Prevalence of \(BRCA2\) mutations in a hospital based series of unselected breast cancer cases

S-W Kim, C S Lee, J V Fey, P I Borgen, J Boyd


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. The breast and ovarian cancer susceptibility genes \(BRCA1\) and \(BRCA2\) were isolated in 1994 and 1995, 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, and a substantial, yet still poorly defined, proportion of such breast cancers. With respect to breast cancer, estimates of \(BRCA2\) 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. 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, and account for approximately 30% of the early onset breast cancers and 12% of all breast cancers in this population. 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. 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. 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 \(BRCA2\). 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. 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.

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, unselected breast cancer cases and 10% of ovarian cancer cases in the general population are attributable to one or more autosomal dominant susceptibility alleles. The breast and ovarian cancer susceptibility genes \(BRCA1\) and \(BRCA2\) were isolated in 1994 and 1995, 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, and a substantial, yet still poorly defined, proportion of such breast cancers. With respect to breast cancer, estimates of \(BRCA2\) 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.

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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%.

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

**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 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 MgCl₂, 50 mmol/l KCl, 10 mmol/l Tris-HCl, pH 8.3, 0.5 U of AmpliTaq 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-33P]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°C, 20 seconds at Ta, and 30 seconds at 72°C, with a 7 minute extension at 72°C following the last cycle. The entire reaction volume was then diluted into 30 μl of denaturing loading buffer consisting of 95% formamide, 0.5 mmol/l EDTA, 0.02% xylene cyanol, and 0.02% bromophenol blue, heated at 95°C 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°C, 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.

**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 post-menopausal. 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.

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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.
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 actual prevalence observed in this study (0.25%) is likely to for less than 0.5% of all invasive breast cancers. However, the sequence analysis in this study.

Table 2 BRCA2 sequence variants

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Variant</th>
<th>Age (years)</th>
<th>Eth*</th>
<th>Family history†</th>
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<td>70</td>
<td>J</td>
<td>0 0 0</td>
</tr>
<tr>
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<td>6174delT</td>
<td>54</td>
<td>J</td>
<td>0 0 0</td>
</tr>
<tr>
<td>208</td>
<td>6174delT</td>
<td>41</td>
<td>J</td>
<td>0 0 0</td>
</tr>
<tr>
<td>307</td>
<td>9132delC</td>
<td>45</td>
<td>W</td>
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</tr>
<tr>
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<td>J</td>
<td>2 0 0</td>
</tr>
<tr>
<td>461</td>
<td>6174delT</td>
<td>41</td>
<td>J</td>
<td>3 0 1</td>
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</table>

Varians of uncertain significance

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<th>Variant</th>
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<th>MB</th>
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<td>219</td>
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<tr>
<td>246</td>
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<td>V3091S</td>
<td>30</td>
<td>W</td>
<td>0 0 0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Eth, ethnicity; A, 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.

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 actual prevalence observed in this study (0.25%) is likely to for less than 0.5% of all invasive breast cancers. However, the sequence analysis in this study.

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