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EPIDEMIOLOGY

BRCA1 and *BRCA2* mutation carriers in the Breast Cancer Family Registry: an open resource for collaborative research

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Abstract The Breast Cancer Family Registry is a resource for interdisciplinary and translational studies of the genetic epidemiology of breast cancer. This resource is available to researchers worldwide for collaborative studies. Herein, we report the results of testing for germline mutations in *BRCA1* and *BRCA2*. We have tested 4,531 probands for mutations in *BRCA1* and 4,084 in *BRCA2*. Deleterious mutations in *BRCA1* and *BRCA2* were identified for 9.8% of probands tested [233/4,531 (5.1%) for BRCA1 and 193/4,084 (4.7%) for BRCA2]. Of 1,385 Ashkenazi Jewish women tested for only the three founder mutations, 17.4% carried a deleterious mutation. In total, from the proband and subsequent family testing, 1,360 female mutation carriers (788 in *BRCA1*, 566 in *BRCA2*, 6 in both *BRCA1* and *BRCA2*) have been identified. The value of the resource has been greatly enhanced by determining the germline *BRCA1* and *BRCA2* mutation statuses of nearly 6,000 probands.

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Introduction

The Breast Cancer Family Registry (Breast CFR), composed of six international registries and collaborating institutions, was established by the National Cancer Institute (USA) in 1995 to create a resource to facilitate collaborative interdisciplinary and translational studies of the genetic epidemiology of breast cancer [1]. The Breast CFR is available to researchers worldwide for collaborative studies (http://epi.grants.cancer.gov/CFR/). Over 40,000 participants from more than 13,000 families have been enrolled. These include incident breast cancer cases and their relatives ascertained through population-based cancer registries (population-based case families), families with strong cancer histories identified through cancer family clinics and community outreach (clinic-based families), and unaffected women and their relatives sampled from the populations (population-based control families). In addition, there was a specific recruitment of Ashkenazi Jewish women with a personal and/or family history of breast cancer. Population-based families were recruited from the San Francisco Bay area, California (California Breast CFR), the Province of Ontario, Canada (Ontario Breast CFR); and Melbourne and Sydney, Australia (Australian Breast CFR). Clinic-based families were recruited from Philadelphia (Philadelphia Breast CFR), New York City (New York Breast CFR), Utah (Utah Breast CFR), Ontario, Canada (Ontario Breast CFR), and Melbourne and Sydney, Australia (Australian Breast CFR). Specific recruitment of Ashkenazi-Jewish families was conducted by the New York, Philadelphia, Ontario and Australian Breast CFRs. All registries used the Breast CFR's standardized questionnaires and protocols to collect family history information, epidemiological and clinical data, and biological specimens (blood and/or buccal samples and tumor tissue), with quality control measures throughout the collection, processing, and storing of data and samples. Families are continuing to be followed for new cancer diagnoses and treatment and disease-free survival for breast cancer cases.

Information on the germline *BRCA1* and/or *BRCA2* mutation status of individuals is needed for many research projects that use the Breast CFR. For example, some studies specifically require mutation carriers only, other studies exclude these carriers as far as is possible, while other studies compare specific characteristics of carriers with those of non-carriers. To facilitate these endeavors, we report the results of a collaborative study through the Breast CFR to identify *BRCA1* and *BRCA2* mutation carriers.

Materials and methods

Subjects

Details of the enrollment criteria were previously described in detail [1]. In brief, the enrollment criteria were as follows:

(a) *Population-based families* The California Breast CFR recruited population-based case probands younger than 65 years at diagnosis through the SEER cancer registry of the Greater San Francisco Bay area. A two-stage sampling scheme was used with an over-sampling based on age at diagnosis and cancer family history. More recently, recruitment has been limited to African-American and Hispanic breast cancer cases. The Ontario Breast CFR recruited population-based case probands diagnosed before the age of 70 years using a two-stage sampling scheme, with over-sampling based on age at diagnosis and cancer family history, through the Ontario Cancer Registry, a voluntary cancer registry that includes 98% of breast cancer cases diagnosed in Ontario. The Australian Breast CFR recruited case probands, stratified by age at diagnosis and unselected for family history, through the Victorian and New South Wales Cancer Registries, for which registration of all cases is mandated by law. Unaffected women were sampled from the population-based sites to be used as population-based control families. The Australian Breast CFR also enrolled family members of control probands. No mutation testing for control probands is described in this paper.

(b) *Clinic-based multiple-case families* The Philadelphia Breast CFR recruited affected probands with a family history of breast and/or ovarian cancer from the Fox Chase Network of community hospitals, and Cooper Hospital/University Medical Center in Camden New Jersey, and unaffected probands with a family history of breast cancer from the Family Risk Assessment Programs (FRAP) at these institutions. The New York Breast CFR similarly recruited affected and unaffected probands with a family history of breast and/or ovarian cancer from hospitals and through community-based outreach to local organizations and breast cancer support groups. The Utah Breast CFR recruited families with three or more cases of breast or ovarian cancer, especially if at least one of the cancers occurred before the age of 45 years, from local clinicians, the Family Cancer Assessment Clinic at Huntsman Cancer Institute, and another ongoing research study. The Australian Breast CFR recruited affected and unaffected probands with at least two

affected relatives through cancer family clinics in Victoria and New South Wales, and through physician referral.

(c) *Ashkenazi-Jewish families* The New York, Philadelphia, Ontario and Australian Breast CFRs recruited Ashkenazi-Jewish women, predominantly those with a personal or family history of breast cancer, through clinics and the local communities [2].

BRCA1 and *BRCA2* mutation testing

Testing for *BRCA1* and *BRCA2* mutations was not supported as part of the original Breast CFR funding beginning in 1995, and therefore mutation testing was performed through multiple funding sources including NCI (a supplement) using several methodologies. The majority of mutational analyses of *BRCA1* and *BRCA2* were undertaken by laboratories closely associated with the Breast CFRs and funded from local sources. A validation study was conducted for the methods used between 1997 and 2000, including two-dimensional gel (2-D gel) scanning, denaturing high performance liquid chromatography (DHPLC), enzymatic mutation detection (EMD) and protein truncation tests (PTT) [3]. Specificity and sensitivity of the methods for protein-truncating mutations were comparable to genomic sequencing of all the coding exons in *BRCA1* and *BRCA2*. An additional method EGAN, based on Conformation Specific Gel Electrophoresis (CSGE) [4, 5] has been used for testing of the California samples. EGAN has been validated on a blinded patient set and compared favorably to full sequence analysis results in the same patients (sensitivity of 97.4% in detecting *BRCA1* and *BRCA2* sequence changes) (A. Miron, pers. comm.).

More recently, a substantial proportion of testing, funded by NIH, has been performed by Myriad Genetic Laboratories, Inc., using full sequence analysis (BRCAnalysis) (this was prior to their introduction of testing for large genomic re-arrangements, deletions, and insertions) [6]. The mutation detection method and number of probands tested at each registry are shown in Table 1, excluding those tested for only the three Ashkenazi Jewish mutations. Women recruited because of their Ashkenazi Jewish heritage were screened for the three founder mutations, 185delAG and 5382insC in *BRCA1* and 6174delT in *BRCA2*, primarily funded through a supplement from the NIH. For the population-based families, the proband was tested. For the clinic-based families, the proband was tested if she was affected with breast cancer, else the youngest breast cancer case in the family from whom a blood sample was available was tested. For the purposes of this report, the affected individual tested will be referred to as the proband. In families in which a

Table 1 *BRCA1* and *BRCA2* germline mutation testing for population-based and clinic-based families, excluding those tested for only the Ashkenazi Jewish founder mutations

Testing method	*BRCA1*			*BRCA2*			*BRCA1* and *BRCA2*
Family registry	# Probands tested	# w/Deleterious mutations	Total # Carriers[a]	# Probands tested	# w/Deleterious mutations	Total # Carriers[a]	
Population-based	3,682	149		3,242	121		
Australia	724	31	47	429	16	48	1
Sequencing	140	16		205	11		
2-D Gel	501	10			0		
PTT	83	5		224	5		
Ontario	1,229	67	100	1,219	58	84	1
Sequencing	150	6		150	6		
PTT	1,062	51		1,060	48		
Heteroduplex	17	10		9	4		
N. California	1,729	51	68	1,594	47	80	1
Sequencing	34	2		411	13		
2-D gel	862	29		0	0		
Heteroduplex	833	20		1,183	34		
Clinic-based—sequencing	849	84		842	72		
Philadelphia	241	19	29	236	15	29	
New York	438	30	42	438	27	49	
Utah	170	35	206	168	30	145	
Total	4,531	233 (5.2%)	492	4,084	193 (4.7%)	435	3

[a] Includes tested family members

deleterious mutation was identified, participating family members for whom we had collected blood were tested when warranted for the same mutation. At the Philadelphia and Utah Breast CFRs, family members were only tested after consenting specifically to genetic testing and counseling. In the Australian Breast CFR, the process for and uptake to the offer of genetic test results has been reported previously [7].

Definition of deleterious mutations

The criteria for defining deleterious mutations were those used by the Breast Information Core (BIC; http://research.nhgri.nih.gov/bic/) and Myriad Genetic Laboratories, Inc. For *BRCA1*, any frameshift or nonsense mutation that occurs at or before codon 1,853 was classified as deleterious. For *BRCA2,* any frameshift or nonsense mutation that occurs at or before codon 3,309 was classified as deleterious. Missense changes at the cysteines and the histidine in the ring finger of *BRCA1* were considered deleterious, as well as the R1699W and A1708E substitutions [8]. Missense changes at the first methionine for both genes were also classified as deleterious.

Results

Mutation testing results for non-Ashkenazi Jewish probands are shown in Table 1. For families tested for *BRCA1,* 5.1% (233/4,531) carried deleterious mutations. For families tested for *BRCA2*, 4.7% (193/4,084) carried deleterious mutations. Three probands were compound heterozygotes carrying deleterious mutations in both *BRCA1* and *BRCA2*. The frequencies of mutations identified from clinic-based families were 9.9% for *BRCA1* and 8.6% for *BRCA2*, and from the population-based families were 4.0% for *BRCA1* and 3.7% for *BRCA2*. Differences across registries may reflect the differences in sampling and extent and type of testing across registries and between population-based and clinic-based sites. Testing of family members in carrier families identified an additional 259 *BRCA1* and 242 *BRCA2* carriers.

Of the 1,385 Ashkenazi Jewish probands tested for the three founder mutations, 241 (17.4%) carried a mutation (Table 2). Testing of DNA from available family members identified an additional 155 carriers, for a total of 430 female mutation carriers. Three women were found to be compound heterozygotes, carrying both a *BRCA1* 185delAG and *BRCA2* 6174delT mutation. These 1,385 probands were not included in Table 1 because they were tested only for the three Ashkenazi Jewish founder mutations.

The total number of female *BRCA1* and *BRCA2* mutation carriers, including probands and family members, was 1,360 (788 *BRCA1*, 566 *BRCA2*, and 6 with both *BRCA1* and *BRCA2* mutations) (Table 3). Of the total mutation carriers, 313 of the 788 (39.7%) of the *BRCA1* carriers, 235 of the 566 (41.5%) *BRCA2* carriers and 0 of 6 compound heterozygotes were unaffected with breast cancer at the time of testing. The age distribution of the female carriers by breast cancer status is shown in Table 4. In addition, 169 *BRCA1* and 114 *BRCA2* male mutation carriers have been identified.

The frequency of mutations was also examined by race/ethnicity, age at diagnosis of the proband, and family history of breast or ovarian cancer. The number of probands tested, defined by racial/ethnic group, is shown in

Table 3 Total numbers of *BRCA1* and *BRCA2* germline mutation carriers, including those tested for only the Ashkenazi Jewish founder mutations

	Families	Individuals
BRCA1 mutation carriers	400	788
BRCA2 mutation carriers	265	566
BRCA1 and *BRCA2* mutation carriers	6	6
Total number	671	1,360

Table 2 Ashkenazi Jewish family testing for the three founder mutations

Family registry	# Probands tested	# Carrying *BRCA1* 185delAG		# Carrying *BRCA1* 5382insC carriers		# Carrying *BRCA2* 6174delT		# Carrying both *BRCA1* and *BRCA2*	Total # carrying mutations in any of the three founder mutations	
		Families	Individuals	Families	Individuals	Families	Individuals	Individuals	Families	Individuals
Australia	319	17	18	7	26	24	35	0	48	79
New York	521	54	77	24	41	26	44	1	104	163
California	32	1	1	0	0	1	1	0	2	2
Ontario	361	34	45	8	16	13	25	0	55	87
Philadelphia	131	15	53	5	15	7	25	2	27	94
Utah	11	2	4	0	0	1	1	0	3	5
Totals	1,385	123 (8.9%)	198	44 (3.2%)	98	72 (5.2%)	131	3	241 (17.4%)	430

Table 5. The majority of mutation carriers were non-Hispanic white ($n = 310$), reflecting the demographics of the Breast CFR as a whole. An additional 42 Latino and 31 African–American mutation carriers, as well as 47 carriers from other ethnic groups, have been identified. A detailed family history of breast and other cancers was collected for all families enrolled in the Breast CFR. The vast majority of mutations were identified in probands who had a first-or second-degree relative with breast or ovarian cancer (Table 6). Probands with at least one-first-degree relative diagnosed with breast cancer before age 50 years had the highest frequency of mutations at 12.7% for *BRCA1* and 8.2% for *BRCA2* (Table 6). For those without a family history of breast cancer, the frequency of mutations identified was 2.4% for *BRCA1* and 2.2% for *BRCA2*. Of the mutations identified, there were 132 and 156 distinct mutations in *BRCA1* and *BRCA2*, respectively.

Table 4 Distribution of affected and unaffected female mutation carriers by age and germline *BRCA1* and *BRCA2* mutation status[a]

Affectation status (breast cancer)	Age in years at diagnosis for cases and age at interview for unaffected						
	<30	30–39	40–49	50–59	60+	Unknown	Total
BRCA1							
Affected	43	193	168	51	19	1	475
Unaffected	69	75	71	41	18	39	313
BRCA2							
Affected	21	107	110	65	28	0	331
Unaffected	43	56	48	40	23	25	235

[a] Excluding the 6 compound heterozygotes

Table 5 *BRCA1* and *BRCA2* germline mutation testing by race/ethnicity, excluding those tested only for the Ashkenazi Jewish founder mutations

Race/Ethnicity	*BRCA1* testing		*BRCA2* testing	
	# Probands tested	# w/Mutations	# Probands tested	# w/Mutations
Non-Hispanic white	2,875	172	2,499	138
Hispanic	532	24	520	18
African American	410	14	405	17
Asian/Pacific Islander	525	5	503	13
Other/multiple race	133	11	125	6
Unknown	56	7	32	1
Total	4,531	233	4,084	193

Table 6 *BRCA1* and *BRCA2* germline mutation testing by family history of breast and ovarian cancer, excluding those tested for only the Ashkenazi founder mutations

Family history of breast or ovarian cancer	*BRCA1*		*BRCA2*	
	# Probands tested	# w/Mutations (%)	# Probands tested	# w/Mutations (%)
No family history in 1st or 2nd degree relatives	1,592	38 (2.4)	1,343	30 (2.2)
Unknown/No relatives	126	9 (7.1)	107	8 (7.5)
With family history in a relative				
Breast cancer at age <50 years in at least one 1st degree family relative	856	109 (12.7)	821	67 (8.2)
Breast cancer at age ≥50 years in at least one 1st degree family relative	994	28 (2.8)	923	41 (4.4)
Breast cancer at age <50 years in a 2nd degree relative (no 1st degree relative affected)	309	20 (6.5)	285	19 (6.7)
Breast cancer at age ≥50 years in a 2nd degree relative (no 1st degree relative affected)	535	23 (4.3)	482	26 (5.4)
Ovarian cancer only in any relative	119	6 (5.0)	123	2 (1.6)
Total	4,531	233 (5.1)	4,084	193 (4.7)

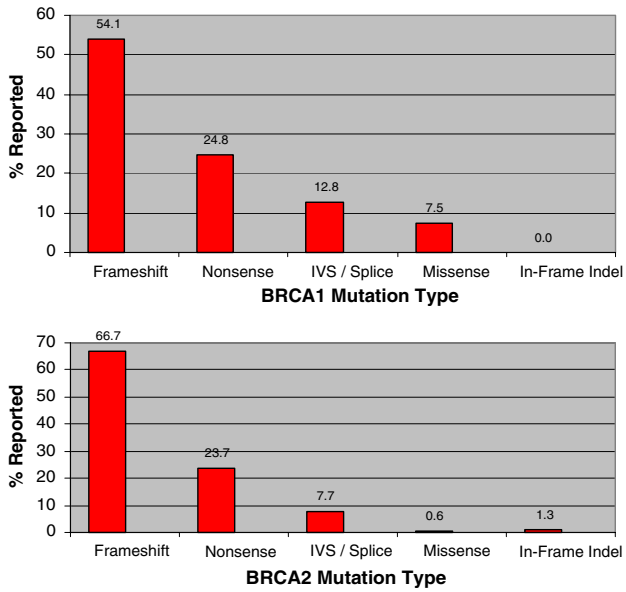

Fig. 1 The frequency of deleterious germline mutation types in *BRCA1* and *BRCA2* observed in the Breast CFR. In-frame indel refers to either insertions or deletions of nucleotides in multiples of three

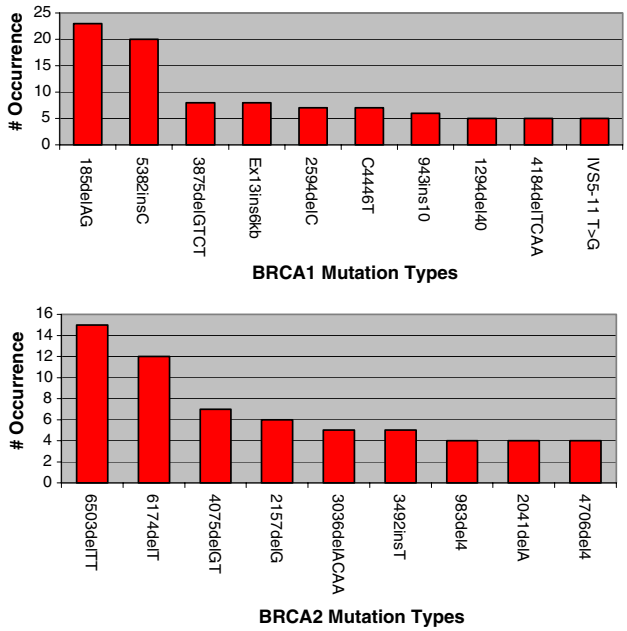

Fig. 2 The most common germline *BRCA1* and *BRCA2* mutations observed in the Breast CFR

The frequencies of each type of deleterious mutation were: 54.1% frameshift, 24.8% nonsense, 12.8% intervening sequence (IVS)/splice, and 7.5% missense for *BRCA1*; and 66.7% frameshift, 23.7% nonsense, 7.7% IVS/splice, 0.6% missense, and 1.3% in-frame insertions/deletions (in-frame indel) for *BRCA2* (Fig 1). The 10 most frequently identified *BRCA1* and *BRCA2* mutations are shown in Fig 2. Reflecting the relatively high proportion of Ashkenazi Jewish families tested, the two Ashkenazi founder mutations were the most commonly found mutations in *BRCA1*. The distribution of distinct mutation types along the three coding segments of *BRCA1* (Exons 1–10; 21.1%, Exon 11; 53.4% and Exons 12–24; 25.6%) and *BRCA2* (Exons 1–10; 23.1%, Exon 11; 50.0% and Exons 12–27; 26.9%) were similar between the two genes.

Discussion

Overall, we have found deleterious mutations in approximately 10% of families screened to date for mutations in *BRCA1* and *BRCA2*, with similar percentages for each gene. The frequency of deleterious mutations identified in clinic-based families was two-fold (18.5%) of that identified in the population-based families (9.4%). The higher frequency of mutations may be due to several possibilities. First, based on the nature of the recruitment, the clinic-based families have a stronger family history than the population-based families, and as such have a higher likelihood of carrying mutations. Second, a proportion of the clinic-based families were known to have a deleterious mutation at enrollment. In particular, approximately 41% of families from the Utah Breast CFR carry deleterious mutations, largely due to referral of families from a research study in which mutations had already been identified in the families. The frequency for the population-based families may be higher than for breast cancer cases in general due to the fact that that the three population-based sites of the Breast CFR over-sampled for younger ages at diagnosis, as well as for family history in Ontario and California.

Of the Breast CFR Ashkenazi families screened for only the three founder mutations, 17.9% carried one of the mutations. This is similar to what was observed by Myriad Genetic Laboratories Inc., who found 20.4% of tested individuals of Ashkenazi Jewish ancestry carried deleterious mutations [6]. In the same report, of 10,000 individuals screened for mutations, deleterious mutations were identified for 17.2% of those screened [6]. The frequency of mutations for individuals screened by Myriad Genetic Laboratories Inc. is higher than for our population-based families, likely reflecting that those screened were clinical samples sent for screening because they had a high prior probability of carrying deleterious mutations in *BRCA1* and *BRCA2*.

The distributions of detected *BRCA1* and *BRCA2* mutations across the genes were similar to what has been reported in the Breast Information Core (BIC) database. For both genes, approximately half the mutations were detected in exon 11 (which constitutes approximately 60% of the coding regions of each gene). The most common type of clinically significant *BRCA1* and *BRCA2* mutations in the Breast CFR

were frameshift mutations (54.9%) followed by nonsense alterations (24.8%). Missense and IVS/splice variants were found less frequently, whereas in-frame mutations were infrequent. For testing performed by cDNA based PTT and 5′ sequencing (as described in [3]), it is possible that some deleterious in-frame deletions that did not cause detectable protein shortening and some missense mutations that were not contained in the 5′ region would have been missed. Furthermore, none of the testing methods used detect large rearrangements/insertions/deletions that have been estimated to account for approximately 12% of mutations in *BRCA1* and *BRCA2* [9]. We had previously reported that in a population-based sample of multiple-case families from the Australian Breast CFR ($n = 66$), 2 of 10 *BRCA1* mutations were large deletions that involved the promoter region of *BRCA1* (large genomic alterations that lie outside the promoter region and *BRCA2* were not studied) [10]. Therefore, it is likely that a small proportion of mutations have not yet been identified in these probands. There is an on-going study to test for large insertions/deletions in *BRCA1* and *BRCA2* in a set of cases selected for family history and breast cancer pathology.

Although the number of unclassified variants (UCVs), including missense and splice variants, identified in the Breast CFR sample is large (data not presented), only a small fraction have so far been shown to be functionally deleterious and thus potentially of clinical significance. Methods to evaluate *BRCA1* and *BRCA2* UCVs to classify them as deleterious or likely neutral have been published [8, 11–14]. These methods are being applied to the Breast CFR unclassified variants and classifications are being entered into the Breast CFR database.

Nearly 6,000 probands in the Breast CFR were tested for mutations in *BRCA1* and *BRCA2*. More than 1,300 *BRCA1* and *BRCA2* mutation carriers have been identified in a total of 671 carrier families, including six compound heterozygotes. Mutation testing of probands for families not yet tested, and the application of new mutation testing strategies [e.g., multiplex ligation-dependent probe amplification (MLPA from MRC-Holland) and regulatory region screening) is ongoing. Once mutations are identified in probands, additional family members for whom DNA samples are available, are tested for the family mutation.

To date, the Breast CFR data on *BRCA1* and *BRCA2* mutation status have been used to estimate the population-based prevalence of *BRCA1* mutations [15–18], the investigation of lifestyle factors [19–21]; and genetic factors [22, 23] that might modify the risk of breast cancer for *BRCA1* and *BRCA2* mutation carriers, and risk of breast and ovarian cancer for relatives of cancer patients with and without *BRCA1* or *BRCA2* mutations [24].

In conclusion, the value of the Breast CFR resource has been greatly enhanced by the testing for germline *BRCA1* and *BRCA2* mutations in probands and in many family members of identified carriers. These data, in combination with the family history, epidemiology, pathology, and follow-up data will facilitate studies of penetrance, genotype–phenotype correlations, gene–gene and gene–environment interactions, modifiers of risk in carriers, risks associated with unclassified variants or polymorphisms, and outcomes, as well as studies of characteristics of *BRCA1*- and *BRCA2*-associated tumors and assist in the efforts to discover novel breast cancer susceptibility genes. The Breast CFR resource is available to all researchers for collaborative, interdisciplinary, and translational studies of the genetic epidemiology of breast cancer. Detailed information on how to access the resource can be found at the URL: http://epi.grants.cancer.gov/CFR/, as well as citations of publications resulting from use of the Breast CFR. The resource will continue to be strengthened by additional knowledge of *BRCA1* and *BRCA2* mutation status and continual follow-up of all families.

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