H, Yao L, Lv M, Bai J, Zhang J, et al. Germline Mutations in Cancer Susceptibility Genes in a Large Series of Unselected Breast Cancer Patients. Clin Cancer Res. 2017;23(20):6113-9.
32. Shah S, Kim Y, Ostrovnaya I, Murali R, Schrader KA, Lach FP, et al. Assessment of SLX4 Mutations in Hereditary Breast Cancers. PLoS One. 2013;8(6):e66961.
33. Li Q, Luo C, Luo C, Wang J, Li B, Ding L, et al. Disease-specific hematopoietic stem cell transplantation in children with inherited bone marrow failure syndromes. Ann Hematol. 2017;96(8):1389-97.

34. Mantere T, Tervasmaki A, Nurmi A, Rapakko K, Kauppila S, Tang J, et al. Case-control analysis of truncating mutations in DNA damage response genes connects TEX15 and FANCD2 with hereditary breast cancer susceptibility. Sci Rep. 2017;7(1):681.
35. Mandelker D, Kumar R, Pei X, Selenica P, Setton J, Arunachalam S, et al. The Landscape of Somatic Genetic Alterations in Breast Cancers from CHEK2 Germline Mutation Carriers. JNCI Cancer Spectr. 2019;3(2):pkz027.
36. Danckert B FJ, Engholm G , Hansen HL, Johannesen TB, Khan S, Køtlum JE, Ólafsdóttir E, Schmidt LKH, Virtanen A and Storm HH. NORDCAN: Cancer Incidence, Mortality, Prevalence and Survival in the Nordic Countries, Version 8.2 (26.03.2019). Association of the Nordic Cancer Registries. Danish Cancer Society; [Acessed on 18.06.2019].

Figures

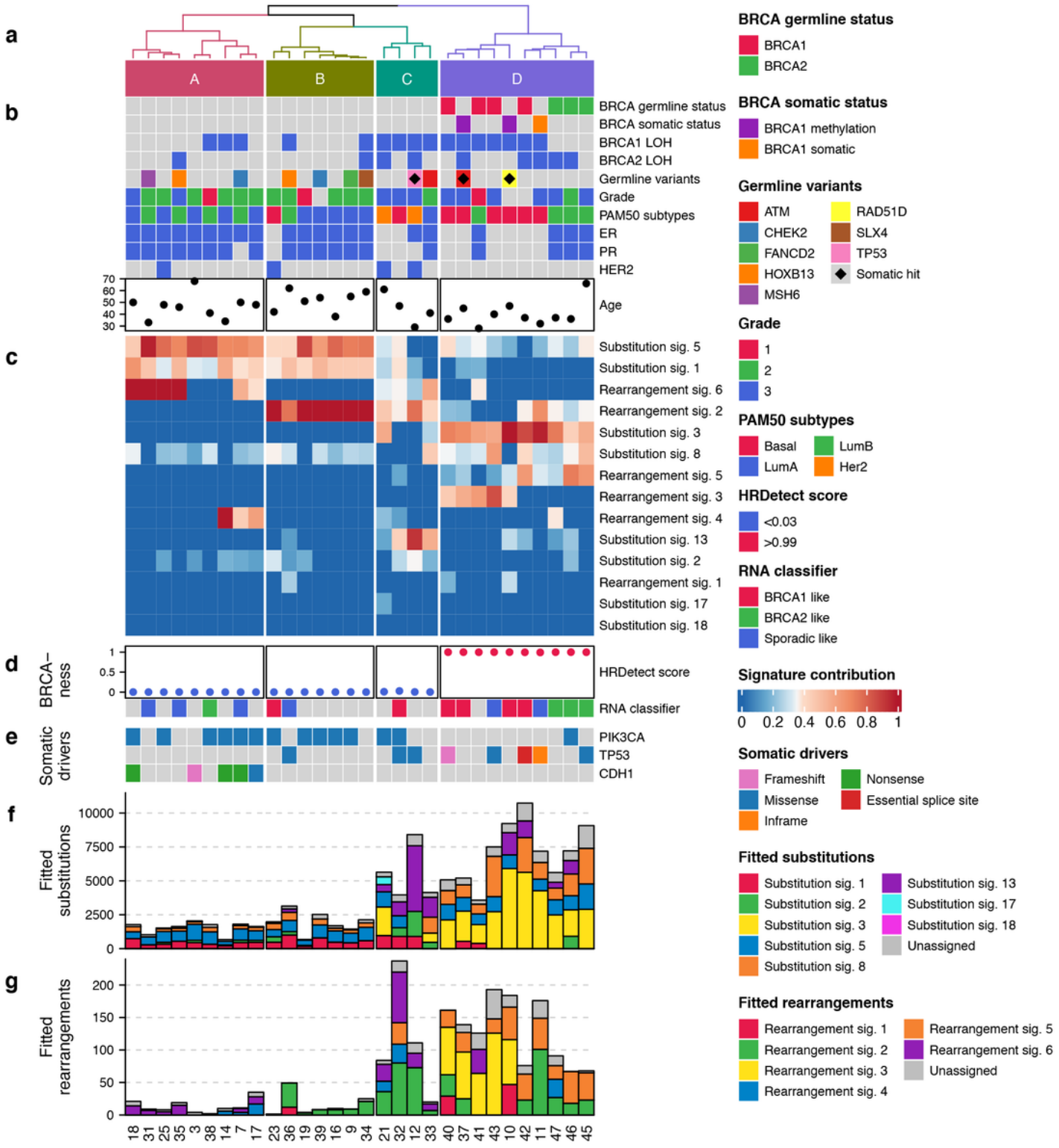

Figure 1

Unsupervised hierarchical clustering based on proportions of mutational signatures in each tumour. (a) Unsupervised hierarchical clustering on substitution and rearrangement signatures revealing four main clusters. (b) Clinical and mutational annotation for each sample. (c) Heatmap of normalised contribution of substitution and rearrangement signatures identified in the cohort. (d) BRCAness predictions: HRDetect prediction score and RNA classifier predictions. (e) Somatic substitution and indel driver mutations

present in more than three samples. (f) Number of substitutions fitted to mutational signatures for each tumour. (g) Number of rearrangements fitted to mutational signatures for each tumour.

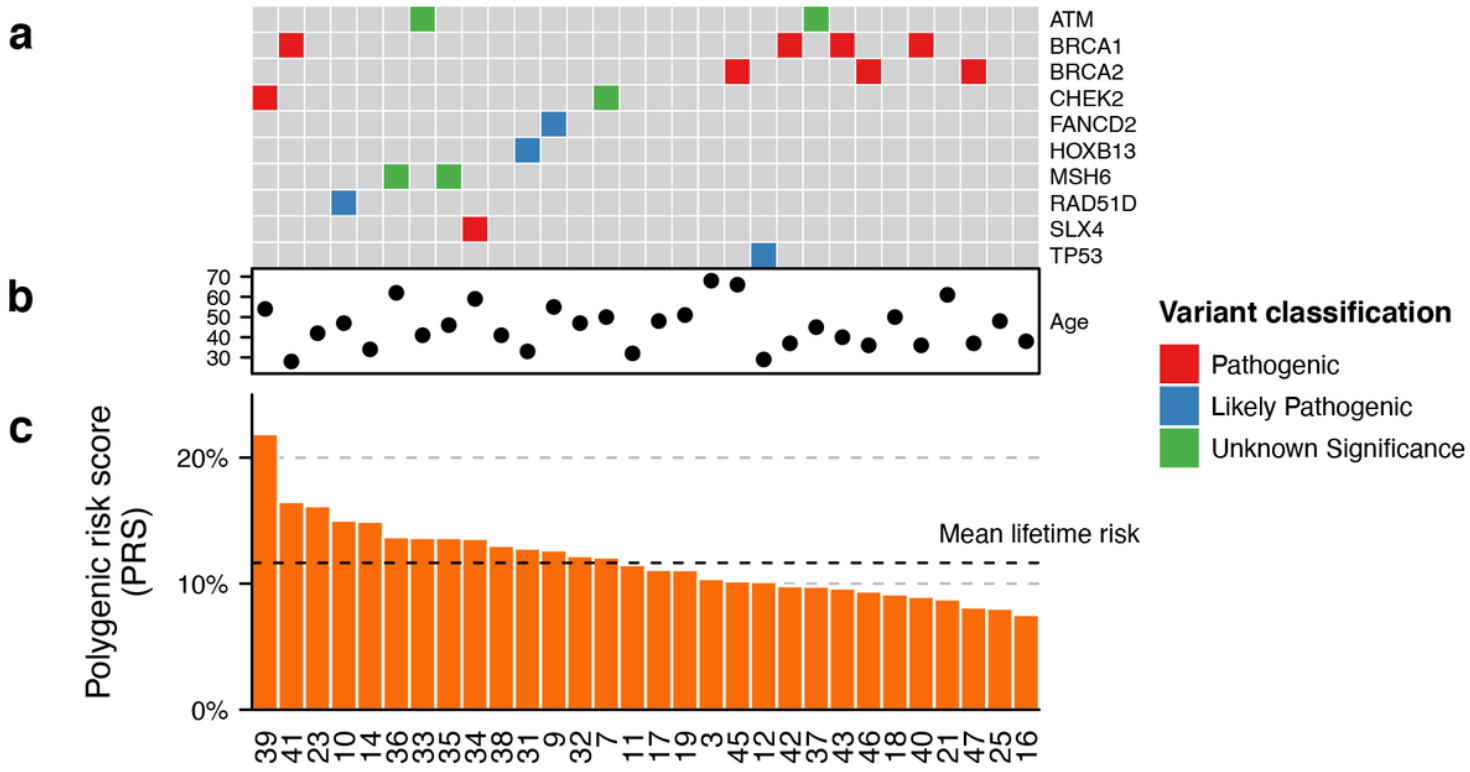

Figure 2

Germline variants and PRS predicted lifetime risk (20-80 years) of breast cancer in high-risk familial breast cancer patients. Samples are ordered according to PRS score. a) Germline variants possibly contributing to increased breast cancer family risk. Potential breast cancer predisposing genes were included if they are reported pathogenic/likely pathogenic in ClinVar. VUS were included for known moderate and high-risk genes. b) Age at diagnosis. c) Predicted lifetime risk of breast cancer by PRS score. The dashed line represents the mean lifetime risk (0-79 years) of breast cancer in the Danish population of 11.7% (36).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFigureS1.docx
- SupplementaryFigureS2.docx
- SupplementaryFigureS3.docx
- SupplementaryFigureS4.docx
- SupplementaryTableS1.xlsx
- SupplementaryTableS2.xlsx

- SupplementaryTableS3.xlsx
- SupplementaryTableS4.xlsx
- SupplementaryTableS5.xlsx
- SupplementaryTableS6.xlsx