Mutation Analysis of BRCA1 and BRCA2 in a Male Breast Cancer Population

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Summary

A population-based series of 54 male breast cancer cases from Southern California were analyzed for germ-line mutations in the inherited breast/ovarian cancer genes, BRCA1 and BRCA2. Nine (17%) of the patients had a family history of breast and/or ovarian cancer in at least one first-degree relative. A further seven (13%) of the patients reported breast/ovarian cancer in at least one second-degree relative and in no first-degree relatives. No germ-line BRCA1 mutations were found. Two male breast cancer patients (4% of the total) were found to carry novel truncating mutations in the BRCA2 gene. Only one of the two male breast cancer patients carrying a BRCA2 mutation had a family history of cancer, with one case of ovarian cancer in a first-degree relative. The remaining eight cases (89%) of male breast cancer with a family history of breast/ovarian cancer in first-degree relatives remain unaccounted for by mutations in either the BRCA1 gene or the BRCA2 gene.

Introduction

Male breast cancer is a rare disease, with ~1,000 cases diagnosed each year in the United States (Hecht and Winchester 1994). Epidemiological studies suggest that the risk factors for male breast cancer are similar to risk factors for female breast cancer, including a positive family history of breast cancer (Olsson et al. 1993; Sasco et al. 1993; Anton-Culver et al. 1996*a*). Approximately 5%–10% of all breast cancer is thought to be due to an inherited predisposition (Newman et al. 1988; Claus et al. 1991). The recently identified breast/ovarian cancer genes, BRCA1 and BRCA2, are estimated to be responsible for 80% of multiple-case breast cancer families with an autosomal dominant pattern of inheritance (Hall et al. 1990; Easton et al. 1993; Miki et al. 1994; Wooster et al. 1994). In women who carry a BRCA1 or BRCA2 mutation, the lifetime risk of developing breast cancer approaches 85%, and men who carry a BRCA2 mutation also have an increased risk of breast cancer (Easton et al. 1993; Wooster et al. 1995). There are two reported BRCA1 families that contain male breast cancer cases, which implies a possible role for this gene in the predisposition to male breast cancer. Each of these two BRCA1 families has multiple cases of female breast cancer and one male breast cancer case (Hogervorst et al. 1995; Struewing et al. 1995). There is much more evidence that inherited BRCA2 mutations increase the risk for developing male breast cancer. To date, 12 breast cancer families with BRCA2 mutations and with cases of male breast cancer have been reported (Wooster et al. 1995; Phelan et al. 1996; Tavtigian et al. 1996; Thorlacius et al. 1996). Additionally, a founder mutation in the BRCA2 gene in Iceland accounted for 40% (12/30) of the male breast cancer cases during the past 40 years (Thorlacius et al. 1996). In a Pennsylvania study of 50 male breast cancer cases, 80% of whom had a positive family history of breast cancer, 14% (7) carried a BRCA2 mutation (Couch et al. 1996). The results of these studies may not be applicable to the general population of male breast cancer, since there is no evidence that ascertainment of cases was population based. In a report by Rosenblatt et al. (1991) of >200 population-based male breast cancer cases, the frequency of first-degree familial cases was 18%. Since male breast cancer is so rare, with only ~1% of all breast cancers occurring in men, no molecular genetic study has been done so far. We analyzed male breast cancer patients from a general population-based study, in order to determine the frequency of BRCA1 and BRCA2 germ-line mutations.

Subjects and Methods

Subject Identification

A protocol for population-based ascertainment of breast cancer has been established. Cases were ascertained retrospectively from all 72 hospitals and other health-care facilities in Orange, San Diego, and Imperial

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Counties, California, where the population base is ~5.1 million, of whom 2.6 million are men. Physicians were notified that their patient(s) would be contacted with regard to study participation. This notification was followed by a letter of introduction sent to the patient. Participation was initiated through telephone contact. Patients were interviewed with regard to family history of cancer, by trained interviewers who elicited information on types of cancer and dates of diagnoses and on births and deaths of all first- and second-degree relatives and first-cousins of the proband, including both affected and unaffected family members. There were 67 male breast cancer patients diagnosed in this area during 1989–94, and we enrolled into the study 54 (81%) of these patients. There are no differences between participants and nonparticipants, with respect to age, race/ethnicity, tumor histology, or family history of breast/ovarian cancer (table 1). The only factor that is significantly associated with study participation is, as one would expect, vital status: 46% of nonparticipants are deceased. Because of these similarities between participants and nonparticipants and because of the unlikelihood that most or all deceased patients had mutations, we believe our data to be representative of the population of all male breast cancer cases. This study was approved by an institutional review board, and all patients signed an informed-consent form.

SSCP/Heteroduplex Analysis (SSCP/HA)

An 18-ml blood sample was drawn from each of 54 patients, and DNA was extracted by standard procedures. Mutation analysis of the small exons of both genes was done by SSCP and heteroduplex analysis, which are estimated to detect ≥80% of single-base-pair substitutions and to detect a higher proportion of small insertion or deletion mutations. The BRCA1 coding exons 2–10 and 12–24 were amplified from genomic DNA as reported in the work of Gayther et al. (1995). The BRCA2 coding exons 2–9 and 12–27 and splice boundaries for exons 10 and 11 were amplified from genomic DNA, with the primers in table 2. Primers were placed ≥40 nucleotides away from the splice boundaries, in order to ensure thorough screening of all splice sites. PCR products were diluted 1:2 in formamide dye (95% formamide/0.0025% xylene cyanole/0.0025% bromphenol blue). Twenty microliters of diluted PCR product was denatured for 5 min at 99°C, then held on ice for ≥15 min to allow single-stranded conformations and homo- and heteroduplexes to form. Two microliters of each sample was electrophoresed through 0.8 × MDE gels (20 cm x 20 cm x 1 mm) at 200–250 V in 0.8 × Tris-borate EDTA at 10°C for 12–14 h. Gels were fixed in a solution of 10% ethanol/0.5% acetic acid, were silver stained, and were dried under vacuum at 80°C. All samples with bandshifts were sequenced.

Table 1

Characteristics of Participants and Nonparticipants in the Male Breast Cancer Study

	No. (%) of	
	Participants	Nonparticipants
Age (years):		
<60	15 (28)	2 (15)
60–69	21 (39)	6 (46)
≥70	18 (33)	5 (38)
Ethnicity:		
Non-Hispanic White	47 (87)	11 (85)
Hispanic, Black, Asian, or other	7 (13)	2 (15)
Histological characteristics:		
Adenocarcinomas, including ductal, lobular, and medullary	46 (85)	11 (85)
Other histological types	8 (15)	2 (16)
Reported family history of breast/ovarian cancer:		
First- or second-degree relative	16 (30)	4 (31)
No family history	38 (70)	9 (69)
Vital status:		
Alive	54 (100)	7 (54)
Deceased	0 (0)	6 (46)

Protein-Truncation Test (PTT)

More than 85% of BRCA1 and BRCA2 mutations that previously have been identified result in truncation of the protein (Friend and Breast Cancer Information Core Steering Committee 1995), and PTT is an efficient way of screening large regions for the presence of truncating mutations. Overlapping PCR products were amplified from genomic DNA in 1–1.3-kb pieces, as described by Gayther et al. (1995), for BRCA1 exon 11, and as described in table 3, for BRCA2 exons 10 and 11. Table 3 shows primer sequences for each BRCA2 PTT fragment, with two forward primers for nested PCR and with one reverse primer, which is not nested. The BRCA2 PTT forward primers include additional 5′ sequence containing the T7 promoter and a start site (ggATCCTAATACgACTCACTATAggACAgACCACCATg). This elongation of the primer sequence is denoted by an asterisk (*) in table 3. PTT using the TNT Rabbit Reticulocyte Lysate (Promega) was performed according to the manufacturer's instructions, incorporating ^{35}S-methionine for detection of the BRCA2 protein. Samples were electrophoresed on 12%–15% acrylamide gels and were exposed to Kodak X-Omat film for 16–72 h. All samples with bandshifts were sequenced.

Sequencing

Each sample revealing a variant band on PTT or SSCP/HA gels was sequenced by the Prism Kit (ABI) and was electrophoresed on an ABI 373, according to the manufacturer's instructions.

Table 2

BRCA2 Primers for SSCP/HA

Exon (Length [bp])	Fragment Length (bp)	Primer Sequence	Annealing Temperature (°C)	Variants (Frequency)
2 (106)	311	Forward: 5′ CCAggAgATgggACTgAATTAg 3′ Reverse: 5′ CTgTgACgTACTgggTTTTTAgC 3′	58	Polymorphism (.22/.78)
3 (249)	423	Forward: 5′ gATCTTTAACTgTTCTgggTCACA 3′ Reverse: 5′ CCCAgCATgACACAATTAATgA 3′	56	
4 (109)	249	Forward: 5′ AgAATgCAAATTTATAATCCAgAgTA 3′ Reverse: 5′ AATCAgATTCATCTTTATAgAACAAA 3′	46	
5 and 6 (51 and 41)	453	Forward: 5′ TgTgTTggCATTTTAAACATCA 3′ Reverse: 5′ CAgggCAAAggTATAACgCT 3′	56	Rare variant (<.01/.99)
7 (115)	214	Forward: 5′ CCTTAATgATCAgggCATTTC 3′ Reverse: 5′ CAACCTCATCTgCTCTTTCTTg 3′	54	
8 (50)	406	Forward: 5′ gCCATATCTTACCACCTTgTgA 3′ Reverse: 5′ AggTTTAgAgACTTTCTCAAAggC 3′	58	Polymorphism (.15/.85)
9 (112)	164	Forward: 5′ CTAgTgATTTTAAACTATAATTTTTg 3′ Reverse: 5′ gTTCAACTAAACAgAggACT 3′	46	
10A (1,116)	393	Forward: 5′ gggTgACTgACCgAAAAATAAA 3′ Reverse: 5′ CAgCgTTTgCTTCATggAAA 3′	52	Rare variant (.04/.96)
10F (1,116)	332	Forward: 5′ AggAggACTCCTTATgTCCAAA 3′ Reverse: 5′ AAACACAgAAggAATCgTCATC 3′	58	
11Ain (4,993)	315	Forward: 5′ ACTgTgCCCAAACACTACCTTT 3′ Reverse: 5′ CAgAgAATCAgCTTCTggggTA 3′	52	
11U (4,993)	270	Forward: 5′ TgAACTgACAgATTCTAAACTgCC 3′ Reverse: 5′ CCATACTCCCCCAAACTgACTA 3′	58	Polymorphism (.28/.72)
12 (99)	391	Forward: 5′ AgTggTgTTTTAAAgTggTCAAAA 3′ Reverse: 5′ ggATCCACCTgAggTCAgAATA 3′	54	
13 (73)	310	Forward: 5′ gCATCCgTTACATTCACTgAAA 3′ Reverse: 5′ ACgggAAgTgTTAACTTCTTAACg 3′	56	
14A (428)	391	Forward: 5′ ACCATgTAgCAAATgAgggTCT 3′ Reverse: 5′ gCTTTTgTCTgTTTTCCTCCAA 3′	58	Polymorphism (.17/.83)
14B (428)	297	Forward: 5′ CACAgAgTTgAACAgTgTgTTAgg 3′ Reverse: 5′ gggCTTTAAAATTACCACCACC 3′	58	Rare variant (<.01/.99)
15 (182)	314	Forward: 5′ ggCCAggggTTgTgCTTTTT 3′ Reverse: 5′ AggATACTAgTTAATgAAATA 3′	46	
16 (188)	395	Forward: 5′ TTTggTAAATTCAgTTTTggTTTg 3′ Reverse: 5′ AgCCAACTTTTTAgTTCgAgAg 3′	54	
17 (171)	306	Forward: 5′ CAgAgAATAgTTgTAgTTgTTgAA 3′ Reverse: 5′ AgAAACCTTAACCCATACTgC 3′	50	Polymorphism (.32/.68)
18A (355)	457	Forward: 5′ gATCCACTATTTggggATTgC 3′ Reverse: 5′ gATCTAACTgggCCTTAACAgC 3′	56	
18B (355)	384	Forward: 5′ gCAgATACCCAAAAAgTggC 3′ Reverse: 5′ TCTggACCTCCCAAAAACTg 3′	56	Rare variant (<.01/.99)
19 (156)	296	Forward: 5′ AAgTgAATATTTTTAAggCAgTT 3′ Reverse: 5′ TATATggTAAgTTTCAAgAAT 3′	46	
20 (145)	296	Forward: 5′ CACTgTgCCTggCCTgATAC 3′ Reverse: 5′ ATgTTAAATTCAAAgTCTCTA 3′	46	
21 (122)	304	Forward: 5′ gggTgTTTTATgCTTggTTCT 3′ Reverse: 5′ CATTTCAACATATTCCTTCCTg 3′	50	
22 (199)	455	Forward: 5′ AACCACACCCTTAAgATgAgC 3′ Reverse: 5′ gggCATTAgTAgTggATTTTgC 3′	56	Polymorphism (.35/.65)
23 (164)	290	Forward: 5′ ACTTCTTCCATTgCATCTTTCTCA 3′ Reverse: 5′ AAAACAAAACAAAAATTCAACATA 3′	50	
24 (139)	365	Forward: 5′ gCAgCgACAAAAAAAACTCA 3′ Reverse: 5′ ATTTgCCAACTggTAgCTCC 3′	56	
25 (305)	426	Forward: 5′ gCTTTCgCCAAATTCAgCTA 3′ Reverse: 5′ TACCAAAATgTgTggTgATgC 3′	54	Rare variant (.02/.98)
26 (147)	379	Forward: 5′ gTCCCAAACTTTTCATTTCTgC 3′ Reverse: 5′ ggAgCCACATAACAACCACA 3′	56	
27A (609 to TAA)	495	Forward: 5′ CTgTgTgTAATATTTgCgTgCT 3′ Reverse: 5′ gCAAgTTCTTCgTCAgCTATTg 3′	58	
27B (609 to TAA)	417	Forward: 5′ gAATTCTCCTCAgATgACTCCA 3′ Reverse: 5′ TCTTTgCTCATTgTgCAACA 3′	54	

Table 3

BRCA2 Primers for PTT

Exon	Primer Location	Length (bp)	PCR Primers
10 Outer forward	Intron 9 bp −171	1,333	5′ ggg TgA CTg ACC gAA AAA TAA A 3′
10 PTT forward	bp 1024–1045	1,181	5′* TTT ggA AAA ACA TCA ggg AAT T 3′
10 Reverse	Intron 10 bp +46		5′ AAA CAC AgA Agg AAT CgT CAT C 3′
11F1 Outer forward	Intron 10 −2 bp	1,475	5′ Agg TTT ATT gCA TTC TTC TgT gAA 3′
11F1 PTT forward	bp 2263–2289	1,348	5′* AAT ACA gTA ATC TCT CAg gAT CTT gAT 3′
11F1 Reverse	bp 3587–3611		5′ GTA AAT TCA AAC TgA CTT CCT gAT T 3′
11F2 Outer forward	bp 3120–3147	1,356	5′ AAT gAC TCT Agg TCA AgA TTT AAA ATC g 3′
11F2 PTT forward	bp 3139–3163	1,337	5′* TTA AAA TCg gAC ATC TCC TTg AAT A 3′
11F2 Reverse	bp 4452–4476		5′ TTg CTC CgT TTT AgT AgC AgT TAA C 3′
11F3 Outer forward	bp 4015–4039	1,329	5′ AgT AAA TgT CAT gAT TCT gTC gTT T 3′
11F3 PTT forward	bp 4030–4055	1,314	5′* TCT gTC gTT TCA ATg TTT AAg ATA gA 3′
11F3 Reverse	bp 5322–5344		5′ TTA TTC TTT CTg gTT gAC CAT CA 3′
11F4 Outer forward	bp 4958–4982	1,313	5′ AAT TAg CAT gTg AgA CCA TTg AgA T 3′
11F4 PTT forward	bp 4993–5017	1,278	5′* ACC ATT gAg ATC ACA gCT gC 3′
11F4 Reverse	bp 6248–6271		5′ ATA CTT Tgg AAA AgA CTT gCT Tgg 3′
11F5 Outer forward	bp 5862–5884	1,207	5′ CgA gAA TAA ATC AAA AAT TTg CC 3′
11F5 PTT forward	bp 5872–5891	1,197	5′* TCA AAA ATT TgC CAA ACg AA 3′
11F5 Reverse	bp 7047–7069		5′ CCA CTA AgA TAA ggg gCT CTC CT 3′

Results

Epidemiology

The 54 population-based cases of male breast cancer in this study have a median age at diagnosis of 65 years, with a range of 44–88 years. The age distribution, ethnic origin, pathology of tumors, and family history are shown in table 4. Among males in the general population who are from the same geographic area and who are of the same age as the cases, the expected proportion having a positive family history of breast or ovarian cancer in first-degree relatives is 7% (Anton-Culver et al. 1996*b*). Seventeen percent of male breast cancer patients in this series reported a history of breast/ovarian cancer in at least one first-degree relative. None of the patients reported any additional cases of male breast cancer in the family.

BRCA1 Mutation Analysis

Mutation analysis of the entire coding region and splice junctions of the BRCA1 gene was done by PTT and SSCP/HA of the 54 male breast cancer patients. Several common polymorphisms were seen, but no mutations.

BRCA2 Mutation Analysis

Mutation analysis of the coding region and splice junctions of BRCA2 revealed several common polymorphisms, as well as rare sequence variants (table 2). Most of the rare sequence variants are in noncoding regions or do not change the predicted amino acid sequence of the gene, and, on this basis, are predicted not to be mutational events that predispose to cancer. Eight of these variants are novel to this study and have been deposited into the BIC database (Friend and Breast Cancer Information Core Steering Committee 1995). The exon 2 polymorphism has been described elsewhere (Couch et al. 1996; Tavtigian et al. 1996; Teng et al. 1996), as has the exon 14A polymorphism (Couch et

Table 4

Characteristics of Population-Based Series of Male Breast Cancer Cases

	No. (%)
Age (years):	
40–49	4 (7)
50–59	11 (20)
60–69	21 (39)
70–79	14 (26)
≥80	4 (7)
Ethnicity:	
Non-Hispanic White	47 (87)
Hispanic	3 (6)
Black	1 (2)
Asian	1 (2)
Other	2 (4)
Histological characteristics:	
Ductal, lobular, and medullary	40 (74)
Other adenocarcinomas	6 (11)
Squamous cell	1 (2)
Other invasive malignancies	2 (4)
Carcinomas, in situ	5 (9)
Reported family history of breast/ovarian cancer:	
First-degree relative	9 (17)
Second-degree but not first-degree relative	7 (13)
No family history	38 (70)

al. 1996). The exon 10A rare variant, Asn289His, was found in 4/54 male patients in our study and elsewhere had been reported in one ovarian tumor (Teng et al. 1996). This missense also was found several times in a control population, indicating that it is a probable polymorphism (Alison Dunning, personal communication).

BRCA2 mutations predicted to lead to a truncated protein were found in the germ line of two patients (table 5). The exon 11 mutation was detected by PTT, and the exon 14 mutation was detected by HA. Both mutations are unique frameshift deletions and are predicted to cause premature truncation of the BRCA2 protein, at 50% and 69% of the total length.

A review was conducted of the clinical and family histories of the two patients in whom a BRCA2 mutation was identified. The first patient, 1259, was diagnosed with high-grade infiltrating ductal carcinoma at age 61 years. He is a non-Hispanic White and has a positive family history of cancer. The patient's mother was diagnosed with ovarian cancer at age 61 years, and a maternal uncle and maternal aunt were diagnosed with malignancies of unknown type and age. The proband's father was diagnosed with lung cancer at age 61 years, and a paternal uncle was diagnosed with bone cancer in his late 60s. The second patient, 2028, was diagnosed with high-grade infiltrating ductal carcinoma at age 59 years. He is U.S. born of Chinese descent. At present he does not appear to have a family history of breast or ovarian cancer. However, his daughter is now being evaluated for breast cancer, with no confirmation as of the writing of this manuscript. The only family member reported to have cancer is a maternal uncle who had throat cancer diagnosed at an unknown age and who had colon cancer diagnosed at age 51 years.

Discussion

In this population-based study of male breast cancer in Southern California, 17% of patients have a family history of breast and/or ovarian cancer in at least one first-degree relative. This is higher than the 7% of men in the general population predicted to have a first-degree relative with breast or ovarian cancer (95% confidence interval 3%–11%) (Anton-Culver et al. 1996*b*), and it supports the conclusion of other epidemiological studies—that a positive family history of breast cancer is a risk factor for male breast cancer (Olsson et al. 1993; Sasco et al. 1993). The 30% of patients with a positive family history of breast or ovarian cancer in first- or second-degree relatives is substantially lower than the 80% reported by Couch et al. (1996). However, ascertainment of patients in the study by Couch et al. was not population based and was biased toward men with a family history of breast cancer. Our results are consistent with those of Rosenblatt et al. (1991), who reported the proportion of breast or ovarian familial male breast cancers to be 18% in first-degree relatives and 10% in second-degree relatives; in our study, the proportions were 17% and 13%, respectively. Both the study by Rosenblatt et al. and our study are population based.

The BRCA1 gene originally was identified in large families with multiple cases of breast cancer, and it has been shown to predispose mutation carriers to female breast cancer and ovarian cancer (Hall et al. 1990; Easton et al. 1993). The evidence suggesting that BRCA1 may be involved in inherited male breast cancer consists of two BRCA1-linked families, each with one case of male breast cancer. In the Dutch BRCA1 family, the male breast cancer case was not available for mutation screening (Hogervorst et al. 1995). In the American BRCA1 family, the male breast cancer patient (who also had prostate cancer) was confirmed to be a germ-line carrier of the BRCA1 mutation (Struewing et al. 1995). In the present study, no male breast cancer patients with BRCA1 mutations were identified, indicating that BRCA1 does not contribute substantially to male breast cancer in the general population.

The BRCA2 gene was first identified in families with young-age-at-onset female breast cancer. BRCA2 mutation carriers were also found to be at an increased risk of ovarian cancer, and male mutation carriers were found to be at an increased risk for male breast cancer (Wooster et al. 1994). In our population study of male breast cancer, 2 (4%) of 54 patients (95% confidence limits 0%–9%) with germ-line BRCA2 mutations were identified. The age at diagnosis of the two male breast cancer BRCA2 mutation carriers (61 years and 59 years) is not appreciably different from that of the remaining male breast cancer non-BRCA2-mutation carriers (median 65 years).

Both of the BRCA2 mutations identified in this population were novel frameshifts. This result is in agreement with previous studies, where no BRCA2 missense mutations were reported in male breast cancer families, only (*a*) mutations that are predicted to truncate the BRCA2 protein and (*b*) one in-frame deletion of one amino acid (Wooster et al. 1995; Couch et al. 1996; Phelan et al. 1996; Tavtigian et al. 1996; Thorlacius et al. 1996). We have shown elsewhere that the location of a BRCA1 mutation may influence cancer phenotype (Gayther et al. 1995). Additionally, an ovarian cancer–cluster region has been identified in the BRCA2 gene (Gayther et

Table 5

BRCA2 Mutations in Male Breast Cancer Population

Patient	Exon	Mutation	Stop Codon
2028	11	5357 deletion, 4 bp	Amino acid 1710
1259	14	7253 deletion, 2 bp	Amino acid 2358

al., in press). To test the probability that mutations in a specific domain of BRCA2 might be associated with male breast cancer, all BRCA2 mutations that have been described in male breast cancer families were correlated with the location of the mutation in the gene (fig. 1) (Wooster et al. 1995; Couch et al. 1996; Phelan et al. 1996; Tavtigian et al. 1996; Thorlacius et al. 1996). The mutations in families with male breast cancer begin in exon 2 and extend to exon 20, and nonmale familial mutations begin in exon 2 and range to exon 23 (Wooster et al. 1995; Phelan et al. 1996; Thorlacius et al. 1996; Couch et al. 1996; Tavtigian et al. 1996). The distribution of mutations among male breast cancer families is not notably different from the distribution of all BRCA2 mutations (Friend and the Breast Cancer Information Core Steering Committee 1995). There is therefore no evidence, to date, for a correlation between the location of mutations in BRCA2 and male breast cancer risk.

One of the patients from this population study who carries a BRCA2 mutation has a family history of ovarian cancer, and the second patient has no family history of breast/ovarian cancer. The lack of family history in one patient with a BRCA2 mutation could be explained if this mutation arose recently in his family or if this mutation segregated through males who often display no cancer phenotype, or it could indicate that this BRCA2 mutation was incompletely penetrant. The possibility of variable penetrance in BRCA2 mutation carriers also has been suggested for the Iceland population, where 3 of 12 male breast cancer cases who carry the same BRCA2 mutation have no family history of breast cancer (Thorlacius et al. 1996).

Whereas only 3% (1/37) of the male breast cancer cases with no family history carry a BRCA2 mutation, 11% (1/9) of the male breast cancer cases with an affected first-degree relative carry a BRCA2 mutation. Eight (89%) of nine male breast cancer cases with a positive family history of breast/ovarian cancer in a first-degree relative are unaccounted for by germ-line mutations in either BRCA1 or BRCA2. In six of the eight families with no detectable mutation, there are two breast or ovarian cancers in first-degree relatives. One family has four breast/ovarian cancers in first-degree relatives, and one family has five breast/ovarian cancers in first-degree relatives. These results are similar to those in the previously reported BRCA2-mutation study of selected families, where 34 (85%) of 40 male breast cancer patients with a positive family history had no BRCA2 mutations, although the average number of affected relatives was higher, at 5.5/family (Couch et al. 1996). This lack of mutations in male breast cancer cases with a family history could be due to several reasons. The methods used for mutation screening are probably not completely sensitive, so some mutations, including possible noncoding mutations, may have been missed. A positive family history of breast cancer may be chance clustering due to the high frequency of sporadic female

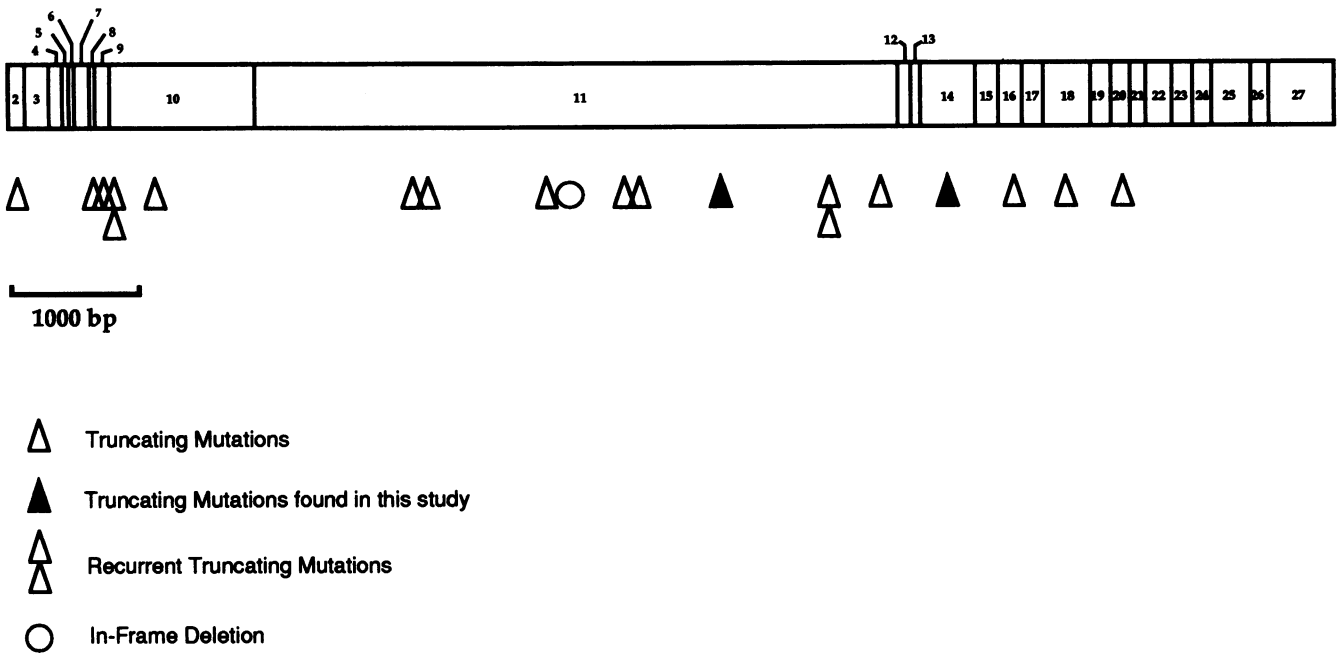

Figure 1 Mutations in BRCA2 gene. Mutations identified in this study are represented by blackened triangles; all other mutations have been identified in other studies of male breast cancer families (Wooster et al. 1995; Couch et al. 1996; Phelan et al. 1996; Tavtigian et al. 1996; Thorlacius et al. 1996). There does not appear to be, in the BRCA2 gene, a specific domain that predisposes mutation carriers to male breast cancer.

breast cancer cases in the general population. Alternatively, there may be additional inherited breast cancer–susceptibility genes that confer a risk of male and female breast cancer.

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