Genetics in Medicine Open (2024) 2, 100849

Genetics in Medicine OPEN
An Official Journal of the ACMG
www.journals.elsevier.com/genetics-in-medicine-open

ARTICLE

Population-based germline testing of *BRCA1*, *BRCA2*, and *PALB2* in breast cancer patients in the United Kingdom: Evidence to support extended testing, and definition of groups who may not require testing

D. Gareth Evans[1,2,3,4,*], Emma R. Woodward[1,2], George J. Burghel[1], Sophie Allen[5], Beth Torr[5], Monica Hamill[5], Grace Kavanaugh[5], Mike Hubank[5], Stephen Bremner[6], Christopher I. Jones[6], Helene Schlecht[1], Susan Astley[7], Sarah Bowers[3], Sarah Gibbons[3], Helen Ruane[3], Caroline Fosbury[3], Sacha J. Howell[3,4,8], Claire Forde[1], Fiona Lalloo[1], William G. Newman[1,2], Miriam J. Smith[1,2], Anthony Howell[3,4,8], Clare Turnbull[5], Ashu Gandhi[3,4,8]

ARTICLE INFO

Article history:
Received 25 February 2023
Received in revised form
13 November 2023
Accepted 13 November 2023
Available online 25 November 2023

Keywords:
BRCA1
BRCA2
Breast cancer
Manchester score
PALB2 population testing

ABSTRACT

Purpose: To assess the contribution of germline pathogenic variants (PVs) in population-based series of breast cancers and the best strategy to improve detection rates.

Methods: Three cohort studies were utilized, including a hospital-based series identified from new UK mainstream testing criteria (group-1), offering testing to all women (group-2-BReast CAncer [BRCA]-DIRECT), and a Greater Manchester cohort study recruited from the mammography screening population (group-3-Predicting Risk of Cancer at Screening). DNA samples from women with breast cancer were sequenced for PVs in *BRCA1*, *BRCA2*, and Partner and Localiser of BRCA2 (*PALB2*). The Manchester score (MS) was used at different points thresholds. Current mainstream criteria include women diagnosed <40 years and all triple negative <60 years or an MS ≥15.

Results: Thirty-six PVs (*BRCA1* = 9, *BRCA2* = 18, *PALB2* = 9) were identified among 1061 women with breast cancer (3.4%). Mainstreaming criteria identified 21 of 36 (58%) of PVs by testing 190 women; detection rate (8.4%), specificity = 83.5%. A better detection rate was found using an MS threshold of 12-points with 66.7% (24/36) sensitivity and 85.7% specificity in 171 women. No PVs were identified in 158 women with grade-1 invasive cancers. The best strategy to detect all PVs was an MS ≥3 with specificity of 32.6%.

Conclusion: In order to detect higher PV rates on a population basis the best strategy is to reduce the MS threshold for genetic testing.

This article was invited and the Article Publishing Charge (APC) was waived.

*Correspondence and requests for materials should be addressed to D. Gareth Evans, Department of Genetic Medicine, Manchester Academic Health Sciences Centre (MAHSC), St Mary's Hospital, University of Manchester, Manchester M13 9WL, UK. *Email address:* gareth.evans@mft.nhs.uk; Gareth.d.evans@manchester.ac.uk
Affiliations are at the end of the document.

doi: https://doi.org/10.1016/j.gimo.2023.100849

Introduction

Current guidelines for germline genetic testing for highly penetrant, high-risk breast cancer genes in most of the world are based on algorithms that assess a likelihood of a woman carrying a germline pathogenic variant (PV) in the genes tested. For breast cancer in Europe, testing strategies are primarily targeted at individuals affected with cancer and testing of relatives is only usually offered if a PV is identified in an index relative.[1,2] More recently updated European guidelines are now strongly driven by the potential for gene-level driven management strategies, such as Poly (ADP-ribose) polymerase (PARP) inhibitors (PARPi) in the person with breast cancer.[2] Testing in England was updated in April 2022 to make it more widely available in women with breast cancer and it now includes all women diagnosed at <40 years, except those with grade-1 disease (https://www.england.nhs.uk/wp-content/uploads/2018/08/rare-and-inherited-disease-eligibility-criteria-v4.pdf). Testing at a population level in Israel has been started based on health economic data and the known 2.5% frequency of founder variants in *BRCA1/2* in the Ashkenazim.[3-5] Despite advanced plans to roll this out in England, it is likely to be some time before total population testing for breast cancer predisposition genes is implemented outside of specific populations. Until the issue of variants of uncertain significance is resolved, there remains some reluctance, among geneticists in particular, to role this out. In view of the increasing evidence of efficacy for PARPi in earlier stage breast cancer,[6] further expansion of testing criteria is likely to be implemented. The main question is whether this should be provided to all people with breast cancer or whether a threshold should be used, based on overall likelihood of *BRCA1/2* and *PALB2* variants, perhaps incorporating likely future benefit from PARPi. In view of this, we have assessed population testing of *BRCA1/2* and *PALB2* variants in North-West England in over 1000 affected women with breast cancer.

Materials and Methods

Patient cohorts

The population-based patient cohorts were drawn from 3 sources:

1. Women diagnosed with breast cancer meeting NHS England Mainstreaming criteria (Table 1) at Manchester NHS Foundation Trust (MFT) and where samples were available for genetic testing between October 2021 and November 2022.
2. All women diagnosed with breast cancer in follow-up after primary treatment at MFT consenting to the BRCA-DIRECT study[7] between December 2021 and August 2022.
3. Women identified from the Predicting Risk of Cancer at Screening (PROCAS) population-based study of women in the NHS Breast Screening Programme.[8] This included prevalent cases at recruitment (2009-2014) and incident cases after recruitment in women aged 46 to 79 years at recruitment.

The combination of group 1 and 2 means that all women with breast cancer were eligible.

Clinical or research consent was given for testing of breast cancer associated genes [Research Ethics Committee, reference 09/H1008/81 (PROCAS) and 08/H1006/77 and Trust Committee for Clinical Research, reference CCR5234, and the Research Ethics Committee, reference 20/LO/1200].

Genetic testing

For group 1, DNA was extracted from lymphocytes and tested using a combination of targeted sequencing and multiplex ligation-dependent probe amplification.[9] All participants underwent testing of *BRCA1*, *BRCA2*, *CHEK2, ATM,* and *PALB2*. *CHEK2* and *ATM* were only tested from April 2022 and are not reported here.

For group 2, women had DNA extracted from saliva samples. Samples were tested as part of the BRCA-DIRECT study using a bespoke sequencing panel for the *BRCA1, BRCA2*, and *PALB2* genes, as previously described.[7]

For group 3, women had DNA extracted from saliva samples. Samples were tested as part of the Breast cancer Risk after Diagnostic GEne Sequencing (BRIDGES) study by direct sequencing of a 34 gene panel, including *BRCA1*, *BRCA2*, *CHEK2*, *ATM*, and *PALB2*, as previously described.[9,10]

Additional testing from referrals to Genomic Medicine (MFT) were utilized to assess Manchester scores (MS) on larger numbers, particularly for sporadic breast cancers.

Variants were classified in accordance with the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG-AMP)[11] and Cancer Variant Interpretation Group guidelines CanVIG.[12] Only those reported as "pathogenic" or "likely pathogenic" were included as positive findings and, along with copy number variants, we refer to all these categories as PVs.

Tumor pathology information was obtained for each case, when available, through hospital records, and cancer registries, as previously described.[8,9,12,13] The likelihood of a *BRCA1/2* PV was determined using the MS for each affected individual.[14] The MS provides a points score to each cancer in the individual and direct family lineage and adjusts for tumor pathology, including a score of minus 6 points for HER2+ breast cancer and plus 6 for grade 3 triple-negative breast cancer (Supplemental Table 1).[14] An MS of 15 to 19 equates to a 10% likelihood of a *BRCA1/2* PV, and MS 20 to 24 to a 20% likelihood, with a MS ≥40 equivalent to a >75% likelihood.[14]

Table 1 NHS England mainstream testing criteria since April 2022 for breast cancer for R208

Living affected individual (proband) with breast or ovarian cancer where the individual +/− family history meets 1 of the criteria. The proband has the following:
a. Breast cancer (age < 40 years, excluding grade 1 breast cancers), OR
b. Bilateral breast cancer (age < 50 years), OR
c. Triple-negative breast cancer (age < 60 years), OR
d. Male breast cancer (any age), OR
e. Breast cancer (age <45 years) and a first-degree relative with breast cancer (age <45 years), OR
f. Pathology-adjusted Manchester score ≥15 or CanRisk score ≥10%
g. Ashkenazi Jewish ancestry and breast cancer at any age

Statistics

Where relevant, 2-sided χ^2 testing with Yates correction was used.

Results

For this research, we initially assessed at a 5% MS threshold for identifying either a *BRCA1* or *BRCA2* PV. This was the threshold at which there would be at least a 5% detection rate in the lowest risk group among patients diagnosed with breast cancer through all MFT genetic testing for *BRCA1/2* (n = 5410). The 5% threshold was surpassed with a MS of 12 points with *BRCA1/2 PV* seen in 22 to 340 (6.47%) (Supplemental Table 2). The threshold was not reached in those with MS = 10, (7/256 [2.73%]) or in those with MS = 11, (10/231 [4.33%]).

A total of 1061 samples have been submitted for population testing: group 1 mainstreaming (Table 1) n = 97 (*BRCA1* = 7; *BRCA2* = 6; *PALB2* = 1, overall detection rate 14.7%, mean age of cohort = 46.6 years), group 2: BRCA-DIRECT n = 416 (*BRCA1* = 1; *BRCA2* = 4; *PALB2* = 4, overall detection rate 2.2%, mean age cohort = 56.0 years), and from group 3:PROCAS n = 548 (*BRCA1* = *1*; *BRCA2* = *8*; *PALB2* = 4, overall detection rate 2.4%, mean age cohort = 59.1 years). Mean age at breast cancer was 56.7 years (median = 56.9, IQR = 50.5-64.4) and range was 26.3 to 90.4 years. As expected, age was strongly predictive of a PV in *BRCA1/2* +/− *PALB2* (P < .0001; χ^2 test for trend) (Table 2). Overall, 27 PVs were found in *BRCA1/2* in 2.54% *(BRCA1 = 9; BRCA2 = 18)* and 9 in *PALB2* (0.85%), with an overall detection rate of 3.39%. PV detection rate reduced from 12.9% in women aged <40 years (27% with a first-degree relative family history-FDRFH) to 0/96 in all women diagnosed >70 years, irrespective of FDRFH. A 5% threshold for identifying a *BRCA1/2* PV, based on age alone, was met for women diagnosed <50 years and those with a FDRFH aged 50 to 59 years. The relatively high rate in women with sporadic breast cancers aged 40 to 49 years was driven by 4 of 30 (13.3%) with triple-negative tumors because detection rate in the remaining pathologies for all 3 genes was only 7 of 143 (4.9%).

Mainstreaming criteria (Table 1) were met in 190 women, with PVs found in 8.4% in *BRCA1/2* and 11% when PVs in *PALB2* are included (Table 3). In those meeting mainstreaming criteria with an MS <15, the detection rate was 7 of 122 (5.7%) for *BRCA1/2* PVs. The MS was strongly predictive for both *BRCA1/2* PVs and the addition of *PALB2* PVs (Table 3). In particular, the PV detection rate for women with an MS <7 (n = 666) was only 0.60% for *BRCA1/2* PVs and only rose to 0.75% by including *PALB2* PVs (P < .0001 for all PVs MS ≥15 vs <7).

However, the PV yield from those who met mainstream testing criteria but had an MS of <12 was only 1 of 69 (1.4%), with no BRCA1/2 PVs. In contrast, among 50 women with an MS of 12 to 14 that did not meet mainstreaming criteria, 3 *BRCA2* PVs were identified (6.0%, 1 from each cohort). As such, using an MS of ≥12 was more sensitive and specific than mainstream testing criteria (Table 3).

Likewise, tumor pathology was also predictive with an overall detection rate of 8% for *BRCA1/2* in women with triple-negative breast cancer rising to 8.8% with the inclusion of *PALB2,* but this dropped to 0 in 23 in those with triple-negative tumors aged >60 years compared with 9 of 50 (18%) <50 years (P = .049) (Table 4). Women with high-grade estrogen receptor (ER) + HER2- breast cancer (n = 152) had the next highest detection rate at 4.6% (*BRCA1/2* only) and 7.2% for the combined 3 genes. Notably, no PVs were identified in 156 women with grade 1 invasive breast cancers and 96 with invasive lobular breast cancers. Unfortunately, pathology reports could not be obtained for 52 retrospective (prevalent) PROCAS cases.

We next added all sporadic breast cancers from other testing at MFT (n = 5410) to those without a family history of breast/ovarian cancer and without a contralateral breast cancer or ovarian cancer to those identified from population testing (Table 5). We assessed the detection rates for *BRCA1/2* PVs alone, and with the addition of *PALB2,* in 10-year age cohorts in these cases of an isolated breast cancer with no relevant family history. We specifically assessed the rates in triple-negative and all other pathologies combined. The PV detection rates in women with triple-negative tumors exceeded 5% for breast cancers <60 years for *BRCA1/2* PVs, but for women with non-triple-negative tumors, the rate did not exceed 5%. However, by adding *PALB2 PVs*, the rates approached 5% in women with non-triple-negative tumors aged <40 years. We did not have sufficient numbers to stratify non-triple-negative tumors by histological subtype. If we included additional testing for grade 1 invasive cancers with an MS <12 then the detection rate was 0 in 246 women. Nonetheless, for lobular cancer, additional testing revealed that 3 of 90 women with MS <12 had PVs (1 each in *BRCA1, BRCA2,* and *PALB2*). Thus, 3 of 186 (1.6%) women with lobular cancer and MS <12 had a PV in 1 of the 3 genes. In women with HER2+ breast cancer, testing a further 132 samples revealed an additional

Table 2 Population based testing of breast cancer by age and family history

Age (yrs)	Number	% All BC	BRCA1	BRCA2	BRCA1/BRCA2	%	PALB2	Any PV	% Any PV
<40	70	6.6%	5	2	7	10.0%	2	9	12.9%
FH+	11		2	1	3	27.3%	0	3	27.3%
FH-	59		3	1	4	6.8%	2	6	10.2%
40-49	186	17.5%	3	7	10	5.4%	3	13	6.9%
FH+	43		2	1	3	7.0%	0	3	7.0%
FH-	143		1	6	7	4.9%	3	10	7.0%
50-59	396	37.6%	1	7	8	2.0%	4	12	3.0%
FH+	90		0	4	4	4.4%	3	7	7.8%
FH-	306		1	3	4	1.3%	1	5	1.6%
60-69	315	29.8%	0	2	2	0.6%	0	2	0.6%
FH+	67		0	1	1	1.5%	0	1	1.5%
FH-	248		0	1	1	0.40%	0	1	0.4%
70+	96	9.0%	0	0	0	0.00%	0	0	0.0%
FH+	28								
FH-	66								
Total FH+	239	22.5%	4	7	11	4.6%	3	14	5.8%
Total FH+	822	77.5%	5	11	16	1.9%	6	22	2.7%
Mean age									56.74
Median age									56.89
IQR									50.48-64.43

BC, breast cancer; *FH+*, first degree family history of BC; *PV*, pathogenic variant.

PALB2 and *BRCA2* PV with MS <12, providing a combined rate of 3 of 185 (1.6%).

Finally, we assessed changing the threshold for genetic testing using additional criteria rather than just using MS. We looked at women fulfilling the following 3 criteria: (1) meeting current mainstream testing criteria and (2) those diagnosed with breast cancer aged <50 years, and (3) those FDRFH 50 to 59 years. This group comprised 38.7% (411/1061) of the study cohort; yet, testing would identify 83.3% (30/36) of all PV.

Alternatively, reducing the MS threshold to 7 points identifies 31 of 36 (86.1%) of PVs in only 395/1061 (37.2%) of the population (Table 3). Thus, using MS is, again, more sensitive and specific than tweaking the mainstream criteria. To identify all PV heterozygotes, an MS of ≥3 would have been required with testing an additional 364

Table 3 PV rate by Manchester score (MS) and mainstreaming criteria

MS	Number	BRCA1	BRCA2	BRCA1/2	%	PALB2	Any PV	%
20+	25	2	3	5	20.00%	1	6	24.00%
15-19	43	2	2	4	9.30%	2	6	13.95%
12-14	103	5	5	10	9.71%	2	12	11.65%
7-11	222	0	4	4	1.80%	3	7	3.15%
<7	668	0	4	4	0.60%	1	5	0.75%
3-7	364	0	4	4	1.10%	1	5	1.37%
<3	334	0	0	0	0.00%	0	0	0.00%
Total	1061	9	18	27	2.54%	9	36	3.39%

	Mainstream Criteria Met								Sensitivity	Specificity
Yes	190	9	7	16	8.42%	5	21	11.05%	21/36 (58%)	83.5%
Yes MS <15	122	5	2	7	5.74%	2	9	7.38%	9/36 (25%)	-
Yes MS <12	69	0	0	0	0.0%	1	1	1.4%	-	-
No MS 12-14	50	0	3	3	6.0%	1	4	8.0%	-	-
No	871	0	11	11	1.26%	4	15	1.72%	-	-
	MS thresholds									
MS ≥15 10%threshold	68	4	5	9	13.2%	3	12	17.6%	12/36 (33%)	94.5%
MS ≥12 5%threshold	171	9	10	19	11.1%	5	24	14.0%	24/36 (66.7%)	85.7%
MS ≥7 2%threshold	395	9	14	23	5.8%	8	31	7.8%	31/36 (86.1%)	64.5%
MS ≥3 1%threshold	727	9	18	27	3.1%	9	36	4.95%	36/36 (100%)	32.6%
Current mainstreaming + All BC <50 or FDRFH or TNT 50-59 years	411	9	13	22	5.4%	8	30	7.3%	29/36 (80.5%)	62.8%

FDRFH, first-degree relative family history; *MS*, Manchester score; *PV*, pathogenic variant; *TNT*, triple negative tumor.

Table 4 Pathogenic Variant (PV) rate by breast cancer pathology

Pathology	Total	*BRCA1*	*BRCA2*	*PALB2*	Total PV	%	Total *BRCA1/2*	%*BRCA1/2*
G3 BC ERpos her2-	152	1	6	4	11	7.24%	7	4.61%
G2 BC ERpos her2-	301	0	5	1	6	1.99%	5	1.66%
G1BC	156	0	0	0	0	0.00%	0	0.00%
DCIS	138	0	2	2	4	2.90%	2	1.45%
Lobular	96	0	0	0	0	0.00%	0	0.00%
HER2+	53	0	1	0	1	1.89%	1	1.89%
TNT	113	7	2	1	10	8.85%	9	7.96%
IDC grade NOS	52	1	2	1	4	7.69%	3	5.77%
Total	1061	9	18	9	36	3.39%	27	2.54%

BC, breast cancer; *G1BC*, grade 1 invasive ductal carcinoma; *G2BC*, grade 2 invasive ductal carcinoma; *G3BC*, grade 3 invasive ductal carcinoma; *DCIS* -disseminated carcinoma *in situ*; *IDC*, invasive ductal carcinoma; *NOS*, not otherwise stated; *TNT*, triple negative tumor; *PV*, pathogenic variant.

women to detect the remaining 3 *BRCA2* PVs and 1 *PALB2* PV. Because of tumor pathology, 123 women had a 0 or negative MS. Given the 0 rate of PV seen in women with grade 1 cancers, adding in another 42 women with scores of 3 to 11 would improve specificity to 346 in 1025 (33.8%). This would still be a better strategy than testing all women diagnosed with grade 2 or grade 3 breast cancer aged below 70 years, which would only have identified 245 not requiring testing (specificity 24%).

The variant of uncertain significance (VUS) rate was not available for PROCAS or BRCA-DIRECT because these were not part of the protocol. Thus, women receiving results from BRCA-DIRECT, nor their clinicians were aware of any VUS. Current reporting of variants since the beginning of 2021 from clinical testing in our center excludes reporting VUS with no evidence to support pathogenicity (cold VUS).

Table 5 Detection rate of BRCA1/2 PVs in sporadic breast cancer by age group

Age BC	Sporadic	TNT	Other
<30	Number tested	48	61
	BRCA1/2	6	2
	%	12.50%	3.28%
	PALB2	1	1
	Combined %	14.5%	4.9%
30-39	Number tested	193	92
	BRCA1/2	15	3
	%	7.77%	3.26%
	PALB2	3	1
	Combined %	9.3%	4.3%
40-49	Number tested	137	114
	BRCA1/2	11	2
	%	8.03%	1.75%
	PALB2	1	2
	Combined %	8.8%	3.5%
50-59	Number tested	159	237
	BRCA1/2	8	5
	%	5.03%	2.11%
	PALB2	4	0
	Combined %	7.5%	2.1%
>60	Number tested	15	237
	BRCA1/2	0	1
	%	0.00%	0.42%

BC, breast cancer; *TNT*, triple negative tumor.

There was no VUS reported in the 97 women in group 1 tested with mainstreaming. If we include all breast cancer testing since the beginning of 2021 only 8 of 952 (0.84%) had a *BRCA1/2* VUS with none in *PALB2*. This was similar for those samples referred by surgeons/oncologists for mainstreaming 5 of 614 (0.81%).

Discussion

We have carried out genetic testing for the 3 most important germline high-risk genes in a population-based sample of breast cancers in North-West England. The age range and pathology are consistent with the UK population apart from lower rates of HER2+ breast cancer and women aged >70 years. From Cancer Research UK statistics for the UK (2016-2018)[15] the number of women affected with invasive breast cancer was 46,993 with 5.1% (n = 2403) occurring in women aged <40 years and 16% (n = 7533) occurring in women aged 40 to 49 years, which is similar to the 6.6% and 17.5% tested in our study. However, only 9% (n = 83) of women were aged 70 to 79 years (9%) compared with an expected 10,739/46,993 22.9%. The main over-representation was therefore in the 50 to 59 years age group at 37.6% versus UK statistics of 27%. The proportion of our cohort with a FDRFH was 22%, in the middle of the range of 15% to 28.3% in the cohorts in the Cancer Risk Estimates Related to Susceptibility (CARRIERS) population-based-study[16] (7 cohorts above 8 below). The rate of 2.54% for identification of *BRCA1/2* PVs is consistent with other population-based cohorts with no upper age limit.[10,16,17] Only 2.15% in the CARRIERS study[16] had a *BRCA1* or *BRCA2* PV. The very low rate of *BRCA1/2* PVs in women over age 70 years and the relative under representation of this age group means the true figure for *BRCA1/2* PV is likely closer to 2% in our population of women with breast cancer. These low figures for outbred populations are much lower than stated in many reviews of genetic testing, which quote ranges of 3% to 6% or even higher.[18-20] These reviews have likely selected older, more selected populations (at least by age) in these testing studies. The prevalence of *PALB2* PVs in our study at 0.84% compares with 0.46% in the US-based CARRIERS study[15] and 0.56% in the international BRIDGES study.[10] This difference is not

significant even against the CARRIERS study ($\chi^2 = 2.54$; $P = .11$), although the high frequency of *PALB2* c.3113G>A; p.(Trp1038Ter), c.3116del; p.(Asn1039IlefsTer2), and c.3549C>G; p.(Tyr1183Ter) accounting for over 50% of *PALB2* PVs may mean there is a founder effect in our region.[12]

To our knowledge, this study is the first to assess new testing criteria in the United Kingdom aimed at simplifying the availability of testing with all breast cancers, except in women with grade 1 invasive breast cancer, being eligible for testing <40 years and women with triple-negative tumors <60 years. We have shown that, in those not meeting a 10% threshold for identification of *BRCA1/2* PVs of an MS ≥15 points, the detection rate is only 5.7% for *BRCA1/2* PVs with a detection rate of 0 for 69 women with an MS <12. Rather than withdraw the availability of testing, we suggest increasing availability by using a 5% threshold of an MS ≥12 points, which has a greater sensitivity and specificity than the current mainstreaming criteria. Mainstreaming criteria were met by 18% of our population and identified 58% of the PVs in the 3 genes, as opposed to using an MS of ≥12 points, which identifies 66.7% of the PVs from only 16% of the population. The exclusion of grade 1 breast cancers is further justified by our study because 0 in 246 women with grade 1 breast cancers and an MS <12 had a PV identified in any of the 3 genes. It is highly unlikely that a grade 1 breast cancer will require PARPi treatment and the extremely low level of grade 1 breast cancers with *BRCA1/2* PVs suggests that many of the grade 1 breast cancers that do occur in the context of a PV in *BRCA1/2* or *PALB2* may not be driven by loss of homologous repair function but represent sporadic breast cancers. Further, loosening of the criteria for testing, short of proposing universal testing, may suggest a 2% threshold for genetic testing in all women with breast cancer aged <50 years (except grade 1 with an MS <12) and anyone with a breast cancer aged <60 years with a FDRFH (except grade 1 with an MS <12). In order to detect all PVs, the best strategy was to use an MS threshold of 3, which would mean 304 of 1061 (28.7%) women would not require testing, increasing to 346 if excluding grade 1 with an MS of 3 to 11. A simpler strategy of testing all women <70 years, except grade 1 cancers, would have only identified 245 women not requiring testing. Although the MS requires a few minutes work, it is being used by all the breast units sending samples to MFT using the new mainstreaming criteria. The 70 years age threshold might also be a concern for oncologists for triple-negative cancers. These would all qualify using a MS threshold of 3 because even grade 2 tumors over age 60 years would score 2 (for combined *BRCA1* and *BRCA2* age score) + 4 (for triple negative) for a total of 6.

The current study has further justified the utility of the MS for *PALB2* PV. We have shown that women with a *PALB2* PV are more likely to have high-grade breast cancers[12] and, along with others, develop triple-negative disease.[10,12,16] Along with the higher penetrance of *PALB2* PVs compared with *CHEK2* and *ATM*, all of these factors contribute to a higher likelihood of a higher MS.

This study has some limitations. As stated above, it falls just short of a fully representative population cohort with an under representation of women aged >70 years but is not dissimilar to many of the other population-based cohorts in this respect. By offering genetic testing to all women in MFT with breast cancer, either through BRCA-DIRECT (group 2) or mainstream testing (group 1), around 75% of women in breast cancer follow-up were able to opt for testing, regardless of age or family history or pathology. Testing for copy number variants was not used in the BRIDGES study for PVs in PROCAS. Because only 1 *BRCA1* PV was identified in cases, this is unlikely to have had a major effect on our figures. We have chosen not to report *ATM* and *CHEK2* variants because these were not tested in the 416 women in the BRCA-DIRECT study. Identifying PVs in these 2 genes has much less actionability than the 3 high-risk genes and is not predictive of benefit for PARPi treatment. We also have not reported VUS in the full cohort. Very few of these would be reclassified as a PV and the current UK practice is only to report VUS that have evidence pushing toward pathogenicity. We show a detection rate using current reporting criteria of these VUS of only 0.84%, which is almost 10 times lower than the 7.8% detection rate of PVs using 2% threshold in Table 3. Indeed, a new population screening program for the UK Jewish population in England is screening all of *BRCA1/2* but not reporting back any VUS.[21]

In conclusion, this study has shown that 1 in 6 women who came forward for population-based testing were eligible for germline genetic testing, and this identified 56% of the PVs. Using an MS of ≥12 points, in addition to mainstream criteria, identified 4 additional PVs by testing an extra 50 women, increasing sensitivity to 69% by testing 20.7% of the population. We have also identified further strategies to loosen criteria that would identify 80.6% of PVs (29/36) in only 35.2% of the population (374/1061). Alternatively, an MS threshold of 7 has a higher sensitivity of 86% by testing 16 less women. An MS threshold of 3 (excluding grade 1 with an MS = 3-11) would have detected all PVs identified in this study, with 346 (33%) women not requiring testing. As most of the "missing" population are likely to have very low rates of germline PVs, it is likely that the 80% sensitivity figure using the expanded loosened criteria and/or an MS ≥12 would remain.

Data Availability

Data are stored for the studies on the university of Manchester and ICR data resources. Source data are available on request.

Acknowledgments

The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR, or the Department of Health. We acknowledge help in data entry and recruitment from Karen Connolly, Chloe Newton, Jerin Valliyaparambil James, Georgina Fogarty, and Hani Ali.

Funding

MFT research was funded by the Manchester National Institute for Health Research Biomedical Research Centre (IS-BRC-1215-20007). BRCA-DIRECT was supported by Cancer Research UK (C61296/A29423). PROCAS was supported by the National Institute for Health Research under its Programme Grants for Applied Research programme (reference number RP-PG-0707-10031: "Improvement in risk prediction, early detection and prevention of breast cancer" and the Prevent Breast Cancer (references GA10-033 and GA13-006).

Author Information

Conception and Design: D.G.E., C.T.; Acquisition of Data: D.G.E., S.Bo., S.G., H.F., S.A., B.T., M.Ha., G.K., M.Hu.; Analysis and Interpretation of Data: D.G.E., C.T., S.Br., C.I.J., B.T.; Drafting: D.G.E., C.T.; Manuscript Review: D.G.E., E.R.W., G.J.B., S.Al., B.T., M.H., G.K., M.H., S.B., C.I.J., H.S., S.As., S.B., S.G., H.R., C.F., S.J.H., C.F., F.L., W.G.N., M.J.S., A.H., C.T., A.G.; Approval of Final Submission: D.G.E., E.R.W., G.J.B., S.Al., B.T., M.H., G.K., M.H., S.B., C.I.J., H.S., S.As., S.B., S.G., H.R., C.F., S.J.H., C.F., F.L., W.G.N., M.J.S., A.H., C.T., A.G.

Ethics Declaration

Manchester Research Ethics Committee, reference (09/H1008/81 [PROCAS] and 08/H1006/77). BRCA-DIRECT: Approved by The Institute of Cancer Research/Royal Marsden NHS Foundation Trust Committee for Clinical Research, reference CCR5234, and the Research Ethics Committee, reference 20/LO/1200. Each participant provided informed consent before participation in the study.

Conflict of Interest

D. Gareth Evans, non-executive director, Everything Genetic Ltd. D. Gareth Evans has interests not related to this work. Consulting or Advisory Role: AstraZeneca, Springworks, Travel, Accommodations; Other Expenses: AstraZeneca. All other authors declare no conflicts of interest.

Additional Information

The online version of this article (https://doi.org/10.1016/j.gimo.2023.100849) contains supplementary material, which is available to authorized users.

Affiliations

[1]Manchester Centre for Genomic Medicine, Manchester University Hospitals NHS Foundation Trust, Manchester, United Kingdom; [2]Division of Evolution and Genomic Sciences, School of Biological Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester Academic Health Science Centre, Manchester, United Kingdom; [3]Prevent Breast Cancer Centre, Wythenshawe Hospital Manchester Universities Foundation Trust, Wythenshawe, Manchester, United Kingdom; [4]Manchester Breast Centre, The Christie NHS Foundation Trust, Wilmslow Road, Manchester, United Kingdom; [5]The Institute of Cancer Research, 15 Cotswold Road, Sutton, Surrey, United Kingdom; [6]Brighton and Sussex Clinical Trials Unit, Brighton and Sussex Medical School, Brighton, United Kingdom; [7]Division of Informatics, Imaging and Data Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester Academic Health Science Centre, Manchester, United Kingdom; [8]Division of Cancer Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester Academic Health Science Centre, Manchester, United Kingdom

References

1. McIntosh A, Shaw C, Evans G, et al. Clinical Guidelines and Evidence Review for the Classification and Care of Women at Risk of Familial Breast Cancer; 2017. London: National Collaborating Centre for Primary Care/University of Sheffield NICE Guideline cg014:2004 (updated. 2013/2017. 2006(CG41):CG184). Accessed June 17, 2018. http://www.nice.org.uk
2. Pujol P, Barberis M, Beer P, et al. Clinical practice guidelines for BRCA1 and BRCA2 genetic testing. *Eur J Cancer*. 2021;146:30-47. http://doi.org/10.1016/j.ejca.2020.12.023
3. Manchanda R, Legood R, Burnell M, et al. Cost-effectiveness of population screening for BRCA mutations in Ashkenazi Jewish women compared with family history-based testing. *J Natl Cancer Inst*. 2015;107(1):380. http://doi.org/10.1093/jnci/dju380
4. Manchanda R, Patel S, Antoniou AC, et al. Cost-effectiveness of population based BRCA testing with varying Ashkenazi Jewish ancestry. *Am J Obstet Gynecol*. 2017;217(5):578.e1-578.e12. http://doi.org/10.1016/j.ajog.2017.06.038
5. Patel S, Legood R, Evans DG, et al. Cost effectiveness of population based BRCA1 founder mutation testing in Sephardi Jewish women. *Am J Obstet Gynecol*. 2018;218(4):431.e1-431.e12. http://doi.org/10.1016/j.ajog.2017.12.221
6. Geyer CE Jr, Garber JE, Gelber RD, et al. Overall survival in the OlympiA phase III trial of adjuvant olaparib in patients with germline pathogenic variants in BRCA1/2 and high-risk, early breast cancer. *Ann Oncol*. 2022;33(12):1250-1268. http://doi.org/10.1016/j.annonc.2022.09.159
7. Torr B, Jones C, Choi S, et al. A digital pathway for genetic testing in UK NHS patients with cancer: BRCA-DIRECT randomised study internal pilot. *J Med Genet*. 2022;59(12):1179-1188. http://doi.org/10.1136/jmg-2022-108655
8. Evans DGR, van Veen EM, Harkness EF, et al. Breast cancer risk stratification in women of screening age: incremental effects of adding mammographic density, polygenic risk, and a gene panel. *Genet Med*.

2022;24(7):1485-1494. http://doi.org/10.1016/j.gim.2022.03.009: S1098-3600(22)00699-2

9. Evans DG, van Veen EM, Woodward ER, et al. Gene panel testing for breast cancer reveals differential effect of prior BRCA1/2 probability. *Cancers (Basel)*. 2021;13(16):4154. http://doi.org/10.3390/cancers13164154
10. Breast Cancer Association Consortium, Dorling L, Carvalho S, et al. Breast cancer risk genes – association analysis in more than 113,000 women. *N Engl J Med*. 2021;384(5):428-439. http://doi.org/10.1056/NEJMoa1913948
11. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17(5):405-424. http://doi.org/10.1038/gim.2015.30
12. Woodward ER, van Veen EM, Forde C, et al. Clinical utility of testing for PALB2 and CHEK2 c.1100delC in breast and ovarian cancer. *Genet Med*. 2021;23(10):1969-1976. http://doi.org/10.1038/s41436-021-01234-6
13. Moran A, O'Hara C, Khan S, et al. Risk of cancer other than breast or ovarian in individuals with BRCA1 and BRCA2 mutations. *Fam Cancer*. 2012;11(2):235-242. http://doi.org/10.1007/s10689-011-9506-2
14. Evans DG, Lalloo F, Cramer A, et al. Addition of pathology and biomarker information significantly improves the performance of the Manchester scoring system for BRCA1 and BRCA2 testing. *J Med Genet*. 2009;46(12):811-817. http://doi.org/10.1136/jmg.2009.067850
15. Breast cancer incidence (invasive) statistics. Cancer Research UK. Accessed June 1, 2023. https://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-bycancer-type/breast-cancer/incidence-invasive
16. Hu C, Hart SN, Gnanaolivu R, et al. A population-based study of genes previously implicated in breast cancer. *N Engl J Med*. 2021;384(5):440-451. http://doi.org/10.1056/NEJMoa2005936
17. Li J, Wen WX, Eklund M, et al. Prevalence of BRCA1 and BRCA2 pathogenic variants in a large, unselected breast cancer cohort. *Int J Cancer*. 2019;144(5):1195-1204. http://doi.org/10.1002/ijc.31841
18. Yoshida R. Hereditary breast and ovarian cancer (HBOC): review of its molecular characteristics, screening, treatment, and prognosis. *Breast Cancer*. 2021;28(6):1167-1180. http://doi.org/10.1007/s12282-020-01148-2
19. Armstrong N, Ryder S, Forbes C, Ross J, Quek RG. A systematic review of the international prevalence of BRCA mutation in breast cancer. *Clin Epidemiol*. 2019;11:543-561. http://doi.org/10.2147/CLEP.S206949
20. Petrova D, Cruz M, Sánchez MJ. BRCA1/2 testing for genetic susceptibility to cancer after 25 years: a scoping review and a primer on ethical implications. *Breast*. 2022;61:66-76. http://doi.org/10.1016/j.breast.2021.12.005
21. The NHS Jewish BRCA Testing Programme. Jnetics. Accessed June 1, 2023. https://www.jnetics.org/getting-tested/nhs-brca-screening/