ONLINE MUTATION REPORT

Prevalence of *BRCA2* mutations in a hospital based series of unselected breast cancer cases

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J Med Genet 2005;**42**:e5 (http://www.jmedgenet.com/cgi/content/full/42/1/e5). doi: 10.1136/jmg.2004.025056

Epidemiological data suggest that 7% of breast cancer cases and 10% of ovarian cancer cases in the general population are attributable to one or more autosomal dominant susceptibility alleles.[1] The breast and ovarian cancer susceptibility genes *BRCA1* and *BRCA2* were isolated in 1994[2] and 1995,[3 4] respectively, and since then, a large volume of literature attests to the involvement of these genes in the great majority of ovarian cancers associated with dominant genetic predisposition,[5] and a substantial, yet still poorly defined, proportion of such breast cancers.[6] With respect to breast cancer, estimates of *BRCA* attributable risk are based largely on analyses of populations with founder mutations and those affected by early onset breast cancer, and to a lesser extent, on analyses of unselected population or hospital based series of breast cancer cases.

The *BRCA* genes are very large and subject to a broad spectrum of mutations.[7 8] Thus, population based estimates of the role of *BRCA* genes in breast cancer are more readily accomplished through the study of populations affected by a limited number of founder mutations. For example, the *BRCA1* 185delAG and 5382insC mutations and the *BRCA2* 6174delT mutation are present in 2.5% of Ashkenazi Jews,[9–11] and account for approximately 30% of the early onset breast cancers and 12% of all breast cancers in this population.[12] In Iceland, the founder mutation *BRCA2* 999del5 is present in 0.5% of the population, and accounts for 24% of early onset breast cancers and 8% of all breast cancers.[13 14] The contribution of *BRCA* mutations to breast cancer in outbred populations is difficult to extrapolate from these types of estimates, however. Towards that end, other studies have examined populations of women selected only for early onset breast cancer, with or without a family history. Representative data from this literature indicate that breast cancers in women aged <45 years are attributable to *BRCA1* in 6–13% of cases and to *BRCA2* in 4–5% of cases, suggesting that only 10–18% of early onset breast cancers are attributable to a *BRCA* mutation.[15–20] The largest population based study of *BRCA* mutation in breast cancer contained 1435 cases diagnosed before the age of 55 years in the UK, and found *BRCA* mutations associated with 2% of cases; 0.7% with *BRCA1* and 1.3% with *BRCA2*.[21] In the only population based study of unselected breast cancer cases, *BRCA1* mutations were found in 3/211 American patients (1.4%), and the *BRCA2* mutation was not studied.[22] Several hospital based series of unselected breast cancers implicate *BRCA1* and *BRCA2* in 2–5% and 0–2% of all cases, respectively, but these studies are limited by small sample sizes.[23–26]

Together, these data are consistent with the conclusion that 1–3% of all breast cancers in outbred populations are attributable to *BRCA1*. While it may be inferred from the population based studies of young women that the fraction of all breast cancers attributable to *BRCA2* is smaller than for *BRCA1*, there are insufficient data to support this conclusion directly. The purpose of this study was to determine the prevalence of germline *BRCA2* mutations in a relatively large, hospital based series of unselected breast cancer cases to estimate the fraction of all breast cancers attributable to *BRCA2*. We report here that this frequency appears to be <0.5%.

Key points

- Existing estimates of *BRCA2* mutation prevalence in breast cancer are based on studies of selected populations or small series of unselected cases. The purpose of this study was to determine the prevalence of *BRCA2* mutations in a large hospital based series of unselected breast cancer cases in order to more reliably estimate the fraction of all breast cancers attributable to *BRCA2*.
- The *BRCA2* coding region and exon–intron junctions were screened by single strand conformation polymorphism and sequencing analyses for germline sequence variation in 490 unselected, prevalent cases of breast cancer from a single institution.
- A total of six (1.2%) deleterious mutations were identified in the study population. Of these, five were the Ashkenazi Jewish founder mutation 6174delT, occurring in the subset of 90 Jewish patients (5.6%). One in 400 (0.25%) non-Jewish patients carried a deleterious *BRCA2* mutation (corrected prevalence 0.37%). In addition, 12 distinct rare polymorphic variants or variants of uncertain clinical significance were identified, along with several common polymorphic variants.
- These data suggest that <0.5% of all breast cancers in the general population are attributable to inherited mutations in *BRCA2*.

METHODS

Population

The patient population consisted of a subset of women diagnosed and treated for invasive breast cancer at this institution from 1973–2000. During the period December 1999 to July 2000, blood specimens were obtained from 501 unselected patients from this cohort following informed consent according to a protocol approved by the institutional review board. Data on age at diagnosis, menopausal status, ethnicity, year of diagnosis, histological diagnosis, type of surgery, and personal and family cancer history were obtained retrospectively from medical records. No family history information was available for five of the subjects. Following the attachment of these data to individual cases, all specimens were anonymised by removal of patient identifiers. Genomic DNA was isolated from blood samples using the QIAamp DNA blood maxi kit (Qiagen, Valencia,

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CA, USA), diluted in Tris-EDTA buffer, quantified, and stored at −20℃.

Laboratory analysis

The entire coding region (exons 2–27) and exon–intron junctions of *BRCA2* were analysed by single strand conformation polymorphism (SSCP) analysis, followed by direct sequencing of all potential variants. Coverage of this region was accomplished using 65 PCR primer sets, which generated products ranging from 194 to 315 bp in length. Primer sequences and annealing temperatures (T_A) for PCR amplification of individual products are available upon request. Generally, PCR amplification for SSCP analysis was carried out in a volume of 10 µl containing 50 ng of genomic DNA, 1.5 mmol/l $MgCl_2$, 50 mmol/l KCl, 10 mmol/l Tris-HCl, pH 8.3, 0.5 U of Ampli*Taq* DNA polymerase (Applied Biosystems, Foster City, CA, USA), forward and reverse primers at 0.8 µmol/l each, dATP, dGTP, and dTTP at 200 µmol/l each, 20 µmol/l dCTP, and 0.25 µCi of [α-^{33}P]dCTP (3000 Ci/mmol). Amplification was for 35 cycles in a Perkin-Elmer 9600 thermal cycler, with each cycle consisting of 20 seconds at 94℃, 20 seconds at T_A, and 30 seconds at 72℃, with a 7 minute extension at 72℃ following the last cycle. The entire reaction volume was then diluted into 30 µl of denaturing loading buffer consisting of 95% formamide, 0.5 mol/l EDTA, 0.02% xylene cyanol, and 0.02% bromophenol blue, heated at 95℃ for 10 minutes, and cooled on ice for 10 minutes. Following this, 6 µl of this solution were electrophoresed in gels consisting of 0.5× MDE solution (BMA, Rockland, ME) in 0.6× Tris-borate-EDTA buffer at 6 W for 16 hours at room temperature. Following electrophoresis, gels were dried and exposed to a phosphor screen, which was analysed using a Molecular Dynamics Storm 860 PhosphorImager.

Potential sequence variants identified by altered electrophoretic mobility in SSCP analyses were excised from gels and eluted into 40 µl of water for 24 hours at 4℃, then 2 µl of the eluted DNA sample were used as a template for subsequent PCR amplification using appropriate primers and reaction conditions identical to those described above, except that all dNTPs were at 200 µmol/l, and radiolabelled dCTP was omitted. Products were electrophoresed in low melting point agarose, visualised with ethidium bromide, excised from gels, and purified using the QIAquick gel extraction kit (Qiagen). These purified DNA products were subjected to sequence analysis using an ABI BigDye terminator kit and a Prism 377 automated DNA sequencer (Applied Biosystems). Sequence variants were designated according to recommendations of the HUGO Nomenclature Working Group, using the sequence listed in GenBank accession #U43746 as a reference, and were deposited in the Breast Cancer Information Core Database.[8]

Table 1 Characteristics of study population

	No.	% of total (n = 490)
Age at diagnosis (years)		
25–39	42	8.6
40–49	112	22.9
50–59	148	30.2
60–69	112	22.9
70–85	76	15.5
Menopausal status		
Premenopausal	154	31.4
Postmenopausal	336	68.6
Ethnicity		
White (non-Jewish)	329	67.1
Ashkenazi Jewish	90	18.4
African American	30	6.1
Hispanic	17	3.5
Asian	17	3.5
Unknown	7	1.4
Year of diagnosis		
1973–1994	10	2.0
1995–2000	480	98.0
Type of surgery		
Lumpectomy	323	65.9
Mastectomy	167	34.1
Histopathological diagnosis		
Infiltrating ductal carcinoma	415	84.7
Infiltrating lobular carcinoma	48	9.8
Mixed carcinoma	27	5.5
Personal history of cancer		
Bilateral invasive breast cancer	16	3.3
Ovarian cancer	0	0
Other cancer	37	7.6
Family history of cancer (n = 485)		
Breast cancer	199	41.0
First degree relative	96	19.8
Second degree relative	80	16.5
Third degree relative	23	4.7
Male breast cancer	5	1.0
Ovarian cancer	19	3.9
Breast and ovarian cancer	7	1.4

RESULTS

A total of 501 patients meeting the study entry criteria provided informed consent and a blood specimen, and of these, 11 were excluded because of inadequate quantity or quality of the DNA sample obtained. Clinical and pathological information associated with the remaining 490 cases analysed for *BRCA2* mutation is summarised in table 1. The median age of the study population was 57 years (range 27–85). The majority of patients (approximately 69%) were >50 years and post-menopausal at the time of diagnosis. Two thirds were white, while another 18% were self described as "Jewish", approximately 95% of whom, at this institution, are estimated to be of Ashkenazi (eastern European) descent. Nearly all of the patients had been diagnosed with breast cancer within 5 years of providing a blood specimen. With respect to cancer history, 90% had no previous personal cancer diagnosis (other than breast), while approximately 60% had no stated family history of breast or ovarian cancer. The majority of patients were diagnosed with infiltrating ductal carcinoma and had undergone breast conserving surgery.

Of the 490 cases screened completely for *BRCA2* sequence variants, six (1.2%) were found to harbour clearly deleterious mutations (table 2). Five of these were the founder mutation 6174delT, occurring with a prevalence of 5.6% in the subgroup of 90 Ashkenazi Jewish patients. The one additional mutation, 9132delC, was detected in a non-Jewish white patient and is a recurrent mutation that has been previously reported many times.[8] Of these six patients, two were post-menopausal, none had a personal history of cancer (other than breast), and three had no family history of breast or ovarian cancer.

Twelve additional, relatively uncommon, distinct sequence variants were identified in 15 additional patients (table 2). These variants, none of which are predicted to cause deleterious protein truncation, may be classified as likely polymorphisms or "unclassified variants" depending on the nature of the sequence variation. Of the 12 distinct variants, two nucleotide substitutions (2166C→T and 10338G→A) are designated as polymorphisms based on the absence of an amino acid change, while the remaining 10 are designated as unclassified variants, based on the low probability of a functional effect on the encoded protein and no published evidence to the contrary. Three of the unclassified sequence variants detected in this study (V3091S, IVS9–90A→G, and IVS15–114delAGT) have not previously been reported.[8] In

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Table 2 *BRCA2* sequence variants

Case no.	Variant	Age (years)	Eth*	Family history† FB	MB	O
Deleterious mutations						
50	6174delT	70	J	0	0	0
148	6174delT	54	J	0	0	0
208	6174delT	41	J	0	0	0
307	9132delC	45	W	2	0	0
383	6174delT	45	J	2	1	0
461	6174delT	41	J	3	0	1
Variants of uncertain significance						
43	D1420Y/10323delCins11	70	W	0	0	0
91	2166C→T	53	W	1	0	0
111	IVS9–90A→G	48	W	0	0	0
116	S1172L	58	W	0	0	0
149	D1420Y	61	W	2	0	0
219	I2944F	71	AA	1	0	0
246	10338G→A	61	W	3	0	0
267	T3013I	48	W	2	0	0
307	K2950N	45	W	2	0	0
331	IVS15–114delAGT	79	J	0	0	0
386	I2944F	45	H	0	0	0
475	I2944F	58	AA	0	0	0
482	10338G→A	67	W	0	0	0
516	IVS24–16T→C	38	A	0	0	0
517	V3091S	30	W	0	0	0

*Eth., ethnicity; A J, Ashkenazi Jewish; C, non-Jewish white; AA, African American; H, Hispanic; A, Asian. †Number of first, second, or third degree relatives with female breast (FB), male breast (MB), or ovarian (O) cancers.

addition to the relatively uncommon polymorphic and unclassified sequence variants listed in table 2, several common polymorphisms were also detected, and two (3624A→G and IVS21–66T→C) were characterised by sequence analysis in this study.

DISCUSSION

The results of this study suggest that in the general outbred population, germline mutations of the *BRCA2* gene account for less than 0.5% of all invasive breast cancers. However, the actual prevalence observed in this study (0.25%) is likely to be somewhat of an underestimate, and must be qualified in several respects. Firstly, the screening technique used, SSCP analysis, is an indirect mutation screen with less than complete sensitivity. In a recent evaluation of mutation detection techniques directed toward the *BRCA1* gene, the same protocol for SSCP analysis used in this study, in the same laboratory, was found to have 67% sensitivity.[27] Thus, a corrected prevalence estimate, assuming the same sensitivity for *BRCA2* mutation detection, would be 0.37%. Secondly, all of the polymorphic and unclassified sequence variants detected in this study were assumed to be unrelated to disease. Although there is currently no evidence to support a deleterious effect on *BRCA2* protein function for any of these variants, this assumption may be incorrect for some. Thirdly, the PCR based procedures used in the great majority of mutation screening studies fail to detect large genomic deletions or rearrangements that are known to occur in the *BRCA* genes. For *BRCA2*, however, only one such disease associated mutation, a large deletion affecting exon 3 in one Swedish breast and ovarian cancer family,[28] has been reported to date.

With respect to the population examined in this study, several additional factors could affect the *BRCA2* mutation prevalence estimate. Firstly, this estimate was derived from a hospital based, not population based series of cases. Compared with the breast cancer population in the USA generally, non-Jewish whites were over-represented relative to African American and other ethnic minorities in this study. However, there is no evidence to suggest that the *BRCA2* mutation frequency is substantially different among these various outbred populations. In contrast, as summarised in the introduction, a *BRCA2* founder mutation occurs at relatively high frequency in the Ashkenazi Jewish population and accounts for a proportionately higher fraction of all breast cancers in this group; the frequency of 5.6% observed in this study is consistent with previous estimates based on unselected cases of breast cancer in Ashkenazi Jews.[12 29 30] Secondly, the breast cancer patients in this series were all alive at the time of study entry, and so the proportion of cases attributable to *BRCA2* would be artefactually low if germline *BRCA2* mutation was associated with a substantially shorter survival than all breast cancers. While this may be true for *BRCA1* linked breast cancers, available evidence suggests that this is probably not the case for *BRCA2* linked breast cancers, which more closely resemble their sporadic counterparts in many clinicopathological respects, including hormone receptor expression.[31] Finally, other clinicopathological characteristics of the cases in this study such as age at diagnosis and histological subtype distribution are typical of those for the breast cancer population generally, and are unlikely to have affected the *BRCA2* mutation prevalence estimate.

The *BRCA2* attributable breast cancer incidence estimated in this study of unselected breast cancer cases is consistent with extrapolations from studies of selected groups of breast cancer patients, in which *BRCA2* mutations generally account for a smaller fraction of cases than *BRCA1* mutations.[15–21] The data presented here, together with the population based study of unselected breast cancers attributable to *BRCA1*,[22] suggest that <4% of all breast cancers are associated with inherited mutations in *BRCA1* or *BRCA2*, leaving a considerable proportion (approximately half) of the estimated dominant genetic attributable risk for breast cancer unaccounted for.

While there may exist one or more additional highly penetrant breast cancer susceptibility alleles ("*BRCA3*"), this possibility seems increasingly remote in light of the sustained lack of demonstrable linkage of non-*BRCA* linked breast cancer families to any other genetic locus.[32] Another possibility is that the familial clustering of breast cancer in kindreds without *BRCA* mutations reflects the combined effects of multiple moderate or low penetrance susceptibility alleles—that is, that breast cancer susceptibility represents a polygenic trait.[33] Indeed, there is considerable evidence to suggest that a high proportion, and perhaps the majority, of breast cancers arises in a susceptible minority of women.[34–36] However, case–control studies examining the relative risks associated with individual candidate polymorphic alleles have generally failed to provide strong evidence of low penetrance breast cancer susceptibility genes,[37 38] although the great majority of such studies were probably underpowered to reliably detect modest increases in relative risk.[39] Further progress in this area will likely depend on novel strategies for elucidating the combined effects of multiple low penetrance alleles using non-candidate gene approaches such as genomewide expression profiling.[40]

ACKNOWLEDGEMENTS

This work was supported by grants from the W M Keck Foundation, the Breast Cancer Alliance, and the US National Institutes of Health (R01 CA71840).

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Received 8 July 2004
Accepted 2 September 2004

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