The Relative Contribution of Point Mutations and Genomic Rearrangements in BRCA1 and BRCA2 in High-Risk Breast Cancer Families

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Abstract

The demand for BRCA1 and BRCA2 mutation screening is increasing as their identification will affect medical management. However, both the contribution of different mutation types in BRCA1 and BRCA2 and whom should be offered testing for large genomic rearrangements have not been well established in the U.S. high-risk population. We define the prevalence and spectrum of point mutations and genomic rearrangements in BRCA genes in a large U.S. high-risk clinic population of both non-Ashkenazi and Ashkenazi Jewish descent, using a sample set representative of the U.S. genetic testing population. Two hundred fifty-one probands ascertained through the University of Pennsylvania high-risk clinic, all with commercial testing for BRCA1 and BRCA2, with an estimated prevalence of BRCA1 mutation ≥10% using the Myriad II model and a DNA sample available, were studied. Individuals without deleterious point mutations were screened for genomic rearrangements in BRCA1 and BRCA2. In the 136 non-Ashkenazi Jewish probands, 36 (26%) BRCA point mutations and 8 (6%) genomic rearrangements (7 in BRCA1 and 1 in BRCA2) were identified. Forty-seven of the 115 (40%) Ashkenazi Jewish probands had point mutations; no genomic rearrangements were identified in the group without mutations. In the non-Ashkenazi Jewish probands, genomic rearrangements constituted 18% of all identified BRCA mutations; estimated mutation prevalence (Myriad II model) was not predictive of their presence. Whereas these findings should be confirmed in larger sample sets, our data suggest that genomic rearrangement testing be considered in all non-Ashkenazi Jewish women with an estimated mutation prevalence ≥10%.

Introduction

A woman born in the United States has an average lifetime risk of 13% of being diagnosed with breast cancer (1). Family history is associated with 10% to 20% of breast cancer cases (2), and within that group approximately one half (5–10% of all cases) are strongly hereditary (3). Germline mutations in BRCA1 and BRCA2 are associated with an increased risk of breast and ovarian cancer (4–7). Genetic testing for BRCA1 and BRCA2 mutations has been available for more than a decade (8–10). The usefulness of genetic testing as a medical management tool has become increasingly recognized (11), as effective risk reduction procedures, such as prophylactic oophorectomy, and screening measures, such as breast MRI, are available (12–17). Our ability to offer effective management for patients with BRCA1 and BRCA2 mutations has increased the demand for genetic testing and makes it imperative that we detect all mutations.

Despite our understanding of the clinical phenotypes most predictive of the presence of BRCA1 and BRCA2 mutations (18–22), a significant number of families with both breast and ovarian cancers do not have identifiable mutations (23). An increase in the rate of mutation detection has resulted from the identification of exon duplication(s) or deletion(s), commonly referred to as genomic rearrangements. Genomic rearrangements are not identified using PCR-based methods of mutation screening. Initial studies of genomic rearrangements were limited in various ways—they examined only BRCA1, included small patient populations, and were limited to very high-risk breast/ovarian cancer families or to one ethnicity (24–27). More recently, highly sensitive DNA-based quantitative techniques have been developed to analyze both BRCA1 and BRCA2 for the presence of genomic rearrangements, and as such are useful in large-scale screening. Previous studies, mainly done in homogenous ethnic groups, have suggested that the frequency of genomic rearrangements in BRCA1 in high-risk breast cancer families ranges between 1.3% and 4.4%, accounting for between 8% and 19% of the total mutations, with the number dependent on ethnicity and study eligibility criteria (24–35). To date, the prevalence of genomic rearrangements in BRCA2 is lower than that in BRCA1, with the frequency in high-risk breast/ovarian cancer families ranging from 0% to 2.4% with contribution of large genomic rearrangements to all the BRCA2 mutations between 0% and 11% (29, 32, 33, 36–39). These data have established that genomic rearrangements comprise a significant component of the identifiable mutations in BRCA1 and BRCA2.

Whereas the utility of screening for genomic rearrangement in both BRCA1 and BRCA2 is clear, there remain several crucial unanswered questions, such as the prevalence of genomic rearrangements in the U.S. high-risk population, their contribution to mutations in populations of mixed ethnic backgrounds, and how patients should be selected for screening. Currently, screening for genomic rearrangements is offered only on a standard basis (i.e., incorporated within initial genetic testing, as opposed to being ordered as a separate test) to women with very high-risk personal...
BRCA Mutation Types in a U.S. High-Risk Population

Table 1. Characteristics of all probands

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Family, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethnicity, all probands (n = 251)</td>
<td></td>
</tr>
<tr>
<td>Non-Ashkenazi Jewish</td>
<td>136 (54)</td>
</tr>
<tr>
<td>European</td>
<td>119 (47)</td>
</tr>
<tr>
<td>African American</td>
<td>16 (6)</td>
</tr>
<tr>
<td>Latin American</td>
<td>1 (0)</td>
</tr>
<tr>
<td>Ashkenazi Jewish</td>
<td>115 (46)</td>
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</table>

<table>
<thead>
<tr>
<th>Cancer history</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Ashkenazi Jewish</td>
<td>136 (100)</td>
</tr>
<tr>
<td>Breast only*</td>
<td>90 (66)</td>
</tr>
<tr>
<td>Age at diagnosis (y)</td>
<td></td>
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<tr>
<td>&lt;30</td>
<td>3 (2)</td>
</tr>
<tr>
<td>30–39</td>
<td>33 (25)</td>
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<tr>
<td>40–49</td>
<td>46 (33)</td>
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<tr>
<td>50–59</td>
<td>7 (5)</td>
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<tr>
<td>≥60</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Ovarian only</td>
<td>13 (9)</td>
</tr>
<tr>
<td>Breast and ovarian</td>
<td>11 (8)</td>
</tr>
<tr>
<td>Bilateral breast</td>
<td>14 (11)</td>
</tr>
<tr>
<td>Bilateral breast and ovarian cancer</td>
<td>3 (2)</td>
</tr>
<tr>
<td>Male breast</td>
<td>5 (4)</td>
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<tr>
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<td>115 (100)</td>
</tr>
<tr>
<td>Breast only</td>
<td>71 (62)</td>
</tr>
<tr>
<td>Age at diagnosis (y)</td>
<td></td>
</tr>
<tr>
<td>&lt;30</td>
<td>3 (2.5)</td>
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<tr>
<td>30–39</td>
<td>19 (16)</td>
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<td>50–59</td>
<td>10 (9)</td>
</tr>
<tr>
<td>≥60</td>
<td>4 (3.5)</td>
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<tr>
<td>Ovarian only</td>
<td>13 (11)</td>
</tr>
<tr>
<td>Breast and ovarian</td>
<td>6 (5)</td>
</tr>
<tr>
<td>Bilateral breast</td>
<td>21 (18)</td>
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<tr>
<td>Bilateral breast and ovarian cancer</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Male breast</td>
<td>2 (2)</td>
</tr>
</tbody>
</table>

*Cancer history percentage shown is the percent of total non-Ashkenazi Jewish or Ashkenazi Jewish groups.

or family histories (approximated to an estimated mutation prevalence >30%). The goal of our study was to determine the role of testing for BRCA1 and BRCA2 genomic rearrangements in a U.S. high-risk clinic, representative of the commercial testing population, including women of both Ashkenazi Jewish and non-Ashkenazi Jewish descent.

Materials and Methods

Family ascertainment. Probands were ascertained from the Cancer Risk Evaluation Program at the University of Pennsylvania Health System [Hospital of the University of Pennsylvania (1998–2006) and Pennsylvania Hospital (2001–2006); see Eligibility Criteria Flowchart, Supplementary Fig. S1]. Patients are either self-referred or physician referred based on their personal or family history. All participants provided written consent to participate in an institutional review board–approved study of the genetics of hereditary breast cancer. More than 99% of patients seen in Cancer Risk Evaluation Program participate in a research registry. Only one individual from each family was included in this study. If multiple individuals from the same families were enrolled in our study, the individual affected with breast cancer at the earliest age with commercial screening (Myriad Genetics) performed was considered the proband. Pathology reports and/or medical records for confirmation for age and type of cancer diagnosis were collected from probands and affected relatives whenever possible. Ethnicity was self-reported. The characteristics of the probands are detailed in Table 1.

Study eligibility. Probands were eligible for the current study if they meet all of the following criteria: (a) were affected with breast or ovarian cancer; (b) had full commercial sequencing or Ashkenazi Jewish founder mutation screening (Myriad Genetics); (c) had no prior genetic testing of themselves or any family member; (d) had an estimated mutation prevalence of ≥10% based on the Myriad II model prevalence tables; and (e) had a DNA sample available in the laboratory at the University of Pennsylvania.

Probands were excluded if either they or their relatives had previous genetic testing before being evaluated in Cancer Risk Evaluation Program for two reasons. First, we wanted to exclude mutation positive patients specifically referred for clinical management, so as to limit bias for mutation positivity. Second, we wanted to exclude individuals who were referred to us specifically for research participation after uninformative genetic testing, so as to limit bias for mutation negativity.

The estimated prevalence of BRCA mutations was generated using the Myriad II model, which consists of two mutation prevalence tables, stratified by Ashkenazi Jewish ethnicity.6 The tables were developed following the guidelines initially published by Frank and colleagues (21) and provide a composite mutation prevalence for BRCA1 and BRCA2. The Myriad II model has been shown to perform similarly to BRCAPRO and BOADICA and be the most sensitive in predicting mutations in non-Ashkenazi Jewish families (40). All models use only first- and second-degree relatives. The 507 probands that met criteria a to c were evaluated using the Myriad II model. The predicted prevalence of BRCA mutations was calculated for both the maternal and paternal lineages; the lineage with the highest prevalence was used for analytic purposes. Twelve probands had one parent each of Ashkenazi Jewish and non-Ashkenazi Jewish origin. These probands were grouped with the lineage with the highest estimated mutation prevalence—six Ashkenazi Jewish and six non-Ashkenazi Jewish. Sixty-two probands (90 Ashkenazi Jewish and 22 non-Ashkenazi Jewish) had an estimated mutation prevalence >10%, but did not provide samples. A comparison between the sample and nonsampled groups is included in Results. Two hundred fifty-one probands had both an estimated BRCA mutation prevalence of >10% in either the maternal or paternal lineage and a sample available in the laboratory and thus were included in the study.

Point mutation analysis. DNA was extracted from peripheral blood mononuclear cells by using standard procedures. Either blood or DNA samples were provided to Myriad Genetics Laboratory (Salt Lake City, UT) for mutation detection. After analysis, all patients were classified as having a deleterious mutation, a variant of uncertain significance, or no mutation (see Supplementary Tables S1 and S2). The classification of mutations was done as previously described (21). As detailed in Fig. 1, full sequencing in probands with Ashkenazi Jewish ancestry was done in a total of 44 women because of genetic testing having been done before the availability of the Ashkenazi Jewish founder mutation panel, mixed heritage (probands with non-Ashkenazi Jewish ancestors), high prior probability, or based on patient request.

Screening for genomic rearrangements. Ninety-four probands (72 non-Ashkenazi Jewish and 22 Ashkenazi Jewish) had testing for the presence of five BRCA1 genomic rearrangements included in the commercial screening since 2001 at Myriad Genetics Laboratory. The rearrangement panel includes five specific mutations in BRCA1: 7.1-kb deletion of exons 8 to 9, 3.4-kb deletion of exon 13, 6.2-kb duplication of exon 13, 26-kb deletion of exons 14 to 20, and 510-bp deletion of exon 22 (24, 26, 41–43). Multiplex ligation–dependent probe amplification (MLPA) was used to screen for genomic rearrangements in both BRCA1 and BRCA2 in all the mutation-negative families. MLPA was done according to the instructions provided by the manufacturer (MRC-Holland). The probe mixes MLPA P002

6 http://www.myriadtests.com/provider/brcamutation-prevalence.htm
and P087 were used to screen BRCA1. The probe mix MLPA P045 kit was used to screen BRCA2 and for the CHEK2 1100delC mutation. The fragments were analyzed on ABI 3100 capillary sequencer (Applied Biosystems, Inc.) using Genescan software. Variation in peak height was evaluated by comparing each test sample to the normal control present in the same experiment and by cumulative comparison of four to six samples always from the same experiment. Any sample with variation in peak height was repeated for a total of three times.

Quantitative analysis of MLPA. We used GeneMarker version 1.4 (Softgenetics LLC) to perform data normalization and analysis. GeneMarker normalizes the peak height (fluorescence intensity) of each MLPA fragment with either the internal control probes or the entire population (all the fragments). Samples for which we obtained a height ratio (normalized fluorescence intensity of each individual probe of a patient sample to a control DNA sample) of <0.7 (for deletion) and >1.2 (for duplication) had MLPA repeated. Controls with known genomic deletions were studied and had height ratios of −1.5, 1.0, 0.5, or 0.0 for regions with duplication, the wild-type sequence, or a deletion or absence of the sequence, respectively.

Identification and confirmation of rearrangements. For the rearrangements in BRCA1 and BRCA2, we confirmed the presence of the genomic deletions using MLPA kits for BRCA1 and real-time quantitative PCR for the mutations in BRCA1 and BRCA2. Real-time PCR was done using the LightCycler (Roche Applied Science, v.3.5.3) and SYBR Green I chemistry (LightCycler FastStart DNA Master SYBR Green I). Primers and probes for exons within BRCA1 and BRCA2 were designed as described by Barrois and colleagues (44). We selected one test exon within the deleted region of interest. As an endogenous control, we normalized each sample to ALB (encoding albumin at 4q11-q13). We determined heterozygosity for a deletion when the ratio of ALB/test exon was 2:1. Each assay was run in triplicate; two normal controls (ratio 1:1) and a nontemplate control were included. PCR reactions were set up in a total volume of 20 µL by distributing aliquots of 18 µL of master mix into the capillaries, followed by 2 µL of DNA adjusted to 10 ng/µL. The reaction mixture contained 0.4 µL of each primer, 2 µL of FastStart DNA kit SYBR Green I, and 5 mmol/L MgCl2. The thermal cycling conditions were an initial denaturation step at 95°C for 10 min, 45 cycles at 95°C for 10 s, primer annealing temperature for 5 s, and extension at 72°C for 6 s. (Primers and annealing temperatures are available on request.) A melting curve was produced after each run for every sample. Quantification data were analyzed using the fit point method of the LightCycler software (Supplementary Figs. S2 and S3). In the fit point method for the LightCycler (Roche, Inc.), the relative expression ratio is calculated from the real-time efficiencies and the crossing point (where fluorescence increases above the threshold) deviation of an unknown sample as compared with a control.

All genomic rearrangements identified by MLPA, a subset of which were confirmed by real-time PCR, were sent for the BRACAnalysis Rearrangement Test (BART; Myriad Genetics). All mutations confirmed by real-time

![Figure 1](attachment://image.png)

Figure 1. Analytic strategy of the study and numbers of patients identified in each group. *, mutations are listed in Supplementary Table S1.
PCR were also identified by BART. No mutations unconfirmed by real-time PCR were identified by BART. In three families, additional affected family members were available and cosegregation of the mutation with disease was observed.

**Statistical methods.** Comparisons of discrete and quantitative variables were made by using Fisher’s exact tests and Kruskal-Wallis $\chi^2$ tests, respectively (STATA v.9, StataCorp). The latter was used to test the null hypothesis that there was no difference in the median ages of cancer diagnosis in the mutation-carrying and non–mutation-carrying groups and between the estimated mutation prevalences (Myriad II model) in the proband characteristics of the non-Ashkenazi Jewish and Ashkenazi Jewish probands only in the same individual (%)

Results

**Family characteristics.** Probands from 251 families met the eligibility criteria for inclusion, 244 females and 7 males (Table 1). Of the 251 high-risk families, 115 (46%) were of Ashkenazi Jewish descent and 136 (54%) were of non-Ashkenazi Jewish descent. The proband characteristics of the non-Ashkenazi Jewish and Ashkenazi Jewish families were not significantly different.

The range of estimated prevalence of point mutations in *BRCA1* and *BRCA2* was from 11.2% to 79.3%, with a median of 17.6%; in the non-Ashkenazi Jewish probands, from 11.2% to 79.3% (median, 22.9%), and in the Ashkenazi Jewish probands, from 11.6% to 75% (median, 16%). The characteristics of the non-Ashkenazi Jewish (Table 2) and Ashkenazi Jewish families were very similar: More than 95% of families contained women with unilateral breast cancers, 45% with at least one woman with ovarian cancer, 15% with at least one woman with breast and ovarian cancers, 5% to 7% with at least one man with breast cancer, and 20% with at least one man with prostate cancer.

The familial characteristics differed significantly between non-Ashkenazi Jewish and Ashkenazi Jewish probands only in the percentage of families with women with breast cancer diagnosed under age 50 years, 96% and 86%, respectively ($P = 0.01$). We identified 62 probands who met the study eligibility requirements but did not provide samples; of these, 40 were Ashkenazi Jewish and 22 were non-Ashkenazi Jewish.

**Point mutations and variants of uncertain significance in BRCA1 and BRCA2.** In total, 83 of the 251 (33%) total probands studied were found to have deleterious point mutations, 49 (59%) in *BRCA1* and 34 (41%) in *BRCA2*, respectively (Fig. 1). The 83 mutations are composed of 22 and 15 distinct mutations in *BRCA1* and *BRCA2*, respectively. All mutations are listed in Supplementary Table S1. The three Ashkenazi Jewish founder mutations accounted for 47 (56%) of the mutations detected. Whereas a higher rate of point mutations was found in the Ashkenazi Jewish group (40%)...
than in the non-Ashkenazi Jewish group (26%; \( P = 0.02 \)), the proportions of mutations identified in \( BRCA1 \) (60%) and \( BRCA2 \) (40%) were identical in the two groups.

In the 115 Ashkenazi Jewish probands, 47 mutations (40% of families) were identified, 28 (24%) in \( BRCA1 \) and 19 (16%) in \( BRCA2 \), respectively. Of the identified mutations, 45 were Ashkenazi Jewish founder mutations (\( BRCA1 \) 185delAG, 22; \( BRCA1 \) 5382insC, 5; \( BRCA2 \) -6174delT, 18) and 2 were nonfounder mutations (1135insA in \( BRCA1 \) and 5466insT in \( BRCA2 \)). \( BRCA1 \) 1135insA has been described as an ancient founder mutation in the Norwegian population and previously has been reported in an Ashkenazi Jewish family of Norwegian descent, as our family is (45, 46).

After full sequencing in the probands from the 136 non-Ashkenazi Jewish families, 36 point mutations (26% of families) were identified. Twenty-one (15%) and 15 (11%) mutations were identified in \( BRCA1 \) and \( BRCA2 \), respectively. The only mutations identified more than once were both in \( BRCA1 \), 5382insC in two probands of Polish descent and IVS16+6T>6 in two African American probands. Five of 16 (31%) African Americans had identified mutations as compared with 31 of 120 (25.8%) European Americans. The estimated mutation prevalence for the African American probands ranged from 16.3 to 73.7% with a median of 26.3%.

Twenty variants of uncertain significance were identified in the 251 probands (see Supplementary Table S2). Four variants of uncertain significance were found in patients who also had deleterious mutations. Eight of the variants of uncertain significance were identified in \( BRCA1 \) and 12 in \( BRCA2 \). Of these, \( BRCA1 \) V1688del may be deleterious, but it is not yet formally classified as such (47). Eight of the 20 (40%) variants of uncertain significance were found in probands of African American ancestry.

Comparison of sampled and nonsampled probands. To assess any bias based on sample collection, we evaluated 62 probands who met eligibility criteria but did not provide a sample. Comparing the sampled (251) and nonsampled (62) probands, there was no significant difference in the estimated mutation prevalence (\( P = 0.4 \), Ashkenazi Jewish and \( P = 0.12 \), non-Ashkenazi Jewish). In the 40 Ashkenazi Jewish probands, 12 (29%) had mutations, not significantly different from the 115 Ashkenazi Jewish probands with samples (\( P = 0.12 \)). None of the 22 non-Ashkenazi Jewish probands carried mutations, significantly different than the sampled group (\( P = 0.002 \)). However, the inclusion of the nonsampled probands did not significantly change the rate of point mutations, which decreased from 26% to 23% (\( P = 0.49 \)). Eighty-six percent of the potential non-Ashkenazi Jewish probands were included in the study.

Comparison of testing population with Myriad Genetics testing population. To determine whether we could extrapolate our findings to the larger commercial testing population, we compared the expected number to the observed number of point mutations based on the Myriad II model. The majority of probands had an estimated mutation prevalence between 10% and 20%, comprising 49% and 55% of those studied in the non-Ashkenazi Jewish and Ashkenazi Jewish groups, respectively. In the non-Ashkenazi Jewish group, there was no significant difference between the numbers of observed and expected mutations (Fig. 2A). In the entire Ashkenazi Jewish group, we found a significant difference between the numbers of observed and expected point mutations (\( P = 0.008 \)); this result was entirely due to the considerable difference between the numbers of observed (20) and expected (9) mutations in the group with the estimated mutation prevalence between 11.2% and 16.7% (Fig. 2B). In the 52 Ashkenazi Jewish probands with an estimated mutation prevalence over 20%, there was no significant difference between the numbers of observed and expected point mutations.

Prevalence of genomic rearrangements in \( BRCA1 \) and \( BRCA2 \). In total, we screened 167 probands for genomic rearrangements in \( BRCA1 \) and \( BRCA2 \) using MLPA. Probands were included if they had negative result after full sequencing, the Ashkenazi Jewish founder mutation screen (if Ashkenazi Jewish) or a variant of uncertain significance.

Using MLPA in the 68 Ashkenazi Jewish probands, 4 samples with decreases in peak height as compared with controls were
identified, all of BRCA2 exons 1 to 2. None of the peak height decreases were confirmed as true deletions by real-time PCR of exon 2 of BRCA2 or by BART. In summary, no genomic rearrangements were identified in the 68 Ashkenazi Jewish probands studied. Twenty-five (37%) had an estimated mutation prevalence >10%, which is the cutoff used for non-Ashkenazi Jewish women.

In the 100 non-Ashkenazi Jewish probands without deleterious point mutations, 8 genomic rearrangements were detected: 7 in BRCA1 and 1 in BRCA2, all deletions (Table 3). One large genomic rearrangement (deletion of BRCA1 exons 14–20) was identified by the five-site rearrangement panel (Myriad Genetics). The additional five in BRCA1 and one in BRCA2 were identified by MLPA and confirmed by real-time PCR of one or more of the relevant exon(s) of BRCA1 or BRCA2 (Supplementary Figs. S2 and S3) and BART. A benign polymorphism, BRCA2 2192C>G (P655R), known to be found in African Americans, within the MLPA BRCA2 exon 11 probe binding site (probe 2279-L1770) created a false positive in two samples, which was not confirmed by real-time PCR or BART. Five of these genomic rearrangements have previously been reported (33, 43, 48, 49). We identified two novel large genomic deletions: the deletion of exons 11 to 12 in BRCA1 and that of exons 1 to 13 in BRCA2. We identified a deletion of BRCA1 exons 21, 22, 23, and 24 in two independent families. The deletion of exons 8 to 9 in the African American family presumably is different than the previously reported deletion in women of European origin (42), as it was not detected by the commercially available five-site genomic rearrangement panel. Walsh and colleagues (48) also detected a deletion of exons 8 and 9 in an African American family, so it is possible that it is a founder mutation in this population. In our family, the deletion was in linkage disequilibrium with BRCA1 UCV A102G.

The frequency of genomic rearrangements in all non-Ashkenazi Jewish probands from our clinic based population was 6% (8 of 136), increasing to 8% (8 of 100) in those without identified point mutations. Together the genomic rearrangements constituted 18% (8 of 44) of all mutations identified in the non-Ashkenazi Jewish group; 29% (8 of 28) and 6% (1 of 16) of those in BRCA1 and BRCA2, respectively. One of the genomic rearrangements was found in an African American individual; in this population, it constituted 1 of 6 (16%) of all mutations identified. The estimated mutation prevalences using the Myriad Genetics tables were not predictive of identifying genomic rearrangements in BRCA1 or BRCA2 in the non-Ashkenazi Jewish group (P = 0.27). The median estimated mutation prevalence in the non-Ashkenazi Jewish probands was 22.9%. In families without identified point mutations in BRCA1 or

### Table 3. Description of genomic rearrangements

<table>
<thead>
<tr>
<th>Gene</th>
<th>Exon(s)</th>
<th>Type of genomic change</th>
<th>Myriad II model estimated mutation prevalence*</th>
<th>Phenotype proband</th>
<th>Primary cancer(s) (age at Dx)</th>
<th>Family history (no. cases)</th>
<th>Ethnicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA1</td>
<td>1–2</td>
<td>Deletion</td>
<td>17.6</td>
<td>Breast Ca (45)</td>
<td>1 1 — — —</td>
<td>—</td>
<td>White (Irish/English/German)</td>
</tr>
<tr>
<td>BRCA1</td>
<td>3</td>
<td>Deletion</td>
<td>46.8</td>
<td>Ovarian Ca (54)</td>
<td>2 1 — — —</td>
<td>1</td>
<td>White (Italian)</td>
</tr>
<tr>
<td>BRCA1</td>
<td>8–9</td>
<td>Deletion</td>
<td>17.6</td>
<td>Breast Ca (29)</td>
<td>1 1 — 1 —</td>
<td>—</td>
<td>African American</td>
</tr>
<tr>
<td>BRCA1</td>
<td>Part of 11–12</td>
<td>Deletion</td>
<td>17.6</td>
<td>Breast Ca (39)</td>
<td>1 1 — — —</td>
<td>—</td>
<td>White (Western Europe)</td>
</tr>
<tr>
<td>BRCA1</td>
<td>14–20</td>
<td>Deletion</td>
<td>16.3</td>
<td>Bilateral breast ca (27, 33)</td>
<td>2 2 — — —</td>
<td>—</td>
<td>White (British)</td>
</tr>
<tr>
<td>BRCA1</td>
<td>21–24</td>
<td>Deletion</td>
<td>16.3</td>
<td>Bilateral breast Ca (45, 47)</td>
<td>3 2 — — —</td>
<td>—</td>
<td>White (Irish/German)</td>
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<td>BRCA1</td>
<td>21–24</td>
<td>Deletion</td>
<td>30.7</td>
<td>Breast Ca (29)</td>
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<td>—</td>
<td>White (German/British/Italian/Czech)</td>
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<tr>
<td>BRCA2</td>
<td>1–13</td>
<td>Deletion</td>
<td>11.2</td>
<td>Bilateral breast Ca (55, 55)</td>
<td>6 4 1 1 —</td>
<td>—</td>
<td>White (Scottish/English/German/Norwegian)</td>
</tr>
</tbody>
</table>

Abbreviations: Br Ca, breast cancer; Ov Ca, ovarian cancer.
* Using first- and second-degree relatives.
† Up to fourth-degree relatives included in family history.
‡ Identified by the Myriad Genetics five-site rearrangement screen.
BRCA2, the median estimated mutation prevalence was 17.9%; in eight of these families subsequently identified with a genomic rearrangement, the median estimated mutation prevalence was 17.6%, supporting a family history comparable to those families that did not have identified mutations. Whereas the number of individuals identified with genomic rearrangements was small, those with rearrangements in BRCA1 were more likely to have bilateral breast cancer (37.5% versus 8%, \( P = 0.03 \)) than the mutation-negative probands. There were no other significant differences between the personal or family history of the probands with genomic rearrangements as compared with the mutation-negative probands.

Discussion

Whereas the presence of genomic rearrangements, in addition to point mutations, in both BRCA1 and BRCA2 has been well established, the proportion of women carrying mutations and their relative contribution to the total mutations in a U.S.-based high-risk clinic representative of the commercial testing population have not been determined. In our study, 6% of non-Ashkenazi Jewish women with an estimated mutation prevalence >10% had a genomic rearrangement, comprising 8% of those without an identified point mutation. Of importance, genomic rearrangements accounted for 18% of the mutations identified and their presence did not correlate with the estimated prevalence of BRCA mutation. Specifically, six of the eight women with genomic rearrangements had an estimated mutation prevalence <30%, and thus would not have been eligible for standard screening for genomic rearrangements, which is offered only to women with an estimated mutation prevalence >30% (approximated from the Myriad Genetics criteria). Whereas it is likely that the phenotypic predictors for genomic rearrangements in BRCA1 and BRCA2 are the same as those for point mutations, we did not identify enough rearrangements to fully evaluate mutation predictors. Our data support the consideration of genomic rearrangement screening in BRCA1 and BRCA2 for all non-Ashkenazi Jewish women with an estimated mutation prevalence >10%.

The non-Ashkenazi Jewish group included 16 of 136 (12%) probands of African American ethnicity. Among the African American families with a negative sequencing result, we detected one family with a genomic rearrangement (deletion of exons 8–9) in BRCA1, representing 6% of all African American families and 16% (1 of 6) of all mutations identified in this group. Our data underline the need for larger studies to explore the contribution of genomic rearrangements to the BRCA1 and BRCA2 mutation spectra among minority populations in the United States.

The rate of genomic rearrangements identified in this series is consistent with previous series from Europe and Australia, but lower than a previous U.S.-based series, in which 12% of probands without point mutations were found to have a rearrangement in BRCA1 and BRCA2 (48). The discrepancy is likely due to differences in the study eligibility criteria. The previous study included families with at least four cases of female breast cancer, ovarian cancer, and/or male breast cancer, whereas the current study included patients with an estimated prevalence of >10%, which encompasses families with as few as two cases of female breast cancer. Nonetheless, the conclusion of both studies is the same: Non-Ashkenazi Jewish women without point mutations in BRCA1 and BRCA2 should be offered screening for genomic rearrangements in the clinical setting.

Whereas genomic rearrangements significantly contribute to the total number of mutations in BRCA1 and BRCA2, we did not identify any large genomic rearrangements in the Ashkenazi Jewish probands negative for point mutations. Within the Ashkenazi Jewish group, 45 of 47 (96%) point mutations were founder mutations. Our data are consistent with previous studies showing that the founder mutations account for more than 90% of mutations in Ashkenazi Jewish women (50, 51). In total, as reported in the literature and including our study, 99 Ashkenazi Jewish probands from high-risk families have been negatively screened for genomic rearrangements (48). Thus, founder mutations continue to account for the vast majority of all mutations in this population. Even if 18% of all detectable nonfounder mutations in our Ashkenazi Jewish cohort were genomic rearrangements, they nevertheless would represent very rare events, accounting for 0.72% of mutations based on the prevalence of nonfounder mutations in the Ashkenazi Jewish population. These data suggest that routine screening for large genomic rearrangements does not seem to be warranted in Ashkenazi Jewish women. However, larger studies of this population need to be done.

Empirical predictive models such as Myriad II, Couch, and Manchester Scoring System are intended to estimate the possibility of a BRCA1 or BRCA2 mutation in a woman based on her family history and are used widely in clinical practice (5, 21, 52, 53). Currently, these models do not account for genomic rearrangements and as such underestimate the number of women with identifiable mutations, particularly in BRCA1. In addition, understanding the proportion of mutation that are missed using current methods to identify BRCA1 or BRCA2 is useful when counseling patients about their chances of being undetected mutation carriers. Previous studies from the Breast Cancer Linkage Consortium have suggested that the percentage of linked families with a detectable point mutation may be as low as 65% (23). However, the study was limited because various mutation detection techniques were used, some of which only detect 60% to 65% of mutations as compared with sequencing as a gold standard (54). Nonetheless, these studies did not include genomic rearrangements and thought should be given to repeating them with improved mutation detection techniques and the larger spectrum of mutations now identified, so that women can be accurately counseled about their chances to carry an undetected mutation.

We attempted to limit bias for and against mutations in our population by only including probands with a sample available in the laboratory and excluding any probands who themselves or whose any family member had prior genetic testing before being seen in Cancer Risk Evaluation Program and having commercial testing. Due to our strict eligibility criteria, the number of probands available for study was restricted; as such, these findings will need to be replicated in a larger population. However, any such study will need to include systematic screening of a clearly defined population, so that the findings can be generally applied. Whereas our study is based on a single high-risk clinic population and thus limited, we have shown that the number of observed mutations is not significantly different than that expected in the commercial testing population based on the Myriad II model. The Myriad II model has been shown to perform similarly to BOADICEA and BRCAPRO; all of the models are limited in that they generally under-predict mutations at the lower end of prediction probability and overestimate those at the upper end (40, 55). In the Ashkenazi Jewish population, the models did under-predict the number of mutations at the lower end of the mutation prevalence spectrum.
Most models are limited to first- and second-degree relatives; however, in clinical practice, family history characteristics of more distant relatives are important in evaluating a family. Overall, 80% of potentially eligible probands provided a sample; 86% of the non-Ashkenazi Jewish probands. The sampled Ashkenazi Jewish probands were representative of the potentially eligible Ashkenazi Jewish group. The nonsampled non-Ashkenazi Jewish probands did not differ in their estimated mutation prevalence, but did have fewer point mutations than the sampled group. However, including the nonsampled probands in the overall total did not significantly affect the rate of point mutations. As such, our clinic and the data presented herein are representative of patients seen at high-risk clinics across the United States.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References


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