BRCA1 and BRCA2 Mutations in Women from Shanghai China

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Abstract

Little is known about the frequency of germ-line mutations in the breast cancer susceptibility genes *BRCA1* and *BRCA2* among Asian populations. We investigated the distribution of *BRCA1* and *BRCA2* germ-line mutations and polymorphisms in a cohort of women from Shanghai, China.

Study subjects totaled 1306, and included 645 women with breast cancer, 342 women with benign breast disease, and 319 unaffected controls, born between 1924 and 1958, selected from women enrolled in a randomized trial of Breast Self-Examination in Shanghai, China. Women were selected without regard to family history of breast or ovarian cancer. All of the coding regions and exon-intron boundaries were screened. Data were analyzed with respect to age at diagnosis, and family history of breast and ovarian cancer.

The prevalence of known disease-associated mutations in women with breast cancer was 1.1% each, for *BRCA1* and *BRCA2*. Among breast cancer cases with a family history of breast or ovarian cancer, 8.1% and 2.7% carried likely *BRCA1* and *BRCA2* disease-associated mutations, respectively.

Overall, these results suggest that inherited susceptibility to breast cancer due to germ-line BRCA1/2 mutations among women with a family history of breast cancer is comparable between women from Shanghai and Caucasian women of Western European descent. Most alterations observed appear unique to the Chinese population, suggesting a resource that will be useful for assessing risk among both Chinese women and United States women of Chinese descent.

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Introduction

Although the incidence of breast cancer in China is about one-third that in the United States (1), the overall rate of breast cancer in women from China has been increasing (2). Little is known regarding the role of inherited susceptibility genes in breast cancer risk among Chinese women. To develop genetic screening guidelines for both Chinese women and United States women of Chinese descent, a population-based assessment of *BRCA1* and *BRCA2* germ-line mutation distribution and frequency is needed.

To date, information regarding the frequency of *BRCA1* and *BRCA2* mutations in Chinese women with breast cancer has been derived from two small hospital-based reports. In the first, 76 consecutive breast cancer cases diagnosed before age 40, and 16 women with a family history of breast or ovarian cancer, were screened for germ-line mutations in *BRCA1* (3). Protein truncating mutations were observed in 8.6% of women. In the second study, 3.8% of 130 tumors collected from mastectomy patients in Hong Kong were found to carry protein-truncating mutations in *BRCA1* (4). The prevalence was 8.0% in patients diagnosed under age 45 (4). In the same study, investigators observed a single variant, 589delCT, in three unrelated patients from within a single province of Southern China, suggesting a possible founder effect. However a later more detailed study of 60 early onset breast cancer cases did not reveal any additional carriers (4, 5). Neither study examined the role of *BRCA2* mutations in Chinese women.

Studies of women with ovarian cancer have also contributed to our understanding of the role of *BRCA1* and *BRCA2* mutations in Chinese women. An analysis of 60 ovarian cases from Hong Kong, unselected for family history, revealed that 11.3% and 2.1%, carried germ-line mutations in *BRCA1* and *BRCA2*, respectively (6). A subsequent analysis of 214 consecutive ovarian cases, also from Hong Kong, suggested a founder effect associated with the 1081delG *BRCA1* mutation in women of Southern Chinese descent (7).

In the aggregate, these results suggest a role for inherited susceptibility genes in breast cancer among Chinese women, particularly among women diagnosed early in life. However, none of these prior studies were population based, and, therefore, none provided accurate estimates of the frequency of specific germ-line changes in the *BRCA1* and *BRCA2* genes among unselected women with breast cancer. This study provides important data concerning the frequency and types of *BRCA1* and *BRCA2* mutations in predisposition to breast cancer among Chinese women in Shanghai from a well-defined population.

Subjects and Methods

This study was conducted within a cohort of >266,000 current and former female textile workers in 519 factories in Shanghai who were recruited into a randomized trial of breast self-examination (BSE) between October 1989 and October 1991. The methods for the trial have been reported previously (8, 9). Briefly, nearly all of the women were administered a four-page

optically scannable baseline questionnaire at recruitment. Multiple methods of active and passive case finding were developed to ensure nearly complete identification of women with breast cancer or biopsied benign breast lesions. All of the women in the cohort were followed through July 31, 2000, for vital status, continued employment in the textile industry, and residence in Shanghai, and development of breast diseases. During the course of the trial, independently funded case-control studies nested within the BSE trial cohort were conducted. These provided the opportunity to obtain blood specimens and interviews from variously defined subsets of women who developed breast cancer and benign breast disease, and from randomly selected control women without breast disease (10). All of the diagnoses were confirmed by pathology review in Seattle.

Women with breast cancer included in those case-control studies with sufficient WBCs for DNA extraction and genetic analysis constitute the breast cancer cases in the present investigation. Because breast cancers that occur in young women are more likely to be related to BRCA1 or BRCA2 than breast cancers in older women, a higher proportion of cases diagnosed before age 45 than after were selected for blood draw. A total of 1801 breast cancer cases occurred during the follow-up period (478 under 45 and 1323 age 45 and older) of which 645 (35.8%) are included in the present study. These include 256 (53.5%) of the cases diagnosed before age 45 and 389 (29.4%) cases at $\geq$45 years of age at diagnosis. Similar proportions of the cases that were and were not included reported a history of breast cancer in a mother or sister on the baseline questionnaire. The proportions of cases with such a history in women included and not included in this study were 4.0% and 3.3%, respectively, for all of the women, 5.3% and 3.6% for women under 45, and 3.1% and 3.3% for older women. None of these differences are statistically significant ($P = 0.48$, 0.38, and 0.87, respectively). All of the blood specimens collected from breast cancer cases in this report were obtained between September 1, 1994 and July 31, 2000.

Women in the BSE trial cohort who were diagnosed with benign breast disease at each of three hospitals associated with the Shanghai Textile Industry Bureau between September 1, 1995 and December 16, 1997 were also included in this investigation. Interviews and blood samples suitable for *BRCA1/2* testing were obtained between September 1, 1995 and December 31, 1997 from 342 eligible women. The proportions of women having a family history of breast cancer on the baseline questionnaire were 3.1% among the women who were tested, and 3.5% among the 3466 not included in this study ($P = 0.66$). The proportion of women with benign breast disease that had proliferative fibrocystic conditions was 48.8% in the women included in the study, and 44.8% in those not included.

Unaffected controls (319 women without breast cancer or benign breast disease) were randomly selected for this study from among the controls recruited into the nested case-control studies. Women were chosen so that the distribution of their ages at interview (in 5-year intervals) matched the ages at diagnosis of the women with breast cancer. Interviews and blood samples were obtained from controls between September 1, 1995 and July 31, 2000. Control women who were tested did not differ from the BSE cohort members without breast disease who were not tested with respect to family history of breast cancer on the baseline questionnaire (1.7% *versus* 2.1%, respectively; $P = 0.57$).

A total of 1306 women were included in this study. All of the women who consented were administered a questionnaire by a trained interviewer at or near the time of blood draw. Information on standard risk factors for breast cancer was obtained, and each subject was asked to provide detailed information on any cancer diagnoses for her grandmothers, mother, sisters, and daughters. For each affected relative, the interviewer asked about vital status, age, type of cancer, and age at diagnosis. The Institutional Review Boards at the Fred Hutchinson Cancer Research Center and the Shanghai Textile Industry Bureau approved the study, and informed consent by all of the subjects was obtained before their participation.

Molecular Analyses. Genomic DNA was purified from frozen buffy coats using standard protocols (11, 12). The complete coding regions and exon-intron boundaries for both the *BRCA1* and *BRCA2* genes were screened for DNA sequence variants by single-strand conformation polymorphism assay. Amplification of coding regions used both primers reported previously (13–15) as well as a set of newly designed primers. PCR reactions were carried out in a volume of 12.5 μl, with 25 ng of genomic DNA, 1$\times$ PCR buffer, 1.5 mM Mg^{+2}, 0.048 mM dATP, dTTP, dGTP, 0.0048 mM dCTP, 0.2 units Taq (Bioline), and 0.004 mCi [α-P^{32}] dCTP (Amersham). An initial denaturation of 95°C for 1 min was followed by 35 cycles of amplification (30 s/94°C, 15 s/primer-specific annealing temperature, and 15 s/72°C) and a final elongation (3 min/74°C). Samples were diluted 1:3 in formamide buffer [98% formamide, 10 mM EDTA (pH 8.0), 0.05% bromphenol blue, and 0.05% xylene cyanol], denatured at 99°C for 3 min, and cooled on ice. Multiplex reactions of up to three different PCR products were loaded on 0.5t$\times$ MDE gels. Electrophoresis was performed at room temperature for 16–20 h at 6 W. Exons 6, 11h, and 13 were also run on 3% glycerol gels for 8 h at 8 W. Results were visualized by autoradiography. Amplification and electrophoresis were repeated for confirmation of altered migration patterns of variant bands found in $<$5% of the population. Bands were cut from gels and DNA isolated using protocols published previously (16). DNA was sequenced on an ABI 373, 377, or 3700 fluorescent sequencer with Dye Terminators (PE Applied Biosystems) using the manufacturer's suggested protocols.

All of the DNA sequence changes noted were assigned to one of three groups. Group 1 included variants that are clearly or highly likely to be deleterious, including all of the known protein truncating mutations and splice-site alterations within 2 bp of any exon boundary. Group 2 included variants of unknown significance including missense changes, intronic changes 3–40 bases from the exon boundary, and single amino acid deletions. This category likely included some mutations that are disease-associated, as well as others that do not have functional consequences. Group 3 is unlikely to be of functional significance based on what we currently know about the BRCA1 and BRCA2 proteins. This category included silent changes and intronic changes $>$40 bases from the exon, and for *BRCA2*, any change after the polymorphic stop codon at amino acid 3326. Our rationale for counting *BRCA2* mutations beyond 3326 as group 3 changes stems from the observation that a common polymorphic nonsense change exists at amino acid 3326 within the general population that is observed in 2.2% of normal control individuals (17). Thus, mutations found beyond 3326 are likely to have little functional significance.

Statistical Analysis. Standard χ^2 tests were used to determine whether family history varied between those tested and not tested for *BRCA1* and *BRCA2* mutations. For tested breast cancer cases, the Fisher exact test was used to evaluate differences between frequencies of *BRCA1* or *BRCA2* mutation types by age at diagnosis and family history of breast cancer. The binomial exact 95% confidence interval (CI) was computed for the proportion of cases with each type of BRCA1 or BRCA2

Table 1 Characteristics of breast cancer cases, women with benign breast disease (BBD) and unaffected control women tested for BRCA1 and BRCA2 mutation

	Breast Cancer (n = 645) No. (%)	BBD (n = 342) No. (%)	Controls (n = 319) No. (%)	P value[a]	P value[b]
Age[c]					
<45 years	256 (39.7)	234 (68.4)	126 (39.5)		
≥45 years	389 (60.3)	108 (31.6)	193 (60.5)	0.95	<.001
Family history of breast cancer					
None	594[d] (94.7)	329[e] (96.8)	300[f] (96.2)		
First degree[g]	28 (4.7)	8 (2.3)	7 (2.2)	0.08	0.70
Grandmother only	4 (0.6)	3 (0.9)	5 (1.6)		
Unknown	18	2	7		

[a] P for differences between breast cancer cases and controls based on standard χ^2 tests.
[b] P for differences between women with BBD and controls based on standard χ^2 tests.
[c] Age is age at diagnosis for cases and age at blood draw and interview for women with BBD and for controls.
[d] Includes 112 women with no history of cancer in first-degree female relatives, but history was incomplete for one or both grandmothers; also includes 4 women with no history of cancer in either grandmother, but history was incomplete for 1 or more first-degree female relatives.
[e] Includes 31 women with no history of cancer in first-degree female relatives, but history was incomplete for one or both grandmothers; also includes one woman with no history of cancer in grandmothers or sisters, but mother's history was unknown.
[f] Includes 139 women with no history of cancer in first-degree female relatives, but history was incomplete for one or both grandmothers.
[g] One case has both maternal grandmother and mother with history of breast cancer.

mutation. Where there were zero mutations in a specific group, one-sided 97.5% CIs were computed. Unconditional logistic regression (18) was used to compute odds ratios as estimates of relative risk (RR) of breast cancer and benign breast disease in relation to the various types of *BRCA1* and *BRCA2* mutations. Women with more than one mutation in either BRCA1 or BRCA2 were categorized according to the mutation most likely to be of functional significance. Women with a change in both genes were included in the analyses for each gene. All of the statistical analysis was performed using Stata statistical software (Stata Corporation, College Station, TX) or SAS (SAS Institute Inc., Cary, NC).

Results

Cases were similar in age to controls (39.7% and 39.5%, respectively; had a reference age under 45). However, women with benign breast disease were significantly younger, with 68.4% having a reference age <45 (Table 1). Women with breast cancer reported more first-degree relatives with breast cancer than did women with benign breast disease or normal control women, although the differences were not statistically significant. Few women reported breast cancer in their grandmothers, and no breast cancers were reported in other second-degree relatives. Only 1 woman had multiple relatives with breast or ovarian cancer, a breast cancer case with breast cancer in her maternal grandmother and mother.

DNA sequence changes in the *BRCA1* and *BRCA2* genes were observed in 5% of the study subjects overall. If a sample contained multiple germ-line changes, only the change that was most likely to be disease associated was counted for each woman. No woman had more than one group 1 mutation. Two breast cancer cases had both a group 1 and group 2 *BRCA1* mutation, and 1 control had both a group 2 and group 3 *BRCA1* mutation. One woman with breast cancer had both a *BRCA2* group 1 and group 2 mutation, and 3 had both group 2 and group 3 mutations. Six other women had other combinations of *BRCA1* and *BRCA2* group 2 and 3 mutations. Table 2 shows the distribution and frequency of all of the DNA sequence changes in the *BRCA1* and *BRCA2* genes that were observed. Seven and 9 group 1 mutations were observed in *BRCA1* and *BRCA2*, respectively, as were 96 and 73 group 2 mutations, and 13 and 48 group 3 mutations. Thirty-seven unique events were observed for BRCA1 and 56 for BRCA2, with several events occurring multiple times. Specifically, 54 breast cancer cases, 27 women with benign breast disease, and 30 controls shared 24, 10, and 16 unique mutations, respectively, in BRCA1, whereas 74 breast cancer cases, 25 women with benign breast disease, and 23 controls shared 39, 17, and 19 unique mutations in BRCA2. Twenty-eight (76%) and 31 (45%) of BRCA1 and BRCA2 changes, respectively, have not, to our knowledge, been reported previously.

A total of 13 distinct *BRCA1* or *BRCA2* group 1 mutations were observed in 16 women. (Table 3, *A* and *B*). The 5589del8 change, however, was observed in two separate cases. By comparison, 8 unique *BRCA2* protein-truncating mutations were found in 9 women, 1 who had only benign breast disease and 1 who was a control. The remaining 2 women with a group 1 mutation had the identical *BRCA1* splice-site change, IVS3–2A>G.

Several group 2 missense changes were observed for both genes. Overall, however, such changes were more commonly noted in *BRCA2* than *BRCA1*. In addition, several missense changes were observed more frequently in cases than controls (Table 3, *A* and *B*). For instance, the I1929V change was observed in 5 cases, 1 woman with benign breast disease and 1 control; the K2729N variant was observed in 4 cases, but no women with benign breast disease nor any controls. While provocative, neither of these results was statistically significant.

As expected, there was an increased RR of breast cancer in women with a group 1 mutation (Table 2). For BRCA1, only cases had group 1 mutations; the lower bound of the 97.5% CI was 1.5. For BRCA2, the RR associated with a group 1 change was 3.6 (95% CI, 0.6–68.0). When *BRCA1* and *BRCA2* group 1 mutations were assessed together, the age-adjusted RR, relative to women with no *BRCA1* or *BRCA2* alterations of any type, was 7.2 (95% CI, 1.4–130). RR assessment of BRCA2 group 2 changes revealed a borderline statistically significant result of 1.6 (95% CI, 0.9–3.2). RRs of 1.3 and 1.4 were observed for *BRCA1* and *BRCA2* group 3 changes, respectively, but the results were not statistically significant.

Women with benign breast disease were similar to controls with regard to frequency and type of mutation (Table 2). There was no significant difference between women with different types of benign breast disease and mutation type (data not shown). However, there were two results of note that may merit further study; 1 woman with a *BRCA2* group 1 change

Table 2 BRCA1 and BRCA2 mutations in women with breast cancer, women with benign breast disease (BBD), and unaffected control women

Mutations[a]	Breast cancer (n = 645)		BBD (n = 342)		Controls (n = 319)		RR[b] (95% confidence interval)	RR[c] (95% confidence interval)
	n	%	n	%	n	%		
BRCA1								
None	591	91.6	315	92.1	289	90.6	1.0 (Reference)	1.0 (Reference)
Group 3	8	1.2	1	0.3	3	0.9	1.3 (0.4, 6.0)	0.4 (0.02, 3.4)
Group 2	39	6.1	26	7.6	27	8.5	0.7 (0.4, 1.2)	0.8 (0.4, 1.5)
Group 1	7	1.1	0	0.0	0	0.0	4.7 (1.5, ∞)[d]	—
BRCA2								
None	572	88.7	317	92.7	296	92.8	1.0 (Reference)	1.0 (Reference)
Group 3	25	3.9	8	2.3	9	2.8	1.4 (0.7, 3.3)	0.7 (0.2, 2.0)
Group 2	41	6.3	16	4.7	13	4.1	1.6 (0.9, 3.2)	1.1 (0.5, 2.4)
Group 1	7	1.1	1	0.3	1	0.3	3.6 (0.6, 68.0)	1.0 (0.04, 27.0)
BRCA1/BRCA2								
None	522	80.9	292	85.4	268	84.0	1.0 (Reference)	1.0 (Reference)
Group 3 only	29	4.5	8	2.3	11	3.5	1.4 (0.7, 2.9)	1.6 (0.6, 4.4)
Group 2	80	12.4	41	12.0	39	12.2	1.0 (0.7, 1.6)	1.1 (0.7, 1.8)
Any Group 1	14	2.2	1	0.3	1	0.3	7.2 (1.4, 130)	1.0 (0.04, 28.8)

[a] Group 1: Frameshift and splice-site mutations.
Group 2: Missense changes, single amino-acid deletions and intronic changes ≤40 bases from coding region.
Group 3: Silent changes, intronic changes >40 bases from coding region and changes after the BRCA2 polymorphic stop codon at amino acid 3326.
[b] Relative risks (RR) of breast cancer in relation to type of BRCA1 or BRCA2 mutation. Breast cancer cases compared with unaffected control women.
[c] Age adjusted relative risks (RRs) of BBD in relation to type of BRCA1 or BRCA2 mutation. Women with BBD compared with unaffected control women.
[d] Median unbiased estimate computed where sample size is 0.

(1529del4) was diagnosed with proliferative fibrocystic disease, and a second woman with a *BRCA2* group 3 mutation (4035T/C) was diagnosed with atypical proliferative disease. This group 3 change was also found in 3 cases, but no controls.

The distribution of *BRCA1* mutations, according to disease characteristics and family history features, is summarized in Table 4. Seven cases (1.1%) had a *BRCA1* group 1 mutation, 6 of whom were diagnosed after age 45. There was a significantly higher proportion of group 1 mutations (P = 0.006) among cases with a family history of breast and/or ovarian cancer (3 of 37; 8.1%) compared with cases without a family history (4 of 590; 0.7%).

The single woman with a group 1 mutation who was diagnosed before age 45 also reported a first-degree family history of ovarian cancer, and 2 of the women with a group 1 mutation who were diagnosed at age 45 or older had a family history of breast cancer. One relative was diagnosed with breast cancer before age 45 and the other at age 45 or older. A total of 6.0% and 1.2% of cases had group 2 and group 3 changes, respectively.

Disease characteristics and family history features of women with BRCA2 changes are summarized in Table 5. Seven cases (1.1%) had a *BRCA2* group 1 mutation, 4 of whom were diagnosed under age 45. One woman, diagnosed under age 45, also reported a first-degree relative diagnosed with breast cancer under age 45. Group 2 and group 3 changes were seen in 6.4% and 3.9% of women, respectively, with 4 of 41 women with a group 2 change reporting a family history of breast cancer. All 4 of these women were diagnosed under age 45, 2 with a first-degree relative diagnosed under age 45, 1 at an unknown age, and 1 with a second-degree relative diagnosed at age 45 or older. No women with any *BRCA2* change reported a family history of ovarian cancer.

RRs in relation to *BRCA1* mutations did not differ appreciably when the analyses were restricted to women without any *BRCA2* changes (572 cases, 296 controls, and 317 women with benign breast disease). Nor did RRs in relation to *BRCA2* mutations differ appreciably when analyses were restricted to women without BRCA1 changes (591 cases, 289 controls, and 315 women with benign breast disease).

Discussion

We observed that 1.1% of Chinese women with breast cancer were group 1 *BRCA1* mutation carriers, and 1.1% were group 1 BRCA2 carriers. Our data are most comparable with a population-based study from Britain in which the respective proportions of *BRCA1* and *BRCA2* mutation carriers were 3.1% and 3.0%, in women diagnosed when <50 years of age, and 0.49% and 0.84% in women diagnosed when >50 years of age (19). These results compare well also with the data of Newman *et al.* (14), who reported that 3.3% of white women from a population-based study who were 20–74 years at diagnosis were *BRCA1* mutation carriers. In addition, Southey *et al.* (20) reported a *BRCA1* mutation frequency of 3.8% (95% CI, 0.3–12.6) among Australian women diagnosed when <40 years old. These percentages are similar but larger than those observed in this study. This may reflect a smaller contribution by *BRCA1* and *BRCA2* to breast cancer in Chinese women. Alternatively, the differences could reflect variation in mutation detection methods or be due to chance.

The incidence of breast cancer in the population of women included in the trial of breast self-examination, from which the subjects for this study were selected, is similar to that for women in the general population of Shanghai (9). Furthermore, the women included in this study with breast cancer, benign breast diseases, and no breast disease reported a family history of breast cancer with a frequency similar to comparable women in the trial cohort who were not included in this study. It is quite likely, therefore, that the observed *BRCA1* and *BRCA2* mutation frequencies observed in women with and without malignant and benign breast diseases are reasonable estimates, respectively, for affected and unaffected Han Chinese women in general.

Overall we observe a 1:1 ratio of *BRCA1*:*BRCA2* group 1 mutations. This ratio is distinct from the patterns observed in many studies of high-risk families, where a ratio of 1.5–2.0 is typically observed (21). We note also that 76% of *BRCA1* and 45% of *BRCA2* variants have not been reported previously, and several of the remainder have been observed only in Chinese or Japanese women (4, 22–25). Only two of the five *BRCA1* frameshift mutations (2229delAA

Table 3 Number and distribution of rare[a] sequence changes

A. BRCA1

Sequence changes		Group number[c]	Number of changes			Previously reported[e]
Description[b]	Exon		Cases (Total = 645)	Benign breast disease[d] (Total = 342)	Controls (Total = 319)	
77insTA	5′UTR	3	1			
IVS3-52T>G	Intron 3	3			1	
IVS3-2A>G	Intron 3	1	2			
E85del	6	2	1			
P115L	7	2		1		
IVS8+13delG	Intron 8	2			1	
IVS8+21delT	Intron 8	2		1	1	
IVS8-73delT	Intron 8	3	1			
IVS8-14G>A	Intron 8	2	1		1	
V191I	9	2			1	BIC
IVS9-34T>C	Intron 9	2	4	1		
G275D	11	2	2	2	2	
953T/G	11	3	2			
D330N	11	2	1			
E575K	11	2	1			
1886C/T	11	3	1			
S590del	11	2			1	
2229delAA	11	1	1			BIC
T737A	11	2			1	
R762S	11	2			1	
2430T/C	11	3		1	1	BIC, (20), (23), (24), (25), (27)
I783V	11	2			1	
2615G/A	11	3			1	
Y856H	11	2	27	13	15	BIC, (4)
3389delAC	11	1	1			
3443insA	11	1	1			
P1150S	11	2	1	2		BIC, (4), (24), (27)
A1199V	11	2	1	1	1	
IVS12+22T>A	Intron 12	2	1			
4427T/C	13	3			1	BIC, (20), (27)
M1628T	16	2	2	3	1	BIC
IVS17-8T>A	Intron 17	2	1			
5423C/T	21	3	1			
5429G/A	21	3	1			
IVS22+32A>T	Intron 22	2		2		(4), (25)
5589del8	24	1	2			BIC
5639C/T	24	3	1			

B. BRCA2

Q147R	5	2	1			BIC
D156G	5	2	1			BIC
808delT	7	1	1			
K241R	9	2	1			
N289H	10	2	1			BIC, (27)
C315S	10	2	9	5	3	BIC
1446C/G	10	3	1			
1529del4	10	1	1	1		BIC, (21)
1593A/G	10	3	1	1		BIC, (26), (27)
V465del	10	2		1		
H523R	10	2	1			
1872G/A	10	3	2	1	1	
2000del4	10	1	1			BIC, #
S708T	11	2	1			BIC
2355G/C	11	3	1			
V783I	11	2		1		
3391del4	11	1	1			
3403C/T	11	3			1	
3648T/C	11	3	2			
4035T/C	11	3	3	1		BIC, (26)
M1272V	11	2	1			BIC
N1459S	11	2	1	1		
Q1502R	11	2	1			
K1533N	11	2	1		1	BIC
4965A/G	11	3			1	

Table 3 Continued

B. BRCA2

Sequence changes		Group number[c]	Number of changes			Previously reported[e]
Description[b]	Exon		Cases (Total = 645)	Benign breast disease[d] (Total = 342)	Controls (Total = 319)	
I1607V	11	2			1	
5301insA	11	1	1			BIC
G1700D	11	2			1	
5950delCT	11	1			1	BIC
I1929V	11	2	5	1	1	BIC
S1946P	11	2		1		BIC
S2041T	11	2			1	
V2050I	11	2	1			BIC
V2109I	11	2	1			BIC
6561del5	11	1	1			
6633del5	11	1	1			BIC,(26),(27)
6999C/T	11	3			1	
IVS12-31del6	Intron 12	2	1			
IVS12-25del7	Intron 12	2		1		
7182A/G	13	3		1		
R2336W	13	2		1		
7470A/G	14	3	1			BIC,(22),(26),(27)
IVS14+53C>T	Intron 14	3	10	5	3	BIC,(26)
I2490T	15	2	5	2	1	BIC,(26)
7734T/C	15	3	3		1	
G2508S	15	2	5	1	2	BIC
8314T/C	18	3			1	
K2729N	18	2	4			BIC
V2759M	18	2			1	
A2786T	19	2	1			
G2901D	21	2		1	1	BIC
IVS22-7del4	Intron 22	2	2			
9513C/T	25	3	1			
10092A/T	27	3			1	
E3377D	27	3	3			
I3412V	27	3	1			BIC,(22),(26)

[a] Rare changes are those seen in <5% of study subjects overall.
[b] Sequence change nomenclature as recommended by the BIC.
[c] Group classification descriptions are explained in "Materials and Methods."
[d] Women with benign breast disease.
[e] BIC indicates changes seen in the Breast Cancer Information Core (BIC).
# indicates personal communication from J. Leary, Familial Cancer Service Westmead Hospital (Westmead, Australia)

and 5589del8) had been reported previously, and five of the eight *BRCA2* frameshift mutations (1529del4, 2000del4, 5301insA, 5950delCT, and 6633del5) had been reported previously (Table 3 and references therein). The overall number of carriers, however, is insufficient to determine whether any of these variants represent true founder events, and the samples were not haplotyped for SNPs adjacent to, or within, coding regions.

Three *BRCA2* mutations have been reported frequently from a variety of ethnic groups, suggesting that they are either ancient mutations or that they represent hot spots for mutation (21). These include 1529del4; reported in British and United States women (21), 6633del5, which was observed previously in Austrian, Japanese, and Italian women (26–29,) and is also noted in the Breast Cancer Information Core, an international and voluntary, web-based databank of *BRCA1* and *BRCA2* mutations reported by >20 participating groups (30).[6] Although not highly quantitative, the Breast Cancer Information Core provides a valuable resource for tracking the occurrence of mutations throughout both the *BRCA1* and *BRCA2* genes. Also observed was a 5301insA change in *BRCA2*, reported several times in European women (29, 31, 32) and in women of Near and Middle Eastern descent in the Breast Cancer Information Core.

We observed an increased RR for breast cancer among women with *BRCA2* group 2 mutations, which was of borderline statistical significance (Table 2). Twenty-six of the 30 changes found in *BRCA2* group 2 are missense changes. One such change was of particular interest; G2508S was found in more cases than controls (5 cases, 2 controls, and 1 woman with benign breast disease), including 1 case with a second-degree family history of breast cancer. In addition, the change occurred in a domain conserved in the dog, human, and mouse (33).

Previous reports based on Caucasians have suggested that women with proliferative benign breast disease are at a 1.6–1.9-fold increased risk for breast cancer, with an even stronger association noted (3.6–5.9-fold) among women with atypia (34–36). To date, there are no reports among women of any ethnic background suggesting a role for BRCA1 or BRCA2 in increased susceptibility to benign breast disease, and this study of Chinese women found no such association.

We note two limitations of this study. First, while efficient

[6] Internet address: http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic.

Table 4 Distribution of BRCA1 mutations by disease characteristics and family history in 645 breast cancer cases

Characteristics	Total tested	BRCA1 Mutations						P^e (Fisher's exact test)
		Group 1[a]		Group 2[b]		Group 3[c]		
		n	% (95% CI)[d]	*n*	% (95% CI)[d]	*n*	% (95% CI)[d]	
Age at diagnosis								
<45	256	1	0.4 (0.01, 2.2)	19	7.4 (4.5, 11.3)	2	0.8 (0.09, 2.7)	0.29
≥45	389	6	1.5 (0.6, 3.3)	20	5.1 (3.2, 7.8)	6	1.5 (0.6, 3.3)	
Total cases	645	7	1.1 (0.4, 2.2)	39	6.0 (4.3, 8.2)	8	1.2 (0.5, 2.4)	
Family history of breast cancer[f]								
None[g]	594	5	0.8 (0.3, 2.0)	39	6.6 (4.7, 8.9)	7	1.2 (0.5, 2.4)	0.07
First degree[h]	29	2	6.9 (0.8, 2.3)	0	0.0 (0.0, 11.9)[i]	1	3.4 (0.1, 17.8)	
Grandmother only	4	0	0.0 (0.0, 60.2)[i]	0	0.0 (0.0, 60.2)[i]	0	0.0 (0.0, 60.2)[i]	
Relative had breast cancer <45 yrs[j]	10	1	10.0 (0.2, 4.4)	0	0.0 (0.0, 30.8)[i]	1	0.0 (0.2, 4.4)	0.22
Relative had breast cancer ≥45 yrs	22	1	4.5 (0.1, 22.8)	0	0.0 (0.0, 15.4)[i]	0	0.0 (0.0, 15.4)[i]	
Family history of ovarian cancer[f]								
None[g]	623	6	1.0 (0.4, 2.1)	39	6.3 (4.5, 8.4)	8	1.3 (0.6, 2.5)	0.07
Yes (mother)	4	1	25.0 (0.6, 80.6)	0	0.0 (0.0, 60.2)[i]	0	0.0 (0.0, 60.2)[i]	
Family history of breast or ovarian cancer[f]								
No[g]	590	4	0.7 (0.2, 1.7)	39	6.6 (4.7, 8.9)	7	1.2 (0.5, 2.4)	0.004
Yes	37	3	8.1 (1.7, 21.9)	0	0.0 (0.0, 9.5)[i]	1	2.7 (0.07, 14.2)	

[a] Frameshift and splice-site mutations (no nonsense changes were found in this study).
[b] Missense changes, single amino-acid deletions and intronic changes ≤40 bases from coding region.
[c] Silent changes, intronic changes >40 bases from coding region, and changes after the BRCA2 polymorphic stop codon at amino acid 3326.
[d] 95% confidence interval (CI) for proportion of cases with mutations in this group.
[e] *P* for differences in mutation frequencies among women in different categories of each characteristic.
[f] Family history of cancer was unknown for 18 women; there were no BRCA1 mutations among these women.
[g] Includes 112 women with no family history of cancer in first-degree female relatives, but history was incomplete for one or both grandmothers; also includes 4 women with no family history of cancer in either grandmother, but history was incomplete for one or more first-degree female relatives.
[h] One woman also had family history of breast cancer in maternal grandmother.
[i] One-sided 97.5% confidence interval.
[j] Relative's age at breast cancer was unknown for 1 woman.

and cost effective, single-strand conformation polymorphism may miss 20–30% of sequence alterations, most of which are likely to be single base changes (37–39). This methodological limitation leads to an underestimate of the number of missense and nonsense changes. This is supported by the work of Eng *et al.* (39), who compared several different methodologies for detecting mutations in the *BRCA1* gene in a blinded study involving 58 distinct mutations. Single-strand conformation

Table 5 Distribution of BRCA2 mutations by disease characteristics and family history in 645 breast cancer cases

Characteristics	Total tested	BRCA1 Mutations						P^e (Fisher's exact test)
		Group 1[a]		Group 2[b]		Group 3[c]		
		n	% (95% CI)[d]	*n*	% (95% CI)[d]	*n*	% (95% CI)[d]	
Age at diagnosis								
<45	256	4	1.6 (0.4, 4.0)	17	6.6 (3.9, 10.4)	9	3.5 (1.6, 6.6)	0.77
≥45	389	3	0.8 (0.2, 2.2)	24	6.2 (4.0, 9.0)	16	4.1 (2.4, 6.6)	
Total cases	645	7	1.1 (0.4, 2.2)	41	6.4 (4.6, 8.5)	25	3.9 (2.5, 5.7)	
Family history of breast cancer[f]								
None[g]	594	6	1.0 (0.4, 2.2)	36	6.1 (4.3, 8.3)	24	4.0 (2.6, 6.0)	0.21
First degree[h]	29	1	3.4 (0.09, 17.8)	3	10.3 (2.2, 27.4)	0	0.0 (0.0, 11.9)[i]	
Grandmother only	4	0	0.0 (0.0, 60.2)[i]	1	25.0 (0.6, 80.6)	0	0.0 (0.0, 60.2)[i]	
Relative had breast cancer < age 45 yrs[j]	10	1	10.0 (0.3, 44.5)	2	20.0 (2.5, 55.6)	0	0.0 (0.0, 30.8)[i]	0.08
Relative had breast cancer ≥ age 45 yrs	22	0	0.0 (0.0, 15.4)[i]	1	4.5 (0.1, 22.8)	0	0.0 (0.0, 15.4)[i]	
Family history of ovarian cancer[f]								
None[g]	623	7	1.1 (0.4, 2.3)	40	6.4 (4.6, 8.6)	24	3.8 (2.5, 5.7)	1.0
Yes	4	0	0.0 (0.0, 60.2)[i]	0	0.0 (0.0, 60.2)[i]	0	0.0 (0.0, 60.2)[i]	

[a] Frameshift and splice-site mutations (no nonsense changes were found in this study).
[b] Missense changes, single amino-acid deletions and intronic changes ≤40 bases from coding region.
[c] Silent changes, intronic changes >40 bases from coding region, and changes after the BRCA2 polymorphic stop codon at amino acid 3326.
[d] 95% confidence interval (CI) for proportion of cases with mutations in this group.
[e] *P* for differences in mutation frequencies among women in different categories of each characteristic.
[f] Family history of cancer was unavailable for 18 women; among these women, 1 had a group 2 mutation and 1 had a group 3 mutation.
[g] Includes 112 women with no family history of cancer in first-degree female relatives, but history was incomplete for one or both grandmothers; also includes 4 women with no family history of cancer in either grandmother, but history was incomplete for one or more first-degree female relatives.
[h] One woman also had maternal grandmother with family history of breast cancer.
[i] One-sided 97.5% confidence interval.
[j] Relative's age at breast cancer was unknown for 1 woman.

polymorphism detected 72% of the possible mutations, and most missed were, as expected, single base changes, with a particular preference for missing C or G to T changes (39). In addition, because of limited amounts of DNA, we did not undertake Southern blotting to scan for large genomic deletions, which are known to account for up to 15% of disease-associated mutations reported among Caucasian women (21). If a common founder mutation were to fall in the category of missed changes, it could lead to an underestimate of the overall frequency of mutations in Chinese women.

Second, few women in the study reported a positive family history of breast cancer. This is to be expected in populations with low incidence rates of breast cancer. In addition, this may reflect limitations in the self-reporting system used to obtain a family history, including the fact that some women were unsure of their family history. It is also theoretically possible that the mothers of a subset of study subjects died too early in life to have developed breast cancer, but this is unlikely to be the explanation; 346 cases had mothers who died, of which 322 reported the age at death of the mother. The mean age at death was 68 years, and median was 70 years; thus, mothers who died were relatively long-lived.

Among women with a family history of breast cancer, the contribution of germ-line mutations in the *BRCA1* and *BRCA2* genes appears similar to that observed in women of Western European descent. The types of variants observed, however, are largely unique, suggesting a repertoire of mutations that should be the focus of studies aimed at developing genetic risk profiles for United States women of Asian descent. The frequency of *BRCA* mutations in the study population was low, which limited the power of this study to estimate precisely mutation frequencies. Additional studies involving larger numbers of women are needed to fully assess the frequency of founder mutations, and determine the true population frequency of *BRCA1* and *BRCA2* mutations in Chinese women.

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References

1. Parkin, D. M., Whelan, S. L., Ferlay, J., and Young, J. Cancer incidence in 5 continents. Lyon, France: IARC Scientific Publications, 1997.

2. Jin, F., Devesa, S. S., Chow, W. H., Zheng, W., Ji, B. T., Fraumeni, J. F., and Gao, Y. T. Cancer incidence trends in urban shanghai, 1972–1994: an update. Int. J. Cancer, *83:* 435–440, 1999.

3. Sng, J. H., Chang, J., Feroze, F., Rahman, N., Tan, W., Lim, S., Lehnert, M., van der Pool, S., and Wong, J. The prevalence of BRCA1 mutations in Chinese patients with early onset breast cancer and affected relatives. Br. J. Cancer, *82:* 538–542, 2000.

4. Tang, N. L., Pang, C., Yeo, W., Choy, K., Lam, P. K., Suen, M., Law, L. K., King, W. W., Johnson, P., and Hjelm, M. Prevalence of mutations in the brca1 gene among chinese patients with breast cancer. J. Natl. Cancer Inst., *91:* 882–885, 1999.

5. Tang, N. L., Choy, K. W., Pang, C. P., Yeo, W., and Johnson, P. J. Prevalence of breast cancer predisposition gene mutations in Chinese women and guidelines for genetic testing. Clin. Chim. Acta, *313:* 179–185, 2001.

6. Khoo, U. S., Ngan, H. Y., Cheung, A. N., Chan, K. Y., Lu, J., Chan, V. W., Lau, S., Andrulis, I. L., and Ozcelik, H. Mutational analysis of BRCA1 and BRCA2 genes in chinese ovarian cancer identifies 6 novel germline mutations. Hum. Mutat. (Online), *16:* 88–89, 2000.

7. Khoo, U. S., Chan, K. Y., Cheung, A. N., Xue, W. C., Shen, D. H., Fung, K. Y., Ngan, H. Y., Choy, K. W., Pang, C. P., Poon, C. S., Poon, A. Y., and Ozcelik, H. Recurrent BRCA1 and BRCA2 germline mutations in ovarian cancer: a founder mutation of BRCA1 identified in the Chinese population. Hum. Mutat., *19:* 307–308, 2002.

8. Thomas, D. B., Gao, D. L., Self, S. G., Allison, C. J., Tao, Y., Mahloch, J., Ray, R., Qin, Q., Presley, R., and Porter, P. Randomized trial of breast self-examination in Shanghai: methodology and preliminary results. J. Natl. Cancer Inst., *89:* 355–365, 1997.

9. Thomas, D. B., Gao, D. L., Ray, R. M., Wang, W. W., Allison, C. J., Chen, F. L., Porter, P., Hu, Y. W., Zhao, G. L., Pan, L. D., Li, W., Wu, C., Coriaty, Z., Evans, I., Lin, M. G., Stalsberg, H., and Self, S. G. Randomized trial of breast self-examination in Shanghai: Final results. J. Natl. Cancer Inst., *94:* 2002.

10. Ye, Z., Li, G. D., Qin, Q., Ray, R. M., and Thomas, D. B. Breast cancer in relation to induced abortions in a cohort of Chinese women. Br. J. Cancer, *87:* 2002.

11. Bell, G. I., Karam, J. H., and Rutter, W. J. Polymorphic DNA region adjacent to the 5′ end of the human insulin gene. Proc. Natl. Acad. Sci. USA, *78:* 5759–5763, 1981.

12. Maniatis, T., Fritsch, E. F., and Sambrook, J. Molecular cloning: A laboratory manual. New York: Cold Spring Harbor Laboratory Press, 1982.

13. Friedman, L. S., Ostermeyer, E. A., Szabo, C. I., Dowd, P., Lynch, E. D., Rowell, S. E., and King, M-C. Confirmation of BRCA1 by analysis of germline mutations linked to breast and ovarian cancer in ten families. Nat. Genet., *8:* 399–404, 1994.

14. Newman, B., Mu, H., Butler, L. M., Millikan, R. C., Moorman, P. G., and King, M. C. Frequency of breast cancer attributable to BRCA1 in a population-based series of American women [see comments]. JAMA, *279:* 915–921, 1998.

15. Kirkpatrick, H., Waber, P., Hoa-Thai, T., Barnes, R., Osborne-Lawrence, S., Truelson, J., Nisen, P., and Bowcock, A. Infrequency of BRCA2 alterations in head and neck squamous cell carcinoma. Oncogene, *14:* 2189–2193, 1997.

16. Langston, A. A., Malone, K. E., Thompson, J. D., Daling, J. R., and Ostrander, E. A. BRCA1 mutations in a population-based sample of young women with breast cancer [see comments]. N. Eng. J. Med., *334:* 137–142, 1996.

17. Mazoyer, S., Dunning, A. M., Serova, O., Dearden, J., Puget, N., Healey, C. S., Gayther, S. A., Mangion, J., Stratton, M. R., Lynch, H. T., Goldgar, D. E., Ponder, B. A., and Lenoir, G. M. A polymorphic stop codon in BRCA2. Nat. Genet., *14:* 253–254, 1996.

18. Breslow, N. E., and Day, N. E. The analysis of case-control studies. Statistical methods in cancer research. Lyon, France: IARC, 1980.

19. Peto, J., Collins, N., Barfoot, R., Seal, S., Warren, W., Rahman, N., Easton, D. F., Evans, C., Deacon, J., and Stratton, M. R. Prevalence of BRCA1 and BRCA2 gene mutations in patients with early- onset breast cancer. J. Natl. Cancer Inst., *91:* 943–949, 1999.

20. Southey, M. C., Tesoriero, A. A., Andersen, C. R., Jennings, K. M., Brown, S. M., Dite, G. S., Jenkins, M. A., Osborne, R. H., Maskiell, J. A., Porter, L., Giles, G. G., McCredie, M. R., Hopper, J. L., and Venter, D. J. BRCA1 mutations and other sequence variants in a population-based sample of Australian women with breast cancer. Br. J. Cancer, *79:* 34–39, 1999.

21. Szabo, C. I., and King, M-C. Population genetics of *BRCA1 and. BRCA2.* Am. J. Hum. Genet., *60:* 1013–1020, 1997.

22. Inoue, R., Ushijima, T., Fukutomi, T., Fukami, A., Sugimura, H., Inoue, S., Okonogi, H., Sugimura, T., Matsumoto, Y., and Nagao, M. BRCA2 germline mutations in Japanese breast cancer families. Int. J. Cancer, *74:* 199–204, 1997.

23. Inoue, R., Fukutomi, T., Ushijima, T., Matsumoto, Y., Sugimura, T., and Nagao, M. Germline mutation of BRCA1 in Japanese breast cancer families. Cancer Res., *55:* 3521–3524, 1995.

24. Katagiri, T., Emi, M., Ito, I., Kobayashi, K., Yoshimoto, M., Iwase, T., Kasumi, F., Miki, Y., Skolnick, M. H., and Nakamura, Y. Mutations in the BRCA1 gene in Japanese breast cancer patients. Hum. Mutat., *7:* 334–339, 1996.

25. Matsushima, M., Kobayashi, K., Emi, M., Saito, H., Saito, J., Suzumori, K., and Nakamura, Y. Mutation analysis of the BRCA1 gene in 76 Japanese ovarian cancer patients: four germline mutations, but no evidence of somatic mutation. Hum. Mol. Genet., *4:* 1953–1956, 1995.

26. Wagner, T. M., Hirtenlehner, K., Shen, P., Moeslinger, R., Muhr, D., Fleischmann, E., Concin, H., Doeller, W., Haid, A., Lang, A. H., Mayer, P., Petru, E., Ropp, E., Langbauer, G., Kubista, E., Scheiner, O., Underhill, P., Mountain, J., Stierer, M., Zielinski, C., and Oefner, P. Global sequence diversity of BRCA2: analysis of 71 breast cancer families and 95 control individuals of worldwide populations. Hum. Mol. Genet., *8:* 413–423, 1999.

27. Ikeda, N., Miyoshi, Y., Yoneda, K., Shiba, E., Sekihara, Y., Kinoshita, M., and Noguchi, S. Frequency of BRCA1 and BRCA2 germline mutations in Japanese breast cancer families. Int. J. Cancer, *91:* 83–88, 2001.

28. Santarosa, M., Dolcetti, R., Magri, M. D., Crivellari, D., Tibiletti, M. G., Gallo, A., Tumolo, S., Della Puppa, L., Furlan, D., Boiocchi, M., and Viel, A. BRCA1 and BRCA2 genes: role in hereditary breast and ovarian cancer in Italy. Int. J. Cancer, *83:* 5–9, 1999.

29. Risch, H. A., McLaughlin, J. R., Cole, D. E., Rosen, B., Bradley, L., Kwan, E., Jack, E., Vesprini, D. J., Kuperstein, G., Abrahamson, J. L., Fan, I., Wong, B., and Narod, S. A. Prevalence and penetrance of germline BRCA1 and BRCA2 mutations in a population series of 649 women with ovarian cancer. Am. J. Hum. Genet., *68:* 700–710, 2001.

30. Szabo, C., Masiello, A., Ryan, J. F., and Brody, L. C. The breast cancer information core: database design, structure, and scope. Hum. Mutat., *16:* 123–131, 2000.

31. Warner, E. Fighting the battle against breast cancer. So close and yet so far. Can. Fam. Physician, *45:* 1849–1854, 1999.

32. Bergthorsson, J. T., Ejlertsen, B., Olsen, J. H., Borg, A., Nielsen, K. V., Barkardottir, R. B., Klausen, S., Mouridsen, H. T., Winther, K., Fenger, K., Niebuhr, A., Harboe, T. L., and Niebuhr, E. BRCA1 and BRCA2 mutation status and cancer family history of Danish women affected with multifocal or bilateral breast cancer at a young age. J. Med. Genet., *38:* 361–368, 2001.

33. Szabo, C. I., Wagner, L. A., Francisco, L. V., Roach, J. C., Argonza, R., King, M. C., and Ostrander, E. A. Human, canine and murine BRCA1 genes: sequence comparison among species. Hum. Mol. Genet., *5:* 1289–1298, 1996.

34. Dupont, W. D., and Page, D. L. Risk factors for breast cancer in women with proliferative breast disease. N. Engl. J. Med., *312:* 146–151, 1985.

35. London, S. J., Connolly, J. L., Schnitt, S. J., and Colditz, G. A. A prospective study of benign breast disease and the risk of breast cancer. JAMA, *267:* 941–944, 1992.

36. Byrne, C., Schairer, C., Brinton, L., Wolfe, J., Parekh, N., Salane, M., Carter, C., and Hoover, R. Effects of mammographic density and benign breast disease on breast cancer risk. Cancer Causes Control, *12:* 103–110, 2001.

37. Jordanova, A., Kalaydjieva, L., Savov, A., Claustres, M., Schwarz, M., Estivill, X., Angelicheva, D., Haworth, A., Casals, T., and Kremensky, I. SSCP analysis: a blind sensitivity trial. Hum. Mutat., *10:* 65–70, 1997.

38. Sheffield, V. C., Beck, J. S., Kwitek, A. E., Sandstrom, D. W., and Stone, E. M. The sensitivity of single-strand conformation polymorphism analysis for the detection of single base substitutions. Genomics, *16:* 325–332, 1993.

39. Eng, C., Brody, L. C., Wagner, T. M., Devilee, P., Vijg, J., Szabo, C., Tavtigian, S. V., Nathanson, K. L., Ostrander, E., and Frank, T. S. Interpreting epidemiological research: blinded comparison of methods used to estimate the prevalence of inherited mutations in BRCA1. J. Med. Genet., *38:* 824–833, 2001.