Research Article

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Increased Rate of Phenocopies in All Age Groups in *BRCA1/BRCA2* Mutation Kindred, but Increased Prospective Breast Cancer Risk Is Confined to *BRCA2* Mutation Carriers

D. Gareth R. Evans[1,2], Sarah L. Ingham[2,3], Iain Buchan[3], Emma R. Woodward[4], Helen Byers[1], Anthony Howell[2], Eamonn R. Maher[4], William G. Newman[1], and Fiona Lalloo[1]

Abstract

Background: To establish, if among unaffected noncarrier relatives in a family with an established *BRCA1/2* mutation, there is an increased risk of breast cancer.

Methods: We identified 49 women with breast cancer who were first-degree relatives of a pathogenic mutation carrier among 807 *BRCA1/2* families but who tested negative for the specific mutation. A prospective analysis of breast cancer from date of family ascertainment was performed for first-degree relatives of proven *BRCA1/2* mutation carriers and compared with population-expected incidence rates.

Results: Women who prospectively test negative for *BRCA1/2* mutations showed excess risk of breast cancer to be confined to *BRCA2* noncarriers with an observed:expected (O/E) ratio of 4.57 [95% confidence interval (CI) 2.50–7.67; $P < 0.0001$; O/E in *BRCA1* noncarriers, 1.77]; this dropped to 2.01 for *BRCA2* [relative risk (RR), 1.99; 95% CI, 0.54–5.10] from date of predictive test. Genotyping of 18 breast cancer susceptibility single-nucleotide polymorphisms (SNP) defined an RR of 1.31 for *BRCA2* breast cancer phenocopies with a breast cancer diagnosis at age less than 60 years.

Conclusion: Noncarriers remain at risk in the prospective follow-up of women who tested negative for *BRCA1/2*. Women testing negative in *BRCA2* families may have increased risk of breast cancer compared with population levels, particularly with strong breast cancer history in close relatives. Any increased risk in *BRCA1* families is likely to be insufficient to recommend additional interventions.

Impact: Our work can help with counseling women from *BRCA1/2* families who have tested negative, and could impact on how individual breast cancer risk is related back to these women. *Cancer Epidemiol Biomarkers Prev; 22(12); 2269–76. ©2013 AACR.*

Introduction

Identifying a mutation in either the *BRCA1* or *BRCA2* genes in an individual affected with cancer facilitates cascaded family testing and increased access to evidence-based screening and preventive measures for family members (1–3). However, a proportion of women in families with an identified mutation will develop breast cancer despite testing negative for the familial mutation (4). These women are considered to be phenocopies and raise difficult counseling issues in terms of determining the personal risks of breast and ovarian cancer and how to explain the issues.

Although testing negative for a family-specific mutation, it appears that some women may still be at increased risk of breast cancer if there are multiple members of the family with mutation-related breast cancer, particularly if they were detected at young ages (4, 5). It is likely that these families are enriched for genetic modifiers that may increase the penetrance in both *BRCA1* and *BRCA2* mutations carriers and noncarriers (6–8). Recent data indicates that use of the five most strongly associated single-nucleotide polymorphisms (SNP) of breast cancer risk identified through genome-wide association studies (GWAS) in families with BRCA2 mutations could account for an approximately 2-fold difference in risk between the 5% with the highest risk combination of SNPs compared with the 5% with the lowest risk combination (80%–96% breast cancer risk by age 80, compared with 42%–50%; ref. 9).

It is unclear whether the breast cancer risks in those testing negative for *BRCA1* or *BRCA2* mutations are increased at all ages, in a specific age period, or not at all

Authors' Affiliations: [1]Department of Genetic Medicine, The University of Manchester, Manchester Academic Health Science Centre, St. Mary's Hospital; [2]Genesis Prevention Centre, University Hospital of South Manchester Southmoor Road; [3]Centre for Health Informatics, Institute of Population Health, The University of Manchester, Manchester; and [4]Centre for Rare Diseases and Personalised Medicine, School of Clinical and Experimental Medicine, University of Birmingham College of Medical and Dental Sciences, and West Midlands Regional Genetics Service, Birmingham, United Kingdom

Corresponding Author: D.G.R. Evans, Department of Genetic Medicine, The University of Manchester, Manchester Academic Health Science Centre, St. Mary's Hospital, Oxford Road, Manchester M139WL, United Kingdom. Phone: +44(0)161-276-6206; Fax: +44(0)161-276-6145; E-mail: gareth.evans@cmft.nhs.uk

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(4, 10–15). Indeed, a recent article suggested that all women testing negative for a family mutation were at no greater risk than the average population (14). Without clear information on what risks to provide women testing negative for the family mutation, it is likely that there will be differences in how women are counseled. Therefore, we have reexamined our combined database of families with *BRCA1* and *BRCA2* mutations, to assess the risk of breast cancer in women testing negative for the family-specific *BRCA1* or *BRCA2* mutation after the date of ascertainment of the family and individual (when the first family member joined the Family History Clinic) and after the date of mutation testing.

Materials and Methods

Families with individuals with breast and/or ovarian cancer have been screened for mutations in *BRCA1/2* since 1996 in the overlapping regions of Manchester and Birmingham in Mid-/Northwest England, encompassing approximately 10 million people. Women with a family history of breast/ovarian cancer who attend specialist genetic clinics in these two regions have a detailed three-generation family tree constructed. The date the first family member was referred to the genetic service was considered as the family ascertainment date. If a *BRCA1/2* mutation is identified, further attempts are made to ensure that all individuals relevant to discussions on risk are represented on the family tree. All cases of breast/abdominal cancers are confirmed by means of: hospital/pathology records, Regional Cancer Registries (from 1960) or death certification. When a family-specific pathogenic *BRCA1/2* mutation is identified, predictive testing is offered to all blood relatives.

Details of all tested relatives and first-degree untested female relatives were entered onto a Filemaker Pro-7 database. The initial individual in which a mutation was identified was designated the "index" case, with all other individuals being classified as to their position in the pedigree compared with a proven mutation carrier. All women reaching 18 years were entered on the database even if untested for a mutation. The exception was for mothers of a mutation carrier when it was clear that the mutation was paternally inherited (i.e., there was no maternal family history but a very convincing paternal history of breast/ovarian cancer). A total of 807 index cases were studied. Date of birth and date of last follow-up, breast cancer status, ovarian cancer status, dates of diagnoses, and date of death (if applicable), gene mutation identified in the family, the individual's relationship to a known mutation carrier, and their mutation status were entered. The resultant combined series is referred to as the M6-ICE (Inherited Cancer in England) Study (4).

Women with breast or ovarian cancer who tested negative for the family mutation were defined as phenocopies. In 90% of cases, at least two independent blood draws from every phenocopy have been genotyped to firmly establish negative mutation status. Only first-degree relatives (FDR) of proven pathogenic mutation carriers were included in the study.

An analysis was undertaken assessing prospective breast cancer risk in individuals testing negative for the family mutation using date of ascertainment of the family by the genetic service as the start date. If the reason for family ascertainment was due to an index case in another region or country being identified as a mutation carrier, then the date of mutation report was used as ascertainment date. Standard incidence ratios were derived using age- and year-specific data from the population-based North West Cancer Intelligence Service (NWCIS) as previously described (4). Follow-up was censored at July 1, 2011, or date of breast cancer, date of death, or date of bilateral risk-reducing breast surgery, whichever was the earlier. Person-years at risk analyses were performed to assess expected cancers in the general female population using data from the NWCIS. Observed/expected ratios were assessed for statistical significance using the common method from Clayton and Hills based on the Poisson assumption (16). A subset of women testing negative for the family mutation were part of family history (FH) risk, an assessment program looking at women who have a high familial risk of breast cancer, and is part of the PROCAS program grant, for which we had ethical approval to check details against the NWCIS for cancer incidence. This was carried out in September 2011. A final analysis was carried out using date of testing of unaffected FDRs as start date.

In addition, an assessment of the strength of family history of breast cancer was included by summating the *BRCA2* element of the Manchester scoring system for each affected family member (17). This system scores breast cancers in the direct lineage based on age at diagnosis, giving higher scores for earlier age at diagnoses. In addition, an assessment was made of close breast cancer family history (FDR and second-degree) using diagnosis at less than 40 years in an FDR; less than 50 years in at least two relatives (including an FDR); or at least 3 (including an FDR) diagnosed less than 60 years as a surrogate for increased degree of breast cancer family history.

DNA testing for SNPs

DNA was extracted from blood samples provided by women attending the genetic clinics. In addition to the *BRCA1/2* mutation analysis, women were genotyped for 18 SNPs that have been shown to be associated with breast cancer risk in the general population (7) and a subset of SNPs in familial breast cancer (18). Using the published per SNP ORs and risk-allele frequencies (RAF) from Turnbull and colleagues (e.g. *FGFR2* per allele OR is 1.43 with RAF of 0.42; ref. 7), we calculated the ORs for each of the three SNP genotypes (no risk alleles, one risk allele, and two risk alleles), assuming independence (Table 1). To obtain an overall breast cancer risk score for each woman, we multiplied the ORs for each of her 18 genotypes together.

Table 1. SNP scores taken from the article by Turnbull and colleagues (7)

SNP	Gene	Risk allele	RAF	Weight 0	Weight 1	Weight 2	0 Freq	1 Freq	2 Freq	RR	W*F
rs2981579	FGFR2	T	42	0.72	1.03	1.47	34	49	17	1.43	100
rs10931936	CASP8	C	74	1.20	1.06	0.93	7	38	55	0.88	100
rs3803662	TOX3	T	26	0.86	1.12	1.45	55	38	7	1.3	100
rs889312	MAP3K	C	28	0.89	1.08	1.32	52	40	8	1.22	100
rs13387042	2q	A	49	0.82	0.99	1.20	26	50	24	1.21	100
rs1011970	cdkn2a	T	17	0.97	1.06	1.15	70	27	3	1.09	100
rs704010	10q22	A	39	0.95	1.01	1.08	37	48	15	1.07	100
rs1156287	*COX11*	A	71	0.87	0.96	1.05	8.5	41	50.5	1.1	100
rs11249433	notch	C	42	0.94	1.01	1.09	34	48.5	17.5	1.08	100
rs614367	11q13	T	15	0.96	1.10	1.27	72	26	2	1.15	100
rs10995190	10q21	G	85	0.77	0.90	1.04	2	24	74	1.16	100
rs4973768	3p24 SLC4A7	T	47	0.87	1.00	1.16	28	50	22	1.16	100
rs3757318	*ESR1(6q25.1)*	A	7	0.96	1.25	1.62	86.5	13	0.5	1.3	100
rs1562430	8q24	G	42	1.14	0.97	0.82	33.5	49	17.5	0.85	100
rs8009944	*RAD51L1*	A	75	1.21	1.06	0.94	6	38	56	0.88	100
rs909116	*LSP1*	T	53	0.84	0.98	1.15	22	50	28	1.17	100
rs9790879	*5p12*	C	40	0.92	1.02	1.12	36	48	16	1.1	100
rs713588	*10q*	A	60	1.19	1.02	0.88	16	48	36	0.86	100

Results

Among 809 families (two index cases had a mutation in both *BRCA1* and *BRCA2* providing two families each; Fig. 1) with a proven pathogenic mutation (428 *BRCA1* or 381 *BRCA2*), 290 FDR female relatives with breast cancer have undergone genetic testing following identification of the family mutation; there were also 110 deceased obligate FDR mutation carriers with breast cancer (inferred by other family testing). An additional 383 deceased FDRs with breast cancer were of unknown mutation status as well as 77 living breast cancer FDRs. Forty-nine (17%) FDR relatives with breast cancer tested

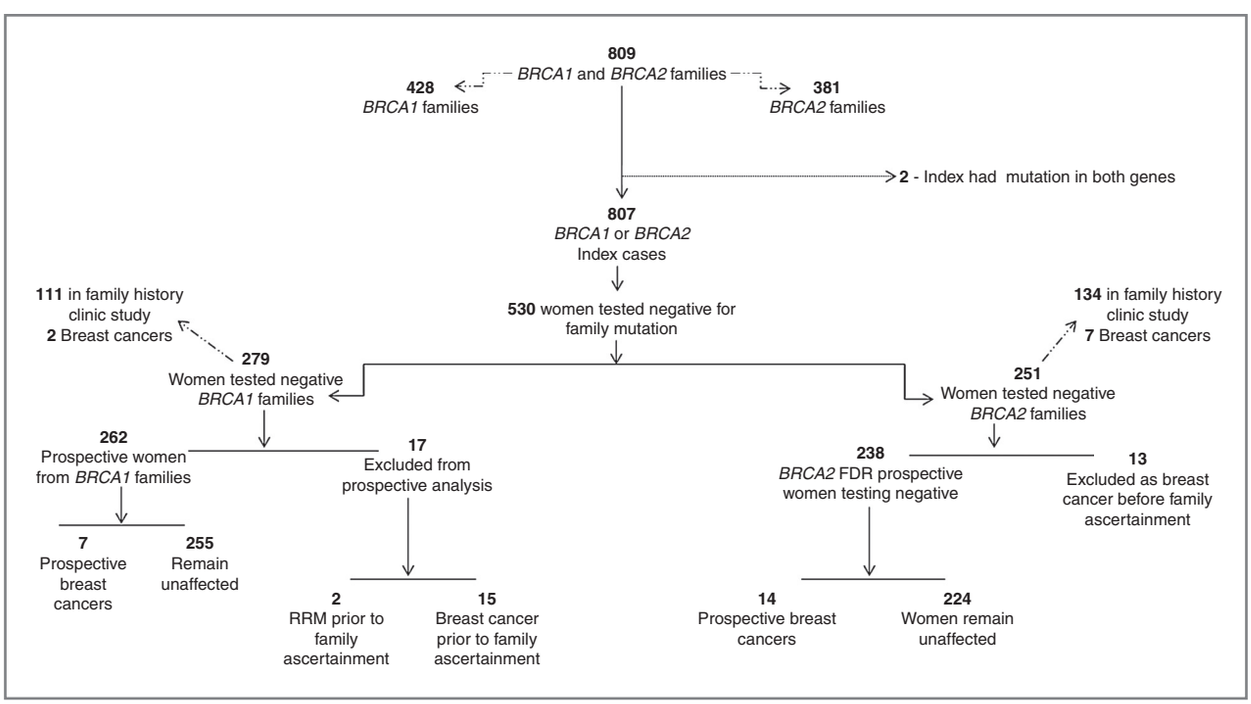

Figure 1. Consort diagram—prospective analysis.

Table 2. Proportion of first-degree relatives with breast cancer of a proven *BRCA1* or *BRCA2* mutation carrier who test negative for the family mutation, by age group

Age range	*BRCA1*	*BRCA2*	*BRCA1* and *BRCA2*	*BRCA1/2* proportion of carriers developing their first breast cancer for each decade in age group	Population breast cancer rates per decade in age group	Ratio of rates in age group compared with population	Proportion of 50% expected to test negative if no other factors involved
18–29	0/8	0/3	0/11	3%	0.05%	600:1	0.016%
30–39	2/43	3/40	5/83 (6%)	15%	0.5%	30:1	3.2%
40–49	6/46	8/48	14/94 (15%)	30%	1.5%	20:1	4.8%
50–59	10/29	9/37	19/66 (28.8%)	15%	2.7%	6:1	15%
60–80	5/15	7/21	12/36 (33%)	25%	5.8%	4:1	18.9%
Total	23/141 (16.3%)	26/149 (17.5%)	49/290				

negative for the family mutation. Ninety-five breast cancers occurred in FDRs after the family ascertainment date and 21 (22%) of these tested negative. Of those who underwent predictive testing for the family mutation as unaffected individuals but who have subsequently developed breast cancer, 8 of 42 (19%) have tested negative. There were only two ovarian cancer phenocopies ages 66 and 71 years, both in *BRCA1* families, and these were not evaluated further.

The proportion of women with breast cancer with a negative test increased with age (Table 2) but leveled off after age 50 years, although 25% of tests were negative above this age. The percentage of retrospective phenocopies is similar in both *BRCA1* and *BRCA2*—16.3% and 17.5%, respectively (Table 2). In each age range (apart from <30 years where there were only 11 cases), there were approximately twice as many cases of breast cancer (phenocopies) than would have been expected in the general population. This proportion may be an underestimate as only recent population incident rates were utilized. Twenty percent of phenocopies (10/49) were diagnosed prior to 1990 when the incidence of breast cancer in the population was lower.

Prospective analysis

In total, 279 female FDRs tested negative for the family *BRCA1* mutation and 251 for *BRCA2* (Fig. 1). Two women (*BRCA1*) who had undergone bilateral risk-reducing breast surgery prior to ascertainment in the genetics service were excluded from the analysis as were 27 women who had developed breast cancer prior to family ascertainment, and one woman who died prior to family ascertainment (*BRCA2*). Thus, 17 breast cancers from *BRCA1* families and 13 from *BRCA2* families were excluded from the prospective analysis. Since family ascertainment, 7 (2.5%) cases of breast cancer occurred in the remaining 262 women testing negative for the familial *BRCA1* mutation and 14 (5.9%) in 238 women testing negative for their familial *BRCA2* mutation (Table 3).

Using a pragmatic recent date of follow-up (June 30, 2011), assuming notification of breast cancers, the rates

Table 3. Prospective rates of breast cancer incidence and ORs in those FDRs testing negative for *BRCA1/2*

Series		Number	Years follow-up (rate per 1,000)	Breast cancers	Expected cancers	OR (95% CI)
All tested negative from family ascertainment	*BRCA1*	262	3,217 (2.17)	7	3.95	1.77 (0.71–3.65)
	BRCA2	238	2,634 (5.3)	14	3.06	4.57 (2.50–7.67)
Family History Clinic group from family ascertainment	*BRCA1* negative	111	1,141.8 (1.76)	2		
	BRCA2 negative	134	1,189.9 (5.9)	7	1.45	4.82 (2.63–8.09)
	BRCA2 untested	52	490.5	0	0.79	
	Adjusted *BRCA2* including untested	162	1,454.8 (4.8)	7	1.98	3.535 (1.42–7.29)
From negative genetic test date	*BRCA1*	262	1,456.8 (2.7)	4	3.80	1.05 (0.28–2.70)
	BRCA2	238	852 (4.7)	4	2.01	1.99 (0.54–5.10)

Table 4. *BRCA1/2* phenocopies occurring after family ascertainment by Manchester score for families with *BRCA2* mutations and degree of breast cancer family history criteria

Gene	Number testing negative	Median age at family ascertainment	Prospective breast cancers	Years follow-up to June 30, 2011, or censoring	Breast cancer rates per 1,000	Expected cancers	OR (95%CI)
BRCA1	262	36.4	7	3,217.8	2.17		
BRCA2	238	35.9	14	2,634.0	5.3		
BRCA1 MS > 10	183	36.1	7	2,401.9	2.91		
BRCA1 MS < 11	79	39.3	0	815.9	0		
BRCA2 MS > 10	149	37.3	11	1,696.8	6.48		
BRCA2 MS < 11	89	33.0	3	937.0	3.2		
***BRCA2* degree of breast cancer family history**	125	35.2	10	1,448	6.9	1.42	7.04 (3.37–12.95)
***BRCA2* no degree of breast cancer family history**	112	35.5	4	1,204	3.3	1.57	2.55 (0.69–6.52)

Abbreviation: MS, Manchester score for *BRCA2* in family.

were 2.17 per 1,000 (in 3,217 years) in *BRCA1* and 5.3 per 1,000 (in 2,634 years) in *BRCA2* noncarriers, with a statistically significant difference between these phenocopy rates ($P = 0.0536$; Table 4). Age at ascertainment was very similar between noncarriers in *BRCA1* (mean age at ascertainment, 38.07 years) and *BRCA2* (mean age at ascertainment, 38.24 years) families, with a P value of 0.9025. Using a person years at risk analysis, 3.95 cancers would have been expected in the cohort of *BRCA1* women and 3.06 in *BRCA2*. The observed:expected ratio was, therefore, 1.77 (95% CI, 0.71–3.65) for the *BRCA1* group and 4.57 (95% CI, 2.50–7.67) for *BRCA2*. The difference between the observed and expected values for *BRCA2* was statistically significant with P value less than 0.0001. This analysis does not allow for any testing bias of those developing breast cancer. We are aware of 21 breast cancers in untested FDRs, post family ascertainment of which three (using the phenocopy rate of 17%) would be expected to have tested negative. There were 9 of 475 untested female FDRs from *BRCA1* families and 12 of 466 from untested female FDRs from *BRCA2* families. A further 514 *BRCA1* FDRs and 471 *BRCA2* FDRs had tested positive. Thus, only 475 of 1,268 (37%) of living female FDRs older than 18 years were untested for their family *BRCA1* mutation and 466 of 1,187 (39%) for *BRCA2*. However, we cannot be sure of being informed of breast cancers in relatives included in this number who were not known to the service, who may be estranged, or live abroad.

Via an ethically approved study, FH risk, 245 of 500 (48%) cases in the present study were confirmed using a cancer registry. These women were all referred as unaffected women to a single family history clinic from 1987, and subsequently tested negative for a family-specific *BRCA1/2* mutation (111 *BRCA1* and 134 *BRCA2*). Confining the analysis to these 245 cases; nine breast cancers occurred in 2,330.68 women years of follow-up, of which seven were in 134 *BRCA2* noncarriers. The rate of breast cancer in these women was 4.29 per 1,000 for women with a median age at entry of 39.5 years as compared with an age-stratified populations 10-year risks of 1.5 per 1,000 annually. The rate in *BRCA1* noncarriers was 1.76 per 1,000 and 5.9 per 1,000 for *BRCA2*. Using population incidence rates for *BRCA2*, only 1.45 breast cancers would have been expected (RR, 4.82; 95%CI, 2.63–8.09). Due to the potential bias in the full dataset of testing those developing breast cancer, an analysis of FDRs who have not been tested was also undertaken for *BRCA2*. Fifty-two untested FDRs with an average age of 40.2 years had 490.5 years of follow-up. None of these had developed breast cancer. It is estimated that, had these women undergone predictive testing, 54% of these would have tested negative (323/600; 53.8% of presymptomatic predictive FDR genetic tests in *BRCA2* at less than 60 years were negative). As such, just over half of 490.5 years (264.8) would be estimated to be contributed by negative tests in the FDR unknown category. Taking into account the potential testing bias, a total of seven breast cancers occurred in 162 predicted *BRCA2* negatively tested FDRs in an adjusted rate of 4.8 per 1,000. Expected breast cancers were 1.55 for a 134 *BRCA2* negative cohort and were 0.79 in the 52 untested FDRs. Taking the same 0.54 proportion of this figure, expected breast cancers were 1.98 compared with seven observed (OR, 3.535; 95% CI, 1.43–7.37). A final analysis was carried out on those testing negative from date of mutation test. It was only possible to ascertain a recent date (within 18 months) of follow-up with known unaffected status for 149 of 238 women testing negative. For the remainder, the last contact from the family file was used. For 132 women, verification of cancer status was possible on the NWCIS registry. For *BRCA2*, a total of four prospective breast cancers occurred in 852 women years of follow-up in the 230 women who had not had risk-reducing mastectomy prior to genetic testing. This gives a rate of 4.7 per 1,000 in women with a median age at

predictive testing of 42.37 years. Expected cancers in this cohort were 2.01 for *BRCA2* (RR, 1.99; 95% CI, 0.54–5.10). There was no increase in risk seen for *BRCA1* families (Table 2).

The Manchester score for the family before mutation screening was calculated to assess the strength of breast cancer history in the full dataset of 500 noncarriers and was dichotomized to scores 11 or more or less than 11 (Table 4). There were no prospective cases of breast cancers diagnosed in mutation-negative women in the *BRCA1* cohort with scores less than 11. The cancer diagnosis rates were similar at 3.2 and 2.9 per 1,000 in the *BRCA2* mutation–negative women from families with a Manchester score less than 11 and *BRCA1* families with a score of 11 or more. However, the highest rate of 6.48 per 1,000 was found in the *BRCA2* cohort with the highest Manchester scores (Table 4). The observed:expected ratio for the three breast cancers in *BRCA2* carriers with scores less than 11 was 2.03 (expected, 1.47; 95% CI, 0.41–5.93) compared with 4.22 for the 11 *BRCA2* noncarrier cancers with family scores of more than 10 (expected, 2.61; 95% CI, 2.10–7.55). A further analysis was carried out on *BRCA2* mutation–negative tested individuals to assess whether those with increased degree of breast cancer family history had a higher rate of breast cancer diagnosis. A total of 126 individuals met the degree of breast cancer family history criteria and 10 developed breast cancer in 1448 years of follow up (6.9 per 1000) and 4 of 112 who did not meet the criteria developed breast cancer in 1204 years of follow up (3.3 per 1000) from family ascertainment. The observed: expected ratio for the degree of breast cancer family history group was 7.04 (expected, 1.42; 95% CI, 3.37–12.95) compared with 2.55 for those who did not meet the criteria (expected, 1.57; 95% CI, 0.69–6.52).

There was no censoring for oophorectomy; in fact, 12 (4.8%) *BRCA2* noncarrier FDRs and 10 (3.6%) *BRCA1* noncarrier FDRs underwent oophorectomy with three *BRCA2* and two *BRCA1* noncarriers subsequently developed breast cancer.

DNA testing for SNPs

Sufficient DNA was available to test 36 FDR phenocopies for the 18 validated SNPs. In addition, testing was carried out on 445 *BRCA2* mutation carriers (280 affected with breast cancer, 165 unaffected) and 462 *BRCA1* carriers (268 affected, 194 unaffected), 185 family history breast cancers testing negative for *BRCA1/2* mutations, and 421 population female controls from the NHS breast screening program in the PROCAS trial (19). The mean RR for the 18 SNPs was 1.27 for 22 FDR *BRCA2* phenocopies (10 before ascertainment and 12 after; range, 0.82–3.17; median, 1.18; 1.31 for 18 diagnosed age less than 60 years) and 1.24 (range, 0.41–2.63; median, 0.96) *BRCA1* for 14 FDRs phenocopies (1.13 for 12 at <60 years). In the 280 affected *BRCA2* mutation carriers, the mean RR was 1.165 (range, 0.29–5.09; median, 1.01), with a RR of 0.993(0.33–3.36; median, 0.86) for the 165 unaffected carriers. In the 268 affected *BRCA1* mutation carriers, the mean RR was 1.07 (range, 0.24–4.35; median, 0.93), with a RR of 1.11 (0.265–3.79; median, 0.956) for the 194 unaffected individuals. Among 185 family history–positive breast cancers without *BRCA1/2* mutations, the RR was 1.24 (range, 0.37–4.62; median, 1.10). The mean score in a series of 421 control samples from the general female population was 1.04 (range, 0.24–4.3; median, 0.93).

Discussion

The results of this analysis suggest higher phenocopy rates in women in families with pathogenic mutations in *BRCA2* than expected in all age groupings. These data suggest that breast cancer genetic modifiers in these families may increase the breast cancer risk even in women who test negative for familial *BRCA1/2* mutations. From the prospective analysis, it would appear that the risk of breast cancer is significantly greater in women who test negative for familial *BRCA2* compared with *BRCA1* mutations. This is consistent with data showing much wider penetrance estimates for breast cancer in *BRCA2* than in *BRCA1* (5, 20–26), and the greater number of SNPs shown to modify *BRCA2* risk (7, 27). It is important to note that eight (six *BRCA2*) of the 21 cases have occurred after our previous report (4). Furthermore, the increased rates of phenocopies among women from *BRCA2* mutation–positive families with high previous incidence of breast cancer as assessed by the Manchester score and degree of breast cancer family history criteria add support to the potential contribution of nonlinked genetic modifiers of breast cancer risk. In this case, unaffected women in *BRCA2* mutation–positive families might be expected to have a protective profile with an SNP RR of less than 1.0. However, we did not find this, suggesting that there is a bias toward higher allele frequencies of risk SNPs in the *BRCA2* mutation–positive families. This then infers that selection of families for mutation screening also selects for higher SNP scores irrespective of the subsequent *BRCA2* mutation status. For *BRCA1* there was little effect of the SNPs with affected carriers having a lower RR than unaffected individuals; this is perhaps unsurprising as many of the common variants influence risk of *ER*-positive disease. The 18 SNPs appear to contribute to the higher rate of breast cancer for those testing negative in *BRCA2* families although there must be other factors involved. At present, the 18 SNPs are considered to account for no more than 15% of the familial component of breast cancer.

Several groups have assessed risk in women testing negative with varied results (10–15, 28). The most compelling case for there not being an increase in risk was provided by a study of 28 families in which 395 female relatives tested negative for the family mutation. Breast cancer incidence was assessed from family/individual ascertainment as opposed to the date of testing negative in the present study. This study reported that the

RR of breast cancer, compared with the population average, was only 0.82 overall, but 1.33 in FDRs of breast cancer cases. This study however, predominantly assessed *BRCA1* carriers (322/395, 81.5%); a cohort in which fewer modifying SNPs have been validated (7). Although the investigators did not present data on *BRCA2* noncarriers, we calculate that the RR in this group would be approximately 1.53 in order to balance the 0.66 found in *BRCA1* noncarriers. A further prospective study from Australia found a nonsignificant increased risk of 1.29 in 442 first/second-degree relatives, but did not separate FDRs or *BRCA1*/*BRCA2* cases and, again, had smaller numbers of women with *BRCA2* mutations (15).

Kurian and colleagues (14) presented modeling data comparing cancer incidence of affected women testing negative for the familial *BRCA1/2* mutations with cancer incidence in affected women from families without *BRCA1/2* mutations. We have previously indicated that this was not a prospective study, but was population based, which would dilute any effect of modifier genes. In addition, this study did not compare risks with the general population (29). The lack of confirmation of cancer status of all individuals against a cancer registry in the North American/Australian studies may result in underestimating the risk of breast cancer. In the present study, 242 of 500 cases were checked against a cancer registry. Confining the analysis in this way, there is clear evidence of a difference in the phenocopy rate between families with *BRCA1* and *BRCA2* mutations. In addition, we have assessed all FDR women testing negative in Manchester and Birmingham, excluding those who developed breast cancer before family ascertainment. A final analysis showed that there was a nonsignificant increased rate of breast cancer in *BRCA2* mutation–negative FDRs, although this analysis was based on shorter follow-up time, and current vital and cancer status could not be confirmed with certainty in approximately 37% of women.

Further recent support for the potential increased risk in FDR noncarriers has just been published. A Dutch study found 17 breast cancers among 464 noncarriers, and estimated a breast cancer risk by 50 years of age of 6.4% (28). They found a statistically significant increase in risk for *BRCA1* noncarriers, but numbers for *BRCA2* were quite small.

A potential weakness in the present study is that breast cancer incidence could be affected by lead time bias from extra mammography compared with the general population. In addition, there may be a screening bias if women with a strong family history of breast cancer have undergone early MRI. In 71 of 242 (37 *BRCA2*) women testing negative for the familial mutation, extra mammography screening at less than 50 years of age is continuing. As such, this may increase breast cancer rates although only half of these women had been screened for less than 5 years. However, the effects may be expected to have had an equal effect on those testing negative for mutations in either gene, but no extra risk of breast cancer was seen in *BRCA1* mutation families.

Many computer models, including BOADICEA (8) and Tyrer–Cuzick (30), give increased risks for women testing negative in the context of a strong early-onset family history of breast cancer, although this imputation has not been directly validated. We believe that genetics specialists should remain cautious about stating that an individual's risk of breast cancer has returned to that of the general population unless they are tested negative for a family *BRCA1* mutation in a family without a strong family history of breast cancer. In the context of a family *BRCA2* mutation, especially when there are multiple close relatives affected with early-onset breast cancer, specialists should advise that breast cancer risks may still be increased compared with the general population. The recent discovery of further genetic loci that alter penetrance predominantly in *BRCA2* carriers (27) may facilitate additional testing for multiple SNPs to accurately predict whether those women negative for the familial *BRCA2* mutation still have an increased genetic risk.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: D.G.R. Evans, A. Howell, F. Lalloo
Development of methodology: D.G.R. Evans
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): D.G.R. Evans, E.R. Woodward, E.R. Maher, F. Lalloo
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S.L. Ingham, I. Buchan, A. Howell, W.G. Newman
Writing, review, and/or revision of the manuscript: D.G.R. Evans, S.L. Ingham, I. Buchan, A. Howell, E.R. Maher, W.G. Newman, F. Lalloo
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): D.G.R. Evans, S.L. Ingham, H. Byers
Study supervision: D.G.R. Evans

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