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Routine germline *BRCA1* and *BRCA2* testing in ovarian carcinoma patients: analysis of the Scottish real life experience

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Running title: *BRCA1* and *BRCA2* sequencing in Scottish ovarian cancer patients

Abstract

Objective

To determine the rate of germline *BRCA1* and *BRCA2* mutations in Scottish ovarian cancer patients before and after a change in testing policy.

Design

Retrospective cohort study.

Setting

Four cancer/genetics centres in Scotland.

Population

Ovarian cancer patients undergoing germline *BRCA1* and *BRCA2* (*gBRCA1/2*) gene sequencing before 2013 ('old criteria'; selection based solely on family history), after 2013 ('new criteria'; sequencing offered to newly presenting non-mucinous ovarian cancer patients) and the 'prevalent population' (who presented before 2013, were not eligible for sequencing under the old criteria but were sequenced under the new criteria).

Methods

Clinicopathological and sequence data were collected before and for 18 months after this change in selection criteria.

Main Outcome Measures

Frequency of germline *BRCA1*, *BRCA2*, *RAD51C* and *RAD51D* mutations.

Results

Of 599 patients sequenced, 205, 236 and 158 were in the 'old criteria', 'new criteria' and 'prevalent' populations respectively. The frequency of *gBRCA1/2* mutations was 30.7%, 13.1% and 12.7% respectively. The annual rate of *gBRCA1/2* mutation detection was 4.2 before and 20.7 after the policy change. 48% (15/31) 'new criteria' patients with *gBRCA1/2* mutations had a Manchester score <15 and would not have been offered sequencing based

on family history criteria. In addition, 20 *gBRCA1/2* patients were identified in the prevalent population. The prevalence of *gBRCA1/2* mutations in patients >70 years was 8.2%.

Conclusions

Sequencing all non-mucinous ovarian cancer patients produces much higher annual *gBRCA1/2* mutation detection with the frequency of positive tests still exceeding the 10% threshold upon which many family history based models operate.

Tweetable abstract:

BRCA sequencing all non-mucinous cancer patients increases mutation detection five fold

Introduction

Ovarian cancer is the fifth most common female cancer in Europe with 65,600 new diagnoses and 42,700 deaths in 2012.[1] In Scotland, this equated to 610 new diagnoses[2] and 383 deaths.[3] There is currently no effective screening programme, symptoms are non-specific and patients usually present with disease that has spread beyond the pelvis. Germline *BRCA1* and *BRCA2* mutations (collectively referred to as *gBRCA1/2* hereafter) confer a high risk of epithelial ovarian and breast cancer. The prevalence of *gBRCA1/2* mutation in studies of populations (of women with epithelial ovarian cancer) with different genetic backgrounds ranges from 3-29% (table 1).[4-16]

Identification of *gBRCA1/2* mutation status in epithelial ovarian cancer has prognostic and predictive benefits for the individual and her family. Mutation carriers with ovarian cancer, have longer progression-free and overall survival compared to non-carriers,[17, 18] and greater sensitivity both to platinum[19, 20] chemotherapy and poly (ADP-ribose) polymerase (PARP) inhibitors. [21-23] Furthermore, identification of a *gBRCA1/2* mutation enables unaffected relatives to be offered testing for the family mutation, and thus risk-reducing surgery or screening for breast and ovarian cancer.

In many countries, the offer of publically-funded *gBRCA1/2* testing is limited on resource grounds to those with a family history that indicates a specified level of risk (for example 10%).[24] In Scotland, as elsewhere, risk thresholds for full *gBRCA1/2* gene testing have fallen over time, as sequencing costs have reduced, from 25% to 10%. Recent studies in a variety of ethnic backgrounds have shown that restricting testing to cases with a family history of breast or ovarian cancer results in 8-54% of mutation carriers being undetected.[19, 25-28] On the basis that the estimated prevalence of *gBRCA1/2* mutations in high grade serous ovarian cancer (HGSOC) may exceed 10% (table 2), routine *BRCA1* and *BRCA2*, sequencing was introduced in Scotland for all patients with non-mucinous ovarian cancer in

2012/13.   The objective of this study was to determine the rates of *BRCA1* and *BRCA2* mutation detection in Scottish epithelial ovarian cancer patients both before and after this change in selection criteria. We also highlight different testing pathways including one where initial counselling is performed by the treating oncology team rather than clinical geneticists.

Methods

Different pathways for delivering testing to all newly diagnosed epithelial ovarian cancer patients were established in the East and West of Scotland (populations 2.9 and 2.5 million respectively). In the East of Scotland (centres in Edinburgh, Dundee and Aberdeen), patients with non-mucinous epithelial ovarian cancer were offered routine testing from 01/11/2012 (Edinburgh), 01/06/2013 (Dundee) and 01/12/2013 (Aberdeen). In Edinburgh and Dundee, patients receive counselling from their medical oncologist, and, after receiving written patient information, give written consent. In Aberdeen, the process is broadly similar with oncologists introducing the test, arranging blood sampling but then referring the patient to clinical genetics for a telephone consultation to complete the consenting process. In the West of Scotland, universal testing started in March 2013. All patients with non-mucinous ovarian cancer are referred to the genetics team, and mutation testing is only initiated following counselling by a genetic counsellor.

DNA testing was performed in CPA accredited laboratories in Aberdeen and Glasgow. DNA was extracted from blood samples, PCR amplified and sequenced bi-directionally using standard Sanger sequencing technology. Sequence data was analysed using Mutation Surveyor (Soft Genetics) and variants assessed for pathogenicity according to the Practice Guidelines for the Evaluation of Pathogenicity and the Reporting of Sequence Variants in Clinical Molecular Genetics (ACGS /VGKL 2013). MLPA (MRC Holland) was used to test for exonic and whole gene deletions. The DNA of patients found not to have a deleterious

BRCA1 or *BRCA2* mutation was then sequenced for *RAD51C* and *RAD51D* mutations. Patients found to have a deleterious mutation or reported variant of uncertain significance in any of the four genes were subsequently referred to clinical genetics for a face to face consultation (as well as any other patients who had a significant family history or who wished a further discussion). Testing for *RAD51C* and *RAD51D* mutations in the West of Scotland was restricted to those with two or more family members with history of ovarian cancer.

Data resulting from the first 18 months of testing following the change in selection criteria were collected from each centre (with the exception of Aberdeen, which instituted the change in practice later and from which 9 months of data were collected) and compared to historical data from the preceding period when access to *BRCA1/2* sequencing was dependent upon family history alone.

For each locality, the number and frequency of positive tests was determined for three specific cohorts:

1) 'Old criteria' patients**:** those patients sequenced before the change in process (when eligibility for testing was based on family history). The period of testing for these patients was from September 1997 until the date of change of selection criteria, as specified above.

2) 'Prevalent population' patients**:** The prevalent population of patients who did not fit previous criteria for sequencing but had a diagnosis of HGSOC and were still being reviewed in clinic following the change in selection criteria.

3) 'New criteria' patients**:** Patients tested under the new selection process during their first line treatment.

Results

A total of 631 patients were offered or referred for genetic testing. Of these, there were 10 active refusers (declined testing at the time it was offered, either by the medical oncologist in the East [n = 3] or the clinical geneticist in the West [n = 7]), 21 passive refusers (patients in the West who were referred to clinical genetics but did not reply to correspondence) and 1 patient who was offered testing but died before it could be performed. 599 remaining ovarian cancer patients were included in the analysis (table 3). This comprised 205 'old criteria' patients and 394 tested following the change in selection criteria (236 'new criteria' patients and 158 'prevalent population' patients). In terms of the different pathways for delivering the genetic sequencing, of the 394 patients sequenced following the change in criteria, 251 were sequenced in the East and 143 in the West of Scotland.

Across Scotland, the frequency of *gBRCA1/2* mutation detection in the old criteria patients was 30.7% in the 'old criteria' patients, 12.7% in the 'prevalent' population and 13.1% in the 'new criteria' population. In the East of Scotland, the *gBRCA1/2* mutation detection rate was 9.4% in the prevalent population and 11.0% in the new criteria patient group. In the West of Scotland, by comparison, the *gBRCA1/2* mutation rate was 17.7% in the prevalent population and 17.3% in the new criteria group. It is worth noting that the frequency of deleterious *gBRCA2* mutations in the West of Scotland patients following the change in selection criteria was strikingly high at 11.2% in the prevalent population and 13.5% in the new criteria patients, compared to 3.1% and 3.9% respectively in the East of Scotland. Overall, the annual rate of *gBRCA1/2* mutation detection was 4.2 and 20.7 patients per year before and after the change in selection criteria respectively. Only two mutations in *RAD51C/D* were identified following the change in testing criteria, one each in the prevalent and new criteria populations (approximate 1% test positivity rate). Variants of Unknown Significance were seen in 5.4% (11 of 205, 4 *BRCA1*, 7 *BRCA2*) patients tested in the old

criteria, 9.4% in the prevalent population (15 of 158, 3 *BRCA1*, 12 *BRCA2*) and 6.4% in the new criteria patients (15 of 236, 4 *BRCA*1, 11 *BRCA2*).

Manchester Score[30] was recorded for all patients identified to have a pathogenic mutation. 48% (15/31) new criteria patients with *gBRCA1/2* mutations had a Manchester score <15(table 4). Thus, if our new patients had been tested on Manchester score alone, almost half the mutation carriers would have been missed. In addition, 20 patients with *gBRCA1/2* mutations in the prevalent population were identified retrospectively over this 18 month period. None of these had previously been identified as eligible for testing, although interestingly 11 of these patients had a Manchester score ≥15 when formally assessed by clinical genetics. Only after sequencing became standard of care were these patients referred. Thus, 39 patients with *gBRCA1/2* mutations were identified across Scotland over this 18 month period who would not have been tested if the new policy had not been implemented.

When the histology of the mutation carriers was considered across all three cohorts (Appendix S1), all the mutation carriers were found to have high grade serous ovarian cancer (102 out of 518, 19.7%) or epithelial ovarian cancer not otherwise specified (largely from the historical 'old criteria' cohort; 12 out of 44, 27.3%). No deleterious *BRCA1/2* mutations were detected in seven patients with carcinosarcoma, nine with clear cell ovarian cancer or 14 with low grade serous ovarian cancer. In addition five patients with mucinous tumours, one with a Brenner tumour and one with a STIC had been sequenced (some in the 'old criteria' cohort). None of these patients harboured deleterious mutations.

The age at diagnosis of all tested patients was recorded. Across all cohorts, 13/114 (11.4%) patients with pathogenic *BRCA1* or *BRCA2* mutations were aged over 70, and the rate of identified pathogenic mutations in this age group was 8.2% (13 pathogenic mutations from 159 patients tested). When the frequency of *BRCA1/2* mutation carriers without a family

history of breast or ovarian cancer was considered by age group across all three cohorts only the 30-39, 40-49, 50-59 and 60-69 age groups had a percentage of mutation carriers in excess of 10% (<30 years 0/6 [0%]; 30-39 years 5/10 [50%]; 40-49 years 5/42 [11.9%]; 50-59 years 20/85 [23.5%]; 60-69 years 12/101 [11.9%]; 70-79 years 3/80 [3.8%]; >80 years 0/13 [0%]).

Discussion

Main findings

Across Scotland, the frequency of *gBRCA1/2* mutation detection was 13.1% in patients presenting with non-mucinous ovarian cancer. The annual rate of *gBRCA1/2* mutation detection was 20.7 compared to 4.2 when selection for sequencing was based solely upon family history criteria. The acceptance of the offer of sequencing was high with only 2% active refusal. In the West of Scotland where a step of active participation in the process was required there were an additional 12% of patients who passively refused sequencing. Analysis of Manchester scores showed that 48% of new mutation carriers would not have been offered sequencing if selection had been based on family history alone. The *gBRCA1/2* mutation rate in the prevalent population was 12.7% which was surprising since these patients had been overlooked for sequencing when selection was based upon family history criteria, although interestingly, 55% of these patients did have a significant family history (Manchester score >15) when formally assessed by the clinical genetics team . Finally, the incidence of *gBRCA1/2* mutations in non-mucinous ovarian cancer patients aged over 70 was 8.2%.

Strengths and limitations

The strengths of this study are its size, the fact that it captured all patients offered sequencing across Scotland (limiting the potential for selection bias in tertiary referral centres) and that the data for the majority of patients were retrieved from prospectively collected clinical databases with case note searching only required in a minority of cases.

The limitations of this study are its retrospective nature, the fact that patient satisfaction was not assessed and the fact that the criteria for selecting patients for sequencing differed slightly between centres (particularly comparing the East to the West) although this did allow the pros and cons of the different mainstreaming techniques to be highlighted.

Interpretation

At a time when *BRCA1/2* mutation status determines access to effective new drugs in the form of PARP inhibitors (olaparib was licensed in 2014 and approved for reimbursement in the UK in 2016), it is imperative that as many ovarian cancer patients as possible are offered the chance of sequencing. In Scotland, all patients diagnosed with non-mucinous ovarian cancer are now routinely offered germline *BRCA1/2* sequencing, an approach enshrined in new national guidelines. [31] Scotland is one of the first countries to offer this service as a national standard of care. The *gBRCA1/2* mutation rate in newly diagnosed Scottish ovarian cancer patients of 13.1% justifies the switch away from family history based patient selection criteria for sequencing.

Two broad models for routine testing have been implemented, with local variation driven by resource and clinician preference. In the West, all patients are referred to Clinical Genetics for counselling and consent before testing, whilst in East Scotland oncologists provide genetic counselling at the first clinic visit, obtain written consent and offer the test. Patients who are subsequently found to harbour a germline mutation return to Clinical Genetics (in the West) or are referred de novo to Clinical Genetics in the East in order that they can receive personal counselling regarding their breast cancer risk and also in order to facilitate

counselling and cascade testing for relatives.   In both test models, we find that testing is acceptable to patients (although patient satisfaction was not assessed in this study) and take-up rates are high. It is noticeable that the take-up rate is lower in the West, where testing requires active patient participation (patients must reply to invitation from Genetics and attend an appointment or have a telephone consultation before testing can commence). . The concept of pre-test counselling being provided by non-geneticists in order to streamline the process so that the genetics team are able to focus on mutation carriers rather than a cohort of patients, many of whom will not carry a mutation is gathering general acceptance with other studies demonstrating high levels of patient satisfaction.[32]

Previous studies have identified higher mutation rates in ovarian cancer patients than identified in East of Scotland, [19, 26, 33] but broadly similar to those in West Scotland. However, many of these previous studies recruited patients retrospectively and were based in tertiary referral centres that were not the sole ovarian cancer care providers in their geographical location. In addition, it was noted that a number of the identified patients could not be enrolled as they had died, thus providing a selection bias towards *gBRCA1/2* mutation carriers who have superior survival to non-carriers. [17] Many of these studies have also been limited by poor uptake of testing giving the potential for further selection bias. In East Scotland, the sequencing uptake rate was 98.8% (3 patients declined testing) suggesting that *gBRCA1/2* mutation rates in genuinely unselected patients may be slightly lower than previously suggested. Nevertheless the case detection rate still met the cost-effectiveness threshold for family benefit, justifying the cost of testing on those grounds alone.

The *gBRCA1/2* mutation rate in the prevalent group (12.7%) was higher than expected. We had hypothesised that patients with a strong family history would have been tested under the

previous schedule, thus reducing the remaining *gBRCA1/2* positive pool in untested patients. An alternative explanation, suggested by the incidence of Manchester scores ≥15 in the patients who tested positive in the prevalent group, is that ascertainment of family history by the oncologists was incomplete. By making sequencing routine, the potential for missing patients due to inaccurate family history assessment is circumvented.

Historically, *RAD51C* and *RAD51D* germ line sequencing has been performed in Scotland because of the increased ovarian cancer risk in carriers.[34, 35] With a frequency of mutation of either gene of around 1%, it could be debated whether this is a cost-effective exercise. However, with evidence emerging regarding the PARP inhibitor sensitivity of ovarian tumours harbouring *RAD51C* mutations in particular[36] the results have implications both for the patients and their families.

The protocol for germ line *BRCA1* and *BRCA2* sequencing of ovarian cancer patients differs across the UK. Many regions of England, Wales and Northern Ireland continue to sequence only patients with a significant family history, a small number sequence all non-mucinous ovarian cancer patients[32] and others impose age restrictions for germline testing. Here, we show that the rate of germline mutation was 8.2% in those over 70 years, and that 11.4% of all mutation carriers were over 70 at the time of diagnosis. The oldest mutation carrier identified was 84 years old. Our data contrast with a recent study from the East of England, where only 1 of 86 women over the age of 70 carried a pathogenic mutation,[37] which led the authors to conclude that testing should be restricted to those under 70 years in the absence of positive family history. Our results are more in line with those of Norquist et al, who identified *BRCA1/2* mutations in over 5% of their population aged 70 – 79.[38] Thus, overall, we advocate a policy whereby testing is offered to patients regardless of their age at diagnosis.

Conclusion

We demonstrate that routine nationwide testing of ovarian cancer patients for *gBRCA1/2* mutations offers prognostic and predictive information and meets cost-effectiveness thresholds already widely in place in UK genetic healthcare. The strategy detects mutation in 13 per 100 cases, 48% of whom would not be identified through case selection on the basis of family history alone. Testing in the oncology setting, without requirement for formal pre-test genetic counselling, or a mixed consent model, is feasible and reduces both the potential for referral bias and the burden upon Clinical Genetics departments. We propose that all women with non-mucinous ovarian cancer should be offered germline *BRCA* mutation testing as part of routine clinical care prior to the commencement of chemotherapy.

Disclosure of interests

MM has sat on advisory boards for Roche and delivered lectures for Boehringer Ingelheim. CG has sat on advisory boards for Roche, AstraZeneca, Nucana, Tesaro, Foundation One and Clovis and has delivered lectures for Roche, Tesaro and AstraZeneca. CG has also received research funding from AstraZeneca, Novartis, Tesaro, Nucana and Aprea. CB has sat on advisory boards for AstraZeneca. The other co-authors have no conflicts of interest to disclose. The ICMJE disclosure forms are available to view online as supporting information.

Contribution to authorship

Study design: KR, CG

Data acquisition: KR, PS, CYT, CB, DS, TFP, RD, MM, FN, RG, NR, AS, MP, TMcG, MF, ZM, IMcN, CG

Data analysis: KR, PS, CB, DS, IMcN, CG

Manuscript preparation: KR, PS, ZM, IMcN, CG

All authors reviewed the manuscript before submission.

Details of ethics approval

We have been informed by South East Scotland Research Ethics Service that studies in BRCA-defective ovarian cancer patients using data obtained as part of usual care do not require NHS ethical review. As such, no independent ethical approval for this study was sought.

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Table 1. Incidence of germline *BRCA1* or *BRCA2* mutations in previous retrospective ovarian cancer studies

Study	Population	Year	Patients	BRCA1	BRCA2	BRCA1&2	Sequencing method
Alsop [19]	Australia	2012	1001	88 (8.8%)	53 (5.3%)	141 (14%)	HRM/MLPA
Malander [25]	Sweden	2004	161	9 (5.6%)	4 (2.4%)	13 (8%)	TS/DHPLC/PTT
Risch [26]	Canada	2006	977	75 (7.7%)	54 (5.5%)	129 (13%)	TS/DGGE/PTT
Stratton [15]	London, UK	1997	374	12 (3%)	-	12 (3%)	HA
Anton-Culver [4]	USA	2000	120	4 (3.3%)	-	4 (3.3%)	TS
Bjorge [6]	Norway	2004	478	19 (4%)	-	19 (4%)	CSCE
Jacobi [8]	Netherlands	2007	85	3 (3.5%)	2 (2.4%)	5 (5.9%)	CSCE
Majdak [10]	Poland	2005	205	18 (8.8%)	0	18 (8.8%)	CSGE
Chetrit [7]	Israel (Non-Askenazi)	2008	174	13 (7.5%)	3 (1.7%)	16 (9.2%)	TS*
Rubin [14]	Philidelphia	1998	116	10 (8.6%)	1 (0.9%)	11 (9.5%)	SSCP
Metcalfe [12]	Canada	2009	416	29 (7%)	11 (2.6%)	41 (9.9%)	TS/DGGE/DHPLC/FM PA
Van der Looij [16]	Hungary	2000	90	10 (11%)	0	10 (11%)	HA/SSCP
Khoo [9]	China	2000	53	6 (11.3%)	1 (2%)	7 (13%)	PTT and sequencing
Menkiszak [11]	Poland	2003	364	49 (13.5%)	-	49 (13.5%)	TS*
Ben David [5]	Israel	2002	896	19.4%	6.7%	234 (26.1%)	TS*
Modan [13]	Israel	2001	840	182 (21.7%)	64 (7.6)	244 (29%)	TS*

HA: heteroduplex analysis; TS: targeted sequencing; CSCE: capillary electrophoresis

CSGE: conformational sensitive gel electrophoresis; SSCP: single-strand conformational polymorphism analysis; DGGE: gradient gel electrophoresis; DHPLC: denaturing high-performance liquid chromatography; FMPA: Fluorescent multiplexed-PCR analysis; PTT: protein truncation test

HRM: high resolution melting; MLPA: multiplex ligation-dependent probe amplification

* Founder mutations only

Table 2. Incidence of germline *BRCA1* or *BRCA2* mutations in previous retrospective serous ovarian cancer studies

Study	Population	Year	Patients	BRCA1	BRCA2	BRCA1&2	Sequencing method
Malander [25]	Sweden	2004	105 (serous)	7 (6.7%)	1 (0.9%)	8 (7.6%)	TS/DHPLC/PTT
Sarantau [39]	Finland	2001	118 (serous)	8 (6.8%)	2 (1.7%)	10 (9%)	TS*/PTT
Rafnar [40]	Iceland	2004	97 (serous)	2 (2%)	8 (8.2%)	10 (10.3%)	TS*
Pal [33]	Florida, USA	2005	121 (serous)	14 (11.6%)	6 (4.96)	20 (16.5%)	TS
Alsop [19]	Australia	2012	709 (serous) (433 HGS)	74 (10%)	44 (6.2%)	18/709 (16.6%) 98/433 (22.6%)	HRM/MLPA
Risch[26]	Canada	2006	610 (serous)	62 (10%)	48 (7.9%)	110 (18%)	TS/DGGE/PTT

TS: targeted sequencing; DHPLC: denaturing high-performance liquid chromatography;

PTT: protein truncation test; HRM: high resolution melting; MLPA: multiplex ligation-dependent probe amplification

* Founder mutations only

Table 3. Frequency of genetic mutations in Scottish ovarian cancer patients.

	East of Scotland			West of Scotland			Combined analysis		
	Old criteria	Prevalent population	New criteria	Old criteria	Prevalent population	New criteria	Old criteria	Prevalent population	New criteria
Number of patients tested	138	96	155	67	62	81	205	158	236
BRCA1 mutation#	33 (23.9%)	6 (6.2%)	12* (7.7%)	8 (11.9%)	4 (6.5%)	3 (3.7%)	41 (20.0%)	10 (6.3%)	15* (6.4%)
BRCA1 VUS	4 (2.9%)	3* (3.1%)	3 (1.9%)	0	0	1 (1.2%)	4 (2.0%)	3 (1.9%)	4 (1.7%)
BRCA2 mutation#	15 (10.9%)	3 (3.1%)	6* (3.9%)	7 (10.4%)	7 (11.2%)	11 (13.6%)	22 (10.7%)	10 (6.3%)	17* (7.2%)
BRCA2 VUS	5 (3.6%)	10** (10.4%)	8 (5.2%)	2 (3.0%)	2 (3.2%)	3 (3.7%)	7 (3.4%)	12 (7.6%)	11 (4.7%)
RAD51C/D mutation#¥	1 (NK)	0	1 (0.7%)	0	1 (16.7%)	0	1 (NK)	1 (1.1%)	1 (0.7%)
RAD51 C/D VUS	2 (NK)	6 (6.9%)	4 (2.9%)	0	0	0	2 (NK)	6 (6.5%)	4 (2.8%)

Mutations known to be pathogenic.

*1 patient had pathogenic mutations in both *BRCA1* and *BRCA2*

**1 patient had a *BRCA1* & 2 VUS.

¥The denominator for *RAD51C/D* mutations and *RAD51C/D* VUS was not the same as the number of patients sequenced for *gBRCA1/2* mutations (see methods). The denominator for patients in the 'old criteria' population from the East of Scotland who received *RAD51C/D* sequencing is not known (NK) because *RAD51C/D* sequencing was introduced after *BRCA1/2* sequencing.

Table 4. Germline *BRCA1/2* mutation breakdown by cohort and Manchester score.

	gBRCA mutation	East		West		Combined	
		Manchester Score ≥15	Manchester Score <15	Manchester Score ≥15	Manchester Score <15	Manchester Score ≥15	Manchester Score <15
Old criteria	BRCA 1	30/33 (91%)	3/33 (9%)	3/8 (38%)	5/8 (62%)	33/41 (80%)	8/41 (20%)
	BRCA 2	13/15 (87%)	2/15 (13%)	3/7 (43%)	4/7 (57%)	16/22 (73%)	6/22 (27%)
Prevalent population	BRCA 1	2/6 (33%)	4/6 (67%)	3/4 (75%)	1/4 (25%)	5/10 (50%)	5/10 (50%)
	BRCA 2	3/3 (100%)	0/3 (0%)	3/7 (43%)	4/7 (57%)	6/10 (60%)	4/10 (40%)
New criteria	BRCA 1	7/12* (58%)	5/12 (42%)	2/3 (67%)	1/3 (33%)	9/15* (60%)	6/15* (40%)
	BRCA 2	3/6* (50%)	3/6 (50%)	5/11 (45%)	6/11 (55%)	8/17* (47%)	9/17* (53%)

*1 'new patient' with Manchester score ≥15 had pathogenic mutations in both *BRCA1* and *BRCA2*