ANATOMICAL PATHOLOGY

Non-*BRCA* familial breast cancer: review of reported pathology and molecular findings

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Summary

The majority of women evaluated for a clinical concern of possible hereditary breast cancer syndromes have no identified pathogenic variants in genes predisposing them to breast cancer. Non-*BRCA1*- or *BRCA2*-related familial breast cancer, also called ‘BRCAX’, thus comprises a sizeable proportion of familial breast cancer but it is poorly understood. In this study, we reviewed 14 studies on histopathology and molecular studies of BRCAX to determine if there were differences between ‘sporadic’ breast cancers and compared to cancers arising in women harbouring variants in known cancer predisposition genes. Across available literature, there was inconsistency on inclusion and exclusion criteria, reported parameters, and use of controls. Cohorts were small, and while several studies reported findings that appeared to distinguish the BRCAX cases from sporadic and/or gene-positive controls, no findings were reported in more than one study. To determine whether the BRCAX families might still contain important genetic subsets awaiting discovery will require prospective ascertainment of a large number of women with familial breast cancer who are screened for all currently established predisposition genes, whose tumours are assessed for multiple parameters in a uniform manner, and in which controls (*BRCA1/2*+ and non-familial ‘sporadic’ cases) are collected from the same population.

Key words: BRCAX; non-*BRCA*; breast pathology; oestrogen receptor; HER2-neu; basal; luminal; familial; family history.

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INTRODUCTION

Prevalence and significance of family history in understanding breast cancer aetiology and risk

Breast cancer is the most commonly diagnosed non-skin cancer among American women. For studies of aetiology and prognosis, breast cancer can be categorised in a number of ways, such as by histology, stage, age at diagnosis, and expression of tumour markers. Family history is also widely recognised as a risk factor for breast cancer within families.^{1–3} Relative risk for breast cancer is doubled if one has a parent or sibling with breast cancer; and quadruples if one has

both a parent and sibling with breast cancer, with risk increasing as age at diagnosis of the affected relative decreases.^{4,5} In a study of 1019 respondents of a random telephone survey of adults aged 25–64 years conducted in the USA in 2001, prevalence estimates for breast cancer among any first-degree relative was 10.9% [95% confidence interval (CI) 8.8–13.3], any second-degree relative was 17.9% (95% CI 15.4–20.1), and for either was 26.4% (95% CI 23.3–29.8).⁶ The same researchers also explored the accuracy of self-reported family history, as that is generally how family history is captured clinically.⁶ For breast cancer, the positive predictive value was about 61% and negative predictive value was more than 95%, indicating that under reporting, not over reporting, was the important factor affecting accuracy. As expected, increasing age was associated with higher prevalence of breast cancer in first-degree relatives. These results were consistent with multiple other similar studies.^{7,8}

In the early 1990s, studies of families with high rates of breast cancer at younger than average ages led to the discovery of the genes *BRCA1*⁹ and *BRCA2*.¹⁰ As these two genes did not explain the majority of families with strong breast cancer histories, it was anticipated that a ‘*BRCA3*’ would also be discovered rapidly. Two decades later, the search is ongoing. A number of other genes that predispose a person to breast cancer have been discovered^{11–13} but pathogenic variants in all of these are rare or of much lower penetrance. With the adoption of next-generation cancer gene panels, multiple studies report that mutations in *BRCA1* or *BRCA2* (hereafter referred to as *BRCA1/2* in this review) still account for the majority of diagnoses made amongst women referred for testing (Table 1). Among individuals seeking clinical evaluation for hereditary breast cancer, pathogenic or suspected pathogenic variants were found in *BRCA1/2* in only 9–29%. Of the 20–40 other predisposition genes on hereditary cancer testing panels, 4–11% of patients had a pathogenic or suspected pathogenic variant in one of the non-*BRCA1/2* genes. Most notable was that 64–86.5% of women evaluated clinically for hereditary breast cancer had no gene mutations found that explained their presentation.¹⁴

Histopathology in breast cancers arising in carriers of pathogenic variants in known genes

BRCA1-related breast cancers have been extensively studied and are known to have a profile that is different from the

Table 1 Description of the inclusion criteria and cohort size in genetic studies of familial breast cancer

Reference	Cohort 1 ¹¹		Cohort 2 ¹¹		12
	13				
Study description	Women referred for clinical BRCA1/2 testing 2002–2012	Referred for commercial BRCA1/2 testing at Myriad Genetics Laboratory due to breast cancer 11/2012–4/2013	BRCA1/2 negative with personal history of breast cancer and family history of breast ± ovarian cancer referred for genetic testing 1998–2013	BRCA1/2 negative with early onset breast cancer (aged <40 years). Negative personal and family history of ovarian cancer	
Number of individuals	198	1781 individuals (1750 women)	377	278	
Number of genes tested	42	25	23	22	
Percentage of germline mutations					
No pathogenic or suspected	64.10%	86.50%	96.30%	89%	
pathogenic mutations found					
BRCA1/2 vs all others	29% vs 11.4%	9.3% vs 3.9%	N/A	N/A	
BRCA1 or BRCA2	29%	9.30%	N/A	N/A	

average breast cancer.^{15,16} The majority of *BRCA1*-related breast cancers are invasive ductal adenocarcinomas (74%); however, medullary-like carcinomas are over-represented (~13%) compared to the general population (~2%). Around 75% of *BRCA1*-related breast cancers are oestrogen receptor (ER) negative, often poorly differentiated high grade tumours with high mitotic counts, and with other features indicating an aggressive phenotype (decreased tubule formation, higher degree of pleiomorphism, and high frequency of lympho-vascular invasion). The immunophenotype is also notable for low expression of progesterone receptors, as well as human epidermal growth receptor 2 (HER-2/neu) compared to non-*BRCA1* cancers, hence there is a much higher rate of being ‘triple negative’/basal-like compared to non-*BRCA1*-related cancers (69% versus 15–20%).

The histopathology of *BRCA2*-related cancers, on the other hand, is very similar to non-*BRCA* cancers. Most (76%) are invasive ductal, with more lobular, tubular, and cribriform types compared to sporadic cancers. Grade is more frequently moderate or poorly differentiated. The immunophenotype for hormone receptors and HER-2/neu is similar to sporadic non-*BRCA*-related cancers: luminal type with over expression of oestrogen and progesterone receptors. Only 16% are triple negative.

Knowledge of the histopathological profile of breast cancers arising in carriers of pathogenic variants in other genes is far more limited and incomplete. Published data are shown for breast cancers among carriers of germline pathogenic variants in *ATM*, *BARD1*, *BLM*, *CDH1*, *CHEK2*, *MUTYH*, *PALB2*, *PTEN*, *RAD51C*, *RAD51D*, and *TP53* (Supplementary Table 1, Appendix A) and for the DNA mismatch repair genes, *MLH1*, *MSH2*, *MSH6*, and *PMS2* (Supplementary Table 2, Appendix A). No details of cancers in *MRE11*, *RAD50*, *SLX4*, and *XRCC2* have yet been published. Across these genes, no clear deviations from sporadic cancers were evident, with three possible exceptions. There is reportedly an increased number of lobular breast cancers in *CDH1* carriers [44–50% in three small series (Supplementary Table 1, Appendix A) compared to 21–22% in Surveillance Epidemiology and End Results (SEER) registries (Table 2)]; *TP53* carriers tended to have high grade tumours [81% grade 3 (Supplementary Table 1, Appendix A) compared to 33–50% in SEER registries (Table 3)]; and *PTEN* carriers were mostly ductal carcinomas but also notable for presence of apocrine tumours and a variety of unusual concomitant benign breast findings qualitatively described including multiple hamartomas, hyaline deposition in lobules, stroma and nodules, adenosis, and intraductal papillomatosis.^{17,18}

However, it remains unclear what proportion of breast cancer cases that are diagnosed at young ages and/or with a strong family history represent a random clustering of a common tumour due to shared multifactorial aetiology (low penetrance polygenic plus shared environmental factors) versus being due to undetected variants affecting moderately or highly penetrant known genes or genes yet awaiting discovery. In 1996, one model suggested that clustering of three first-degree relatives with breast cancer under age 70 years was much more likely to be due to a high penetrance gene,¹⁹ though a more current understanding of ‘sporadic’ breast cancer now includes appreciation that genetic risk is likely a polygenic continuum, not a binary issue. These apparently high-risk non-*BRCA1/2* families are sometimes called ‘BRCAx’ for having a suspected but unknown *BRCA1/2*-

Table 2 Relationship between breast cancer histology and tumour stage, ER/PR status, and grade among women diagnosed either aged 50–89 years or aged 30–49 years in the general population for purposes of comparison with the BRCAX studies^{a,48}

SEER Data ⁴⁸	Percentage ER neg	Percentage PR neg	Stage I	Stage II	Stage III/IV	Grade 1 or 2	Grade 3	Grade 4
Women diagnosed aged 50–89 years								
Ductal (<i>n</i> = 74,798)	22%	33%	53%	38%	9%	62%	36%	2%
Lobular (<i>n</i> = 9387)	8%	25%	44%	43%	13%	76%	21%	3%
Ductal/Lobular (<i>n</i> = 7359)	8%	22%	48%	44%	8%	72%	26%	2%
Mucinous (<i>n</i> = 2730)	5%	17%	68%	28%	4%	93%	6%	1%
Women diagnosed aged 30–49 years								
Ductal (<i>n</i> = 27,665)	34%	38%	40%	50%	10%	47%	50%	3%
Lobular (<i>n</i> = 1888)	11%	15%	36%	48%	16%	74%	22%	3%
Ductal/Lobular (<i>n</i> = 2277)	13%	17%	36%	52%	12%	65%	32%	3%
Mucinous (<i>n</i> = 518)	9%	19%	61%	35%	4%	86%	13%	1%

ER, oestrogen receptor; PR, progesterone receptor

^a Original data showed additional rarer histological subtypes as well.

like genetic causation. We were interested in examining the published literature on such cohorts to evaluate the current evidence for or against these families being different from or the same as sporadic non-familial breast cancers based upon the histopathological or molecular characteristics of the breast cancers that occurred.

METHODS

A literature search using PubMed and Medline was conducted including years 2000–2015, using a variety of search terms including familial breast cancer, BRCAX, non-BRCA, and hereditary breast cancer. References cited in these papers were culled for additional sources. Papers published in English were abstracted when they contained clinical, pathological, or molecular findings on a series of cases (any size) that had a family history of breast cancer but had tested negative for germline BRCA1/2 gene mutations (and other genes, if additional testing was conducted). Information was abstracted on the family history inclusion criteria for each study, the years that patients were ascertained, the method used to exclude BRCA1/2 mutations, country of origin, ages of cases at diagnosis, and type of controls, when applicable. We then recorded the results of the laboratory studies reported on the non-BRCA1/2 cohort. As described herein, the absence of uniform inclusion criteria across studies renders consideration of a meta-analysis infeasible.

RESULTS

Fourteen studies were identified that met our review criteria. Family history criteria varied across the studies. For familial clustering of breast cancer cases in which no BRCA1/2 mutation was detected, a number of studies used the term BRCAX or BRCax to encompass these^{20–34} while others used descriptive terms like non-BRCA families.^{23,35–45} There was overlap but no uniformity in how family history was defined, and the level of detail provided varied from none at all (patients referred for BRCA testing, not otherwise specified) to well-defined criteria (Table 3; Supplementary Table 3, Appendix A). For those providing criteria, elements generally included some reference to young onset diagnosis in the family and having one or more first- or second-degree relatives with breast cancer. In general, these are the types of families that have been recognised as warranting evaluation for BRCA1/2 mutations, based on known characteristics of hereditary cancers, as predicted by the Knudson two-hit hypothesis of cancer predisposition.⁴⁶

Fourteen papers were identified that contained some pathology data on the non-BRCA1/2 families which, for simplicity, will hereafter be called BRCAX families.^{20,21,26–30,32,34,35,37,38,45,47} The inclusion criteria

varied as noted above. Studies included populations from Northern and Southern Europe, Ashkenazim, Australia, and the USA. Years of ascertainment were not provided for 50% of the studies; for those that did provide this information, the range was from 1981 to 2012. All but one study³⁷ was conducted retrospectively. The mean number of BRCAX cases included in these 13 studies was 80 (range 14–391), with only two studies with >200 cases, three studies with 100 to 200 cases, and the remainder below 100 cases. The mean ages at diagnosis of breast cancer in cases was notably younger than in the general population, being approximately age 50 years (range 35–57; not reported in three studies); menopausal status was not generally provided. BRCA1/2 mutations were sought by a variety of methods and in the years following approximately 2007 generally included analyses for large genomic rearrangements when described. The BRCAX cases were compared to both BRCA-mutation positive cases and to sporadic cases in four studies, to BRCA-mutation positive cases only in seven studies, to sporadic cases only in two studies, and no controls were reported in one study.

Ten studies provided breast histology in BRCAX families: 56–86% were ductal carcinomas; 2–15% were lobular carcinomas; 0–17% were medullary carcinomas (Table 4). Table 5 shows comparable figures from a population-based series of 135,157 women with breast cancer per the SEER database of the National Cancer Institute from 1992 to 2001.⁴⁸ Given the ranges across the BRCAX studies, it is not possible to state if these differ from the SEER numbers. Also, limitations of these types of comparisons need to be fully acknowledged. With the SEER data and with all the other studies, there is no information on the concordance of histological classification between pathologists and across different medical centres; therefore, the magnitude of possible misclassification cannot be estimated.

Twelve studies reported on oestrogen and progesterone receptor status and of these, eight reported on HER2 status either determined by immunohistochemistry or by fluorescent *in situ* hybridisation. Across these studies, and not attempting to account for the difference in numbers across these studies, oestrogen receptor was negative in 14–40%; progesterone receptor was negative in 24–70%. HER2 expression was negative in 40–91%; triple negative status was only reported in three studies and was noted in 13.8–34% of cases. The Li *et al.*⁴⁸ study provided numbers

Table 3 Inclusion criteria, number of families, and age at first breast cancer diagnosis in published BRCA1/2 familial breast cancer studies

Reference	Inclusion criteria of mutation-negative 'BRCA1/2' cases studied	Number of mutation-negative cases described	Age at first diagnosis of breast cancer (range) for BRCA1/2 group
47	<ul style="list-style-type: none"> Moderate to strong family history of breast cancer disease; No BRCA1/2 mutations; and Low probability of being attributable to BRCA1/2 per linkage studies Excluded families with ovarian cancer or male breast cancer as likely being BRCA-related	82	Not reported
34	<ul style="list-style-type: none"> Families with early-onset breast cancer (NOS), male breast cancer, breast and ovarian cancer in same family; or An individual with both breast and ovarian cancer 	55	Mean 50 ± 12 years
29	<ul style="list-style-type: none"> Eight families with 2–3 breast cancer cases per family studied Eligibility criteria not described	17	Median 57 years (range 36–79)
45	<ul style="list-style-type: none"> Females with early breast cancer treated with breast conservation surgery; or Genetic testing done selectively on females with two first-degree relatives with cancer, one of which was diagnosed before age 41 years or ovarian cancer at any age; or At least three first- or second-degree relatives from same lineage with invasive breast or ovarian cancer at any age 	107	Not reported
28	<ul style="list-style-type: none"> Eighteen breast cancer cases from 18 families with at least three breast or ovarian cancer cases in first- or second-degree relatives 	18	Mean 46 years (range 32–70)
32	<ul style="list-style-type: none"> Families with breast cancer meeting kConFab^a Category 1 and Category 1B criteria 	132	Median 53 years (range 25–90)
21	<ul style="list-style-type: none"> Females with breast cancer under age 36 years, or Females with breast plus ovarian cancer at any age; or Males with breast cancer at any age, independent of family history; or Three or more first-degree relatives (or second-degree relatives if in paternal lineage) affected with breast or ovarian cancer at any age; or Two first-degree relatives (or second-degree relatives if in paternal lineage) with: <ul style="list-style-type: none"> breast cancer under age 50 years; or breast cancer under age 50 years plus breast cancer bilateral at any age; or breast cancer under age 50 years plus ovarian cancer at any age; or breast cancer under age 50 years plus male breast cancer at any age plus ovarian cancer at any age 	33	Median 44 years (no range reported)
27	<ul style="list-style-type: none"> Families defined by kConFab^a 	14	Not reported
38	<ul style="list-style-type: none"> Females with breast cancer having clinical BRCA testing. Family history criteria not described	151	Mean 53 years (range 24–79)
20	<ul style="list-style-type: none"> Non-BRCA1/2 familial breast cancers not otherwise defined. 	209	Mean 42 years (range 22–64)
30	<ul style="list-style-type: none"> Families with three or more female breast cancers, one under age 50 years; or Families with female breast plus ovarian cancer and at least one male breast cancer 	50	Mean 47 (no range reported)
26	<ul style="list-style-type: none"> Proband with breast cancer from family with three or more females with breast cancer; or Two first-degree relatives with female breast cancer, one under the age of 50 years; or One case of female breast cancer plus ovarian cancer, bilateral breast cancer, or male breast cancer 	14	Median 49 (range 32–66)
35	<ul style="list-style-type: none"> Females with breast cancer seen clinically for BRCA testing No information on criteria for referral for clinical testing.	391	Median 44 (range 21–75)
37	<ul style="list-style-type: none"> Females with breast cancer diagnosed under age 40 years: 47% had a first-degree relative with breast or ovarian cancer; 39% had some family history not otherwise specified 	38	Median 35 (range 23–39)

^a kConFab criteria in [Supplementary Table 3, Appendix A](#).

Table 4 Histopathology of breast cancers across published BRCA1/2 familial breast cancer) studies

Reference	47	34	29	45	37	28	35	32	26	21	20	27	30	38
Histopathology														
Ductal 8503: duct adenocarcinoma	73%	86%	65%	84.60%	N/A	56%	N/A	65.90%	N/A	82%	88%	79%	N/A	78%
Medullary 8513: medullary carcinoma, NOS	1%	4%	6%	1.10%	N/A	17%	N/A	2.30%	N/A	0	0	0	N/A	0
Mucinous or colloid 8483: mucin-producing adenocarcinoma	0%	4%	6%	0	N/A	0	N/A	1.50%	N/A	0	0	0	N/A	0
Lobular 8523: lobular carcinoma	15%	2%	6%	11%	N/A	5%	N/A	10.60%	N/A	3%	2%	7%	N/A	11%
Other or mixed	9%	0	17%	1.10%	N/A	22%	N/A	0.80%	N/A	15%	10%	14%	N/A	12%
Receptor % triple negative	N/A	N/A	N/A	N/A	34%	N/A	13%	N/A	N/A	N/A	N/A	2/8 (25%)	N/A	N/A
Percentage ER negative	N/A	37%	35%	27.50%	34%		23%	31.00%	17%	30%	29%	3/11 (27%)	40%	14%
Percentage PR negative	N/A	32%	41%	21.70%	46%		37%	35.60%	42%	36%	N/A	7/10 (70%)	44%	24%
Percentage HER-2/neu (IHC or FISH) positive	N/A	N/A	N/A	N/A	60%	N/A	14%	N/A	20%	9%	22%	5/9 (56%)	20%	22%
T stage and N stage (M stage not proved for any study)														
T stage	N/A	4% Tis; 67% T1, T2; 2% T3, T4	N/A	79.4% T1, T2; 0% T3	47% Tis, T1; 53% T2, T3, T4	17% T1; 28% T2; 56% T3	N/A	N/A	N/A	N/A	N/A	N/A	N/A	64% T1; 30% T2; 5% T3; 1% T4
N stage	N/A	55% N0; 45% N1	N/A	84% N0; 16% N1	NA	50% N0; 50% N1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	64% N0; 16% N1; 8% N2; 4% N3
Nuclear grade														
1	27%	48%	13%	76%	56%	0%	13%	25.80%	N/A	12%	10%	0%	55%	19%
2	50%		40%			44%	49%	37.90%	N/A	64%	44%	54%	21%	41%
3	23%	52%	47%	23.90%	44%	56%	38%	36.40%	N/A	24%	46%	46%	25%	37%

ER, oestrogen receptor; FISH, fluorescent *in situ* hybridisation; IHC, immunohistochemistry; PR, progesterone receptor.

Table 5 SEER data from 1992–2001 showed the distribution of histological types of breast cancer in the general population⁴⁸

Histology	Women aged 30–89 years (n = 139,310)	Women diagnosed aged 50–89 years (n = 100,028)	Women diagnosed aged 30–49 years (n = 35,129)
Infiltrating ductal	73.60%	74.80%	78.70%
Invasive lobular	8.10%	9.40%	5.40%
Ductal/Lobular	6.90%	7.40%	6.50%
Mucinous (colloid)	2.30%	2.70%	1.50%
Comedo	1.60%	1.40%	2.40%
Inflammatory	1.50%	1.40%	2.00%
Tubular	1.40%	1.60%	1.10%
Medullary	1.20%	0.90%	2.10%
Papillary	0.40%	0.50%	0.30%

on oestrogen receptor, progesterone receptor and HER2 expression from SEER, correlated with histology, which could not be similarly collated from the BRCAX studies (Table 2).

Nuclear grade was reported in 13 studies, with grades 1 and 2 being combined in three of the ten studies. A range of 24–52% was reported to be Grade 3 out of three grades among the BRCAX studies. The Li SEER study used a 1–4 grade scale.⁴⁸ TNM stage was reported in five BRCAX studies. The largest study³⁵ reported that 28.4%, 45.8%, 24.8%, and 1% of non-BRCA cases were TNM stages I, II, III, and IV, respectively. The 25% TNM Stages III plus IV in this study were a higher TNM stage than either the older or younger SEER cases which had 4–13% and 4–16% range, respectively, across the four main histological types.⁴⁸

A variety of additional studies have been reported across families that broadly are defined as non-BRCA familial breast cancer or BRCAX. These are summarised in Supplementary Table 4 (Appendix A). Array comparative genomic hybridisation (CGH) has been reported in five different studies.^{25,26,28,29,42} Fernandez-Ramires *et al.*²⁶ reported that a 21-gene expression signature could distinguish luminal A phenotypes for *BRCA1* versus BRCAX versus sporadic tumours. Didraga *et al.*²⁵ reported that the BRCAX cancer profiles were heterogeneous but a subset showed a gain of whole chromosome 22 (and 10 other loci) not seen in the three control groups which were from women with sporadic breast cancers, *BRCA1*- or *BRCA2*-mutated tumours. In addition, the chromosome 22-gain subgroup tended to run true in families and to have a distinctive pattern (with more ER negative and triple negative, and less TP53 positive) whereas the 22-loss group was indistinguishable from sporadic cancers. Waddell *et al.*⁴² were not able to distinguish BRCAX cancers from *BRCA1* and *BRCA2* but excellent correlation was noted with molecular subtypes (e.g., luminal, basal, etc.) between array CGH and expression data. Hedenfalk *et al.*²⁹ reported being able to recognise two distinct subgroups among the BRCAX tumours with difference in alterations of 8q24, 21q22, and 17q21 and not mapping to known *BRCA1/2* profiles. Gronwald *et al.*²⁸ reported more gains among BRCAX than controls for chromosome 8q22–q24 (in 83% of 18 tumours compared with 39–69% of sporadic tumours reported), 19p, 19q, 20q11–q13; and more losses for 8p23–pter by CGH. Melchor *et al.*³³ used 25 IHC markers to assign molecular subtypes and showed BRCAX had basal-like findings in 7.1%, ERBB2 in 14.3%, luminal B in 25%, luminal A in 46.5%, and unclassified in 7.1%. The 8q24 expression data²⁹ and the

reported 8q22–q24 chromosomal gain data²⁸ may represent a recurrent phenomenon in BRCAX tumours but further work is needed to determine if these different technologies are measuring the same event.

Four studies reported on TP53 expression by IHC in 4–64% of BRCAX breast cancers;^{21,30,34,37} e-cadherin expression was present in 91% (89% in *BRCA1* and 100% in *BRCA2*);²¹ cyclin D1 was expressed in 36–56%^{21,30} compared with approximately 33% in unselected breast cancers;^{49,50} somatic hypermethylation of the *BRCA1* promoter was seen in 42%³⁰ compared with 59% among unselected breast cancers;⁵¹ and p21 was positive in 36.7% versus 18.4% sporadics.³⁰ Honrado *et al.*³⁰ also reported the following were expressed in BRCAX cancers in the same proportion as sporadic controls: Ki-67, BCL2, cyclins D3, E, A, and B1, p16, p27, Skp2, Rb, E2F2, CHEKs, TopoIIalpha, MDM2, CK5/6, CK8, vimentin, survivin, EGFR.

Although not specifically addressed as BRCAX, several studies have concluded that among families with suspicion of hereditary breast cancer (based on ages, family history, variously defined), if *BRCA1* and *BRCA2* mutations have been ruled out, the risk for ovarian cancer does not seem to be elevated.^{52,53} For example, Ingham *et al.*⁵³ studied the risk of ovarian cancer among 8,005 women from a breast cancer family history clinic in the UK. After identifying those with *BRCA1/2* mutations, those remaining had a relative risk of 0.37 (95% CI 0.01–2.03). That is, BRCAX as a group does not seem to have excessive ovarian cancer. On the other hand, the tumour spectrum of 334 BRCAX-type families was investigated in Sweden and endometrial cancer risk was reported to be roughly double the expected, renewing speculation that breast-endometrial cancer might represent a new syndrome⁴³ related to hormonal and reproductive risk factors shared by these two types of cancers.⁵⁴

The risk of contralateral breast cancer in BRCAX patients, as defined by the German Consortium for Hereditary Breast and Ovarian Cancer⁵⁵ was compared to that of *BRCA1* and *BRCA2* by Rhiem *et al.*⁴⁴ and reported overall as 17.2% (95% CI 14.5–19.9). For women with a first breast cancer diagnosed before age 40 years, the cumulative risk after 25 years was 28.4% (far less than for *BRCA1/2*) and for those diagnosed at age 50 years or later, the 25-year cumulative risk for contralateral breast cancer was 12.9%. Overall, these figures are similar to the risk in patients with sporadic breast cancers.

Premalignant proliferative lesions were assessed by Adem *et al.*⁵⁶ in the prophylactic mastectomy cohort at Mayo Clinic. BRCAX cases (*n* = 53) had no major difference in

prevalence of precursor lesions. The neoplastic lesions in BRCA1/2 mutation carriers had higher MIB-1/Ki-67 proliferation-labelling studies compared to BRCAX cases and in sporadic controls.⁵⁶

A combined analysis of four genome-wide linkage screens, including 149 multiple-case breast cancer families (at least three cases per family diagnosed under age 60 years) reported that: 'The highest LOD score obtained in any analysis of the combined data was 1.80 under the dominant model, in a region on chromosome 4 close to marker D4S392. Three further LOD scores over 1 were identified in the parametric analyses and two in the non-parametric analyses. A maximum LOD score of 2.40 was found on chromosome arm 2p in families with four or more cases of breast cancer diagnosed under age 50 years. The number of linkage peaks did not differ from the number expected by chance. These results suggest regions that may harbour novel breast cancer susceptibility genes. They also indicate that no single gene is likely to account for a large fraction of the familial aggregation of breast cancer that is not due to mutations in *BRCA1* or *BRCA2*'.³⁹

DISCUSSION

The majority of women with breast cancer who have a concerning family history for possible hereditary breast cancer are not found to have identifiable germline mutations in breast cancer predisposition genes. These 'BRCAX families' are unexplained today. In the absence of an identifiable underlying genetic cause, clinical management and risk assessment for relatives relies on the family tree. In reviewing the available studies on BRCAX families it is important to note that prior studies screened for *BRCA1* and *BRCA2* but did not screen out those who had pathogenic variants in the rarer genes that now are included on clinical gene panels. Thus, some of the historic BRCAX families can likely be explained by the rare genes but panel testing continues to identify a very large group of women (64–96%) whose families still can be described as BRCAX (Table 1).

Despite having a label or name, BRCAX is certainly not a syndrome or a homogeneous group. Enough studies have been conducted to know that there is not a major 'BRCA3' gene out there awaiting discovery. BRCAX simply describes a group of unknowns (hence the 'X'). There are many potential explanations for the existence BRCAX families. Some, maybe even most, may represent the chance clustering of what is a common tumour (breast cancer). Some families may have variants in known genes that because of their genomic location or nature were missed by current testing methods. Some families may be co-segregating extremely rare genes or not-yet-discovered breast cancer predisposition genes. Some families may have a high genetic burden of multiple low penetrance types of genes as defined by genome wide association studies which in aggregate result in multiple cases within one family. Familial epigenetic factors, perhaps due to shared environmental exposures, may also contribute to some families.

BRCA1-related breast cancers have characteristic histopathological features which set them apart from sporadic breast cancers and from *BRCA2*-related breast cancers. We sought to learn if histopathology findings across multiple BRCAX studies might yield clues to aetiology or highlight important subsets for further study. The studies turned out to

be very limited and of such different designs that interpretation of the findings was inconclusive. Several studies highlighted subtle clues that there may be subsets of familial breast cancer that are more than a simple chance aggregation of a common disease within some families. However, it is hard to know with certainty how much publication bias may be contributing to this impression (wherein positive findings are published and negative findings never see the light of day). None of the findings have been replicated, although two studies suggested some contribution from the 8q24 region.^{28,29}

The current body of literature is quite deficient on many fronts: all studies are retrospective, of modest size, with marked variability of inclusion criteria, lack replication of findings, lack adequate controls, and are composed of genetic testing limited to *BRCA1/2* only. To better gauge whether the non-*BRCA1/2* familial clusters might still contain important single gene subsets awaiting discovery will require much more study. What is needed is a prospectively ascertained cohort of women with breast cancer with collection of detailed family histories, who are screened for all currently known predisposition genes, whose tumours are assessed for multiple parameters in a uniform manner, in which controls (*BRCA1/2*+ and non-familial 'sporadic' cases) are collected from the same population. Only with this sort of rigorous design can one hope to resolve if there exists a subset of families that reflect heretofore undiagnosed specific entities.

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.pathol.2017.03.002>.

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