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Screening for Genomic Rearrangements in Families with Breast and Ovarian Cancer Identifies *BRCA1* Mutations Previously Missed by Conformation-Sensitive Gel Electrophoresis or Sequencing

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The frequency of genomic rearrangements in *BRCA1* was assessed in 42 American families with breast and ovarian cancer who were seeking genetic testing and who were subsequently found to be negative for *BRCA1* and *BRCA2* coding-region mutations. An affected individual from each family was tested by PCR for the exon 13 duplication (Puget et al. 1999*a*) and by Southern blot analysis for novel genomic rearrangements. The exon 13 duplication was detected in one family, and four families had other genomic rearrangements. A total of 5 (11.9%) of the 42 families with breast/ovarian cancer who did not have *BRCA1* and *BRCA2* coding-region mutations had mutations in *BRCA1* that were missed by conformation-sensitive gel electrophoresis or sequencing. Four of five families with *BRCA1* genomic rearrangements included at least one individual with both breast and ovarian cancer; therefore, 4 (30.8%) of 13 families with a case of multiple primary breast and ovarian cancer had a genomic rearrangement in *BRCA1*. Families with genomic rearrangements had prior probabilities of having a BRCA1 mutation, ranging from 33% to 97% (mean 70%) (Couch et al. 1997). In contrast, in families without rearrangements, prior probabilities of having a *BRCA1* mutation ranged from 7% to 92% (mean 37%). Thus, the prior probability of detecting a *BRCA1* mutation may be a useful predictor when considering the use of Southern blot analysis for families with breast/ovarian cancer who do not have detectable coding-region mutations.

Introduction

Numerous studies have estimated the frequency of *BRCA1* (MIM 113705) and/or *BRCA2* (MIM 600185) coding-region mutations in familial breast and ovarian cancer (Narod et al. 1995; Couch et al. 1997; Ford et al. 1998; Moslehi et al. 2000). The results of a heterogeneity analysis based on linkage data from the Breast Cancer Linkage Consortium suggested that 88% of families with at least four cases of breast cancer diagnosed at age <60 years and with at least one case of ovarian cancer are attributable to *BRCA1* or *BRCA2*. In families with at least two cases of ovarian cancer and at least four cases of breast cancer, the percentage of families showing linkage to *BRCA1* and *BRCA2* increased to 100% (Ford et al. 1998). These data suggest that mutations in *BRCA1* and *BRCA2* account for the overwhelming majority of families with hereditary susceptibility to both breast and ovarian cancer. However, PCR-based mutation-detection assays, including direct sequencing, identified *BRCA1* mutations in only 63% of families showing linkage to *BRCA1*. No differences in sensitivity estimates were noted for sequencing, compared with other methods (Ford et al. 1998). It has been suggested that, in part, the discrepancy between linkage data, which predict *BRCA1* or *BRCA2* mutations in almost all carefully defined families with breast and ovarian cancer, and the actual number of mutations detected by PCR-based assays is because a significant proportion of mutations may not be identifiable by these methods.

The recent discovery of several genomic rearrangements within *BRCA1* and its regulatory regions provides evidence that mutations involving several kilobases of genomic sequence in *BRCA1* may account for at least some of this discrepancy (Petrij-Bosch et al. 1997; Puget et al. 1997, 1999*a*, 1999*b*; Swensen et al. 1997; Montagna et al. 1999; Rohlfs et al. 2000). In fact, the exon 13 duplication and the three *BRCA1* genomic rearrangements described by Petrij-Bosch and colleagues are significant founder mutations in United Kingdom and Dutch populations, respectively (Petrij-

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Bosch et al. 1997; The *BRCA1* Exon 13 Duplication Screening Group 2000). However, no studies have evaluated the frequency of genomic rearrangements in American families with breast/ovarian cancer who are seeking genetic testing; therefore, the extent to which these rearrangements are a source of false-negative test results in this setting is unknown.

Almost all of the well-characterized genomic rearrangements in *BRCA1* that have been described to date are thought to result from *Alu*-mediated recombination events that result in deletions, inversions, and duplications (Lehrman et al. 1985; Chen et al. 1989), consistent with data suggesting that *Alu* sequences are found at an increased frequency in *BRCA1* intronic sequences relative to the rest of the genome (Smith et al. 1996). Of the published *BRCA1* genomic rearrangements, three deletions have been described in the 5′ region of *BRCA1* encompassing exons 1 and 2 and upstream regulatory regions (Swensen et al. 1997; Puget et al. 1999*b*). Three different deletions that involve exon 13 and surrounding regions have also been described (Petrij-Bosch et al. 1997; Puget et al. 1999*b*). Two exon 17 deletions have been reported (Puget et al. 1997; Montagna et al. 1999), and other deletions have been found around exons 8, 15, and 22 of *BRCA1* (Petrij-Bosch et al. 1997; Puget et al. 1999*b;* Rohlfs et al. 2000).

Only one genomic rearrangement in *BRCA2,* in which a 5-kb region of the gene was deleted around exon 3, has been described (Nordling et al. 1998). Since *BRCA2* intronic sequence contains fewer *Alu* repeats than does *BRCA1,* it is presumed that genomic rearrangements involving *Alu*-mediated recombination events are less frequent. Peelen et al. (2000) recently reported the results of screening 81 Dutch families with breast and/or ovarian cancer for mutations in *BRCA2,* using Southern blot analysis, and they found no aberrant restriction patterns, providing additional evidence that *BRCA2* genomic rearrangements are infrequent, at least in the Dutch population.

To assess the frequency of *BRCA1* genomic rearrangements in a subset of families seeking genetic testing, 42 American families with breast and ovarian cancer were tested for rearrangements, by use of Southern blot analysis, and were screened for the exon 13 duplication, by use of PCR (Puget et al. 1999*a*). The 42 families had previously been fully analyzed for *BRCA1* and *BRCA2* coding-region mutations, by use of conformation-sensitive gel electrophoresis followed by direct sequencing of variant bands, and all were negative prior to being screened for genomic rearrangements (A.-M. Martin and K. L. Nathanson, unpublished data).

Families, Material, and Methods

Families

Families were ascertained from the Cancer Risk Evaluation Clinic at the University of Pennsylvania (1994–98) or the Breast Cancer Evaluation Clinic at the University of Michigan (1992–94). They were eligible for this study if they had at least one case of breast cancer and one case of ovarian cancer in the same lineage. All families sought evaluation for genetic susceptibility to cancer and provided consent for genetic analysis, although families ascertained before 1994 were aware that genetic testing would take place at an undetermined time in the future, since *BRCA1* and *BRCA2* had not yet been isolated. Families with cases of male breast cancer were included only if there were also a case of female breast cancer and at least one case of ovarian cancer in the same lineage. A DNA sample from at least one family member who was affected with breast and/or ovarian cancer and who was negative for coding-region and splice-site mutations in both *BRCA1* and *BRCA2* was used for Southern blotting and PCR-based genomic-rearrangement testing. When more than one sample was available for testing, the woman with the youngest age at diagnosis of breast cancer was selected. Three probands had breast/ovarian cancers in both the maternal and paternal lineages. A total of 42 families were available for analysis (see table 1).

Exon 13 Duplication Screening

Genomic DNA was isolated from peripheral blood lymphocytes or Epstein-Barr virus–immortalized lymphoblasts, by use of standard procedures. The primer set (Dup13F 5′-GAT TAT TTC CCC CCA GGC TA-3′ and Dup13R 5′-AGA TCA TTA GCA AGG ACC TGT G-3′), which was annealed at 58°C in 1.5 mM ($MgCl_2$), generated a product of ~1.1 kb in the presence of an

Table 1

Families Screened for Genomic Rearrangements

Group and Frequency of Mutation	No. of Families
American (42 families):	
Breast cancers/family:	
0	0
1–3	23
⩾4	19
Ovarian cancers/family:	
0	0
1	28
⩾2	14
Breast and ovarian cancer in one individual/family:	
0	29
⩾1	13

Table 2

PCR Primers for Southern Blot Probes

Exon and Primer Pair	Base Pair	Sequence	Product Size (bp)	Temperature (°C)
3:				
SouEx3F	12386	5′-GTG GAT ATG GGT GAA ACA GC-3′	1,863	58
SouEx3R	14228	5′-CCA GAA AAA TGT ACA TGG CC-3′		58
8:				
SouEx8F	28395	5′-GTT ATC AGA TGT GAT TGG AAT G-3′	768	55
SouEx8R	29142	5′-TCT TTT GCT CCC TTT TTA AA-3′		55
13:				
SouEx13F	46120	5′-ATT TCA TTT TCT TGG TGC CA-3′	1,645	58
SouEx13R	47744	5-′GGG AGA AAA AGG CTC AAA AC-3′		58
14:				
SouEx14F	51444	5′-CCT TCT TGT GCC ATT TCA TC-3′	1,084	58
SouEx14R	52507	5′-ACC ATC AGT TTC CAA GCT TG-3′		58
15:				
SouEx15F	53422	5′-AAA AGG CAG GCA ATA GGG AT-3′	1,211	60
SouEx15R	54632	5′-CCA AGA CTC CCT CAT CCT CA-3′		60
16:				
SouEx16F	57117	5′-AAT TAA TGG GTG AAG AGT ACT CC-3′	1,338	56
SouEx16R	58434	5′-ACA GGG GTG GTA AAC TTC TC-3′		56
17:				
SouEx17F	60380	5′-TTT ATG TCT GCT GAT GTG TAC A-3′	1,124	54
SouEx17R	61503	5′-AGA CTA TCA TCC ATG GTA TGC-3′		54
20:				
SouEx20F	71504	5′-CCT GAA TGC CTT TAA ATA TGA-3′	715	53
SouEx20R	72218	5′-TAA ATT TTA GCT ATT ATT GGC TG-3′		53
23–24:[a]				
SouEx23F	80966	5′-TGA TGA AGT GAC AGT TCC AG-3′	2,209	55

NOTE.—PCR amplification was performed with primers described elsewhere (Friedman et al. 1994), for exons 1–2 (1,522 bp), 5–7 (2,508 bp), 9–10 (1,618 bp), 11–12 (1,657 bp), 18–19 (799 bp), and 21–22 (2,220 bp). In all cases, the forward primer from the first exon was paired with the reverse primer from the second exon.

[a] Data for the 24R primer have been published elsewhere (Friedman et al. 1994).

exon 13 duplication. Because no product is obtained from a normal allele, an internal PCR control amplifying a region of *BRCA1* exon 11 was performed on all genomic DNA before the exon 13 duplication screen. The primers used for exon 11 of *BRCA1* were as follows: 11F 5′-GGG AAA ACC TAT CGG AAG AA-3′ and 11R 5′-AGC CCA CTT CAT TAG TAC TGG AAC-3′. When annealed at 55°C, they generated a 1.7-kb PCR product.

Southern Blot Analysis

Southern blotting was performed using a modified version of the protocol described by Puget et al. (1999*b*). In brief, 10 μg genomic DNA were digested with ~30 units of *Eco*RI, *Hin*dIII, or *Xba*1 for 5 h. Digestions were run overnight on a 25-cm agarose gel (0.8%) at 60 V in 1 × Tris-borate EDTA buffer (Sambrook et al. 1989, pp. 7.01–7.87). Gels were denatured in 0.25 N HCl for 15 min and in 0.5 N NaOH/1.5 N NaCl for 20 min with gentle shaking. Overnight, DNA was transferred to nylon membranes (Hybond N^+ [Amersham]) in the alkali buffer.

To reduce exposure time, a series of longer probes amplified from genomic DNA was designed over several of the exons of *BRCA1* (table 2). These probes were pooled, in the same manner, into one of three hybridization mixtures, and they produced the same banding pattern described by Puget et al. (1999*b*), with the exception of two additional fragments (2.9 kb each) that were detected in introns 3 and 13 when the new probes were used. With the longer probes, the exposure time of the filters was reduced from ~2 wk to 24–48 h.

DNA-containing membranes were prehybridized for 2 h in Church and Gilbert buffer (Church and Gilbert 1984) with denatured human placental DNA (Sigma), 5 mg/ml, at 65°C. Approximately 50 ng of an equimolar mixture of probes from exons 3–10 and 15–19, exons 11–14 and 20–24, or exons 1–2 were labeled with use of the Random Primed DNA Labeling Kit (Roche) and were prehybridized with human placental DNA, 0.1 mg/ml, for 1 h at 65°C. Each labeled probe mixture was

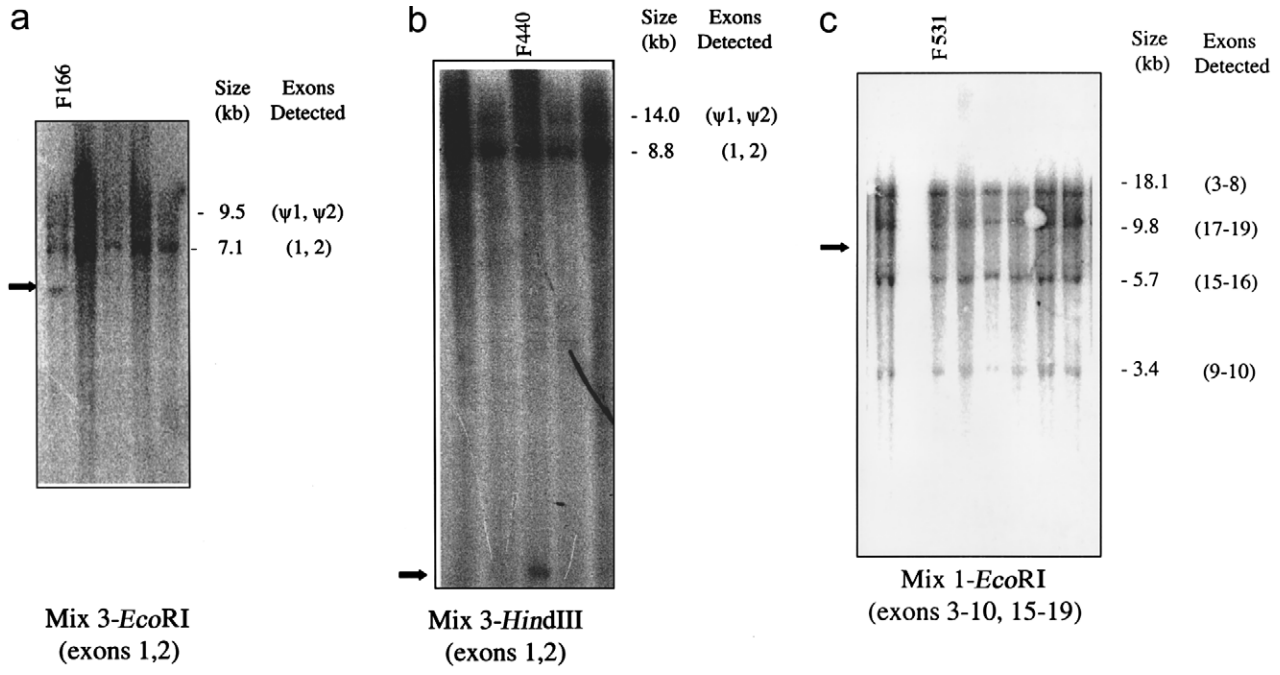

Figure 1 Results of Southern blot analysis, for families 166 (F166), 440 (F440), and 531 (F531)

added to the prehybridization buffer and filter and was hybridized overnight at 65°C.

Filters were rinsed two times in 2 × SSC, 0.1% SDS, and then were washed one time in 1 × SSC, 0.1% SDS, for 15 min at room temperature. The stringency of the washing conditions increased to one wash in 0.2 × SSC, 0.1% SDS, at 65°C for 20 min and one final wash at 0.1 × SSC, 0.1% SDS, at 65°C, if necessary. Filters were exposed to a phosphoimager screen (Molecular Dynamics) for 24–48 h and were analyzed using ImageQuant Software. To hybridize each filter with three separate probe mixtures, filters were stripped of probe by the addition of boiling 0.1 × SSC, 0.1% SDS, for 20 min at room temperature with shaking.

Results

Exon 13 Duplication

Prior to Southern blot analysis, genomic DNA from an affected member from each family was analyzed for the 6-kb duplication encompassing exon 13 of *BRCA1* (Puget et al. 1999*a*). An exon 13 duplication was detected in 1/42 families (data not shown). This family is of English origin, which is consistent with the observation that the exon 13 duplication may be a founder mutation from the Yorkshire region of the United Kingdom (The *BRCA1* Exon 13 Duplication Screening Group 2000).

Southern Blot Analysis

Of the remaining 41 DNA samples that were tested by Southern blot analysis, 4 showed evidence of genomic rearrangements in *BRCA1*. In families 166 and 440, Southern blot analysis showed extra bands in the *Eco*RI and *Hin*dIII digests, respectively, when hybridized with mix 3 probes (exons 1 and 2 of *BRCA1*). In family 166, the additional band was accompanied by a reduction in band intensity corresponding to the *BRCA1* pseudogene and the upstream regulatory region of *BRCA1* on *Eco*RI digestion (fig. 1*a*). In family 440, the extra band was accompanied by a reduction in the band intensity corresponding to exons 1 and 2 of *BRCA1* on *Hin*dIII digestion (fig. 1*b*). The specific breakpoints of the rearrangements in families 166 and 440 are currently being mapped.

An additional rearrangement encompassing exons 17–19 of *BRCA1* was detected in family 531, by use of Southern blotting. Hybridization of mix 1 (exons 3–10 and 15–19) detected, in an *Eco*RI digest, an additional band below the normal band of 9.8 kb (exons 17–19) but above the 5.7-kb band (exons 15–16) (fig. 1*c*). A corresponding reduction in the intensity of the 9.8-kb band in the *Eco*RI digest of this sample was seen. In addition, the *Hin*dIII digest of this sample also provided evidence of a rearrangement after hybridization with mix 1. In the latter case, a reduction in the intensity of the 19.0-kb band (exons 15–19) was detected (data not shown), but no extra bands were detected. The specific

breakpoints of this rearrangement are also currently being mapped.

The final family (family 30), of those in this sample set, to have a genomic rearrangement detected has also been described elsewhere (Rohlfs et al. 2000), since this mutation was detected simultaneously using protein truncation testing (PTT). This family has a 7.1-kb deletion of *BRCA1* flanking exons 8–9. The location of genomic rearrangements detected in this study is summarized in figure 2.

In total, *BRCA1* genomic rearrangements were detected in 5/42 families. Three of the five families had six or more cases of breast cancer and two or more cases of ovarian cancer. Four of the five families included at least one individual with both breast cancer and ovarian cancer (table 3). Completion of the screen for *BRCA1* genomic rearrangements led to the identification of 37 families who tested negative for coding-region, splice-site, and genomic-rearrangement mutations in *BRCA1* and for coding-region and splice-site mutations in *BRCA2*. The "negative" cohort included six (16.2%) families with six or more cases of breast cancer, 11 (29.7%) families with two or more cases of ovarian cancer, and 9 (24.3%) families with at least one individual with both breast cancer and ovarian cancer. Table 4 lists the probability of detecting a *BRCA1* mutation by use of the Couch model (Couch et al. 1997), for all the families tested in the present study.

Discussion

Germline genomic rearrangements have been found to be a cause of disease-associated germline mutations in a variety of human genetic disorders, including several cancer-susceptibility syndromes. In one recent study (Stolle et al. 1998), genomic deletions in the *VHL* gene, which is associated with von Hippel-Lindau disease, were detected in 23 (25%) of 93 carriers of the *VHL* mutation. Deletions of the entire gene were found in 8 (9%) of the 93 mutation carriers, for a combined frequency of 34% for partial and complete genomic deletions (Stolle et al. 1998). Genomic rearrangements also occur in the *APC* gene in association with familial adenomatous polyposis, albeit at a frequency of <5% (van der Luijt et al. 1997; Giarola et al. 1999). As commercial testing for germline mutations in cancer-susceptibility genes becomes a more routine part of medical practice and as effective interventions are developed, genomic rearrangements need to be carefully considered as a cause of false-negative results following PCR-based analysis, including sequencing. However, the frequency of genomic rearrangements in American families with breast/ovarian cancer who are seeking genetic testing for *BRCA1* and *BRCA2* mutations has not been determined.

Two previous studies (Petrij-Bosch et al. 1997; Puget et al. 1999*b*) have screened families for *BRCA1* genomic rearrangements, by means of Southern blot analysis. The first study (Petrij-Bosch et al. 1997), in which a clinically relevant series of Dutch families seeking genetic testing was evaluated, provided evidence that ⩽36% of *BRCA1* mutations could be missed by failure to screen for genomic rearrangements. In the second study, Puget et al. (1999*b*) screened a mixed American/French population and noted a strong founder effect caused by the exon 13 duplication. The American families included in the series screened by Puget et al. were selected from a group of families ascertained primarily for linkage analysis. Thus, the data from this cohort may not provide the information necessary to evaluate the frequency of genomic rearrangements in a more clinically relevant population of women seeking genetic evaluation in the United States. It is also important to know whether a few genomic rearrangements occur repeatedly (as in the Dutch population) or whether many unique rearrangements can be detected (as would be expected in our more heterogeneous population). Finally, it is important to identify individuals who might best be targeted for further analysis after coding-region/splice-site analysis fails to identify a mutation. Therefore, to our knowledge, this is the first study to systematically evaluate the frequency of *BRCA1* genomic

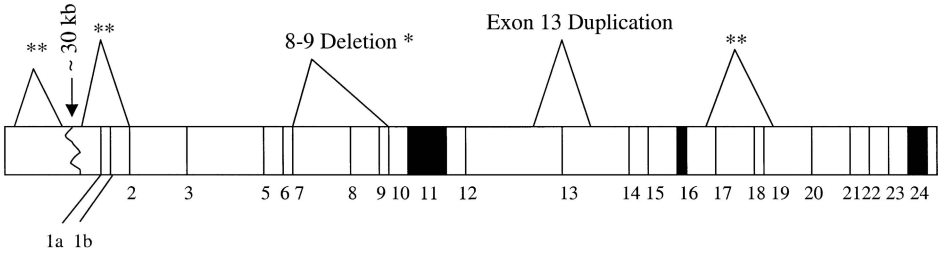

Figure 2 Summary of *BRCA1* genomic rearrangements detected in the present study. A single asterisk (*) indicates screening and mapping of this rearrangement was done as described elsewhere (Rohlfs et al. 2000). A double asterisk (**) denotes genomic rearrangements detected in the present study.

Table 3

Description of Families with Genomic Rearrangements

Family (Genomic Rearrangement)	No. of Cancers (Median Age at Diagnosis [years])					Ethnicity[a]
	Breast	Ovarian	Multiple Primary Breast/ Ovarian	Bilateral	Other	
30[b] (del exons 8–9)	10 (33)	3	2	3	0	Northern European
166 (exons 1–2)	3 (43)	1	1	1	0	Polish
440 (exons 1 and 2)	6 (50)	2	1	1	2[c]	Western European
531 (exons 17–19)	5 (42)	1	0	0	4[d]	Western European
771[e] (dup exon 13)	4 (41)	3	1	0	2[f]	English

[a] Of lineage most likely to carry the mutation.
[b] Described by Rohlfs et al. (2000).
[c] Melanoma and pancreatic.
[d] Endometrial (2), leukemia, and melanoma.
[e] Described by the *BRCA1* Exon 13 Duplication Screening Group (2000).
[f] Colon and cervical.

rearrangements in a cohort of American women from families with breast/ovarian cancer who are seeking genetic testing and who are negative for *BRCA1* and *BRCA2* coding-region mutations. Additional studies are needed, but this study provides a starting point for continued investigation.

In the present study, we analyzed a series of families with breast/ovarian cancer who were seeking genetic testing and who had no detectable coding-region mutations in *BRCA1* or *BRCA2,* and we found genomic rearrangements in 5 (11.9%) of 42 families. The novel genomic rearrangement in family 166 is upstream of the *BRCA1* coding region and appears to involve the promoter as well as the *BRCA1* pseudogene. The duplicated pseudogene region of *BRCA1* is flanked by *Alu* sequences (Brown et al. 1996); therefore, an *Alu*-mediated recombination event between the *BRCA1* pseudogene region and an *Alu* sequence in the 5′ region of *BRCA1* may have generated a deletion of critical binding sites in the *BRCA1* promoter. The genomic rearrangements detected in families 440 and 531 are large deletions of genomic sequence around exons 1–2 and exons 17–19, respectively; these rearrangements possibly remove important functional domains and/or create frameshift mutations. Additional mapping studies of these three novel rearrangements are under way. The rearrangement detected in family 771 is the previously described exon 13 duplication, and the rearrangement (a deletion of exons 8–9) in family 30 has been described by Rohlfs et al. (2000).

This study was designed to address two questions. The first question is whether there is evidence for additional susceptibility genes in families who have multiple cases of breast and ovarian cancer but who do not have detectable *BRCA1* or *BRCA2* coding regions, or whether such cancers in these families are attributable to genomic rearrangements in *BRCA1* that are missed by PCR-based mutation analysis. The second question is which families are most likely to require additional analyses in the setting of a negative *BRCA1/2* coding-region analysis, to maximize sensitivity.

In addressing the question of whether genomic rearrangements in *BRCA1* can be detected in the majority of families with breast/ovarian cancer who do not have coding-region mutations, we detected rearrangements in only 5/42 families. Thus, in our series, the results of Southern blot analysis for genomic rearrangements were negative in 37/42 families. In the present study, all of the families that are large enough to be informative are consistent with autosomal dominant transmission of breast cancer susceptibility; therefore, these data suggest that other susceptibility genes or other as-yet-undetected mutations in *BRCA1* remain to be discovered in at least some of these families. Because linkage analysis is not possible in the majority of these families, as a result of limited numbers of living affected individuals, we cannot distinguish between these possibilities in this cohort.

All relevant studies to date suggest that a discrepancy remains between the estimated 81% of families with breast/ovarian cancer caused by *BRCA1* mutations, as predicted by linkage analysis (Ford et al. 1998), and the mutation frequency estimates of 37%–71% in similar families, even when including genomic rearrangements (table 5). Differences in family ascertainment may account for some of the variability seen in *BRCA1* mutation frequency; however, there remains a proportion of families with breast/ovarian cancer who show evidence of linkage to *BRCA1* in which mutations have not been found.

Families without identifiable *BRCA1* or *BRCA2* mutations after full analysis for coding-region mutations and genomic rearrangements may still harbor *BRCA1*

Table 4

Prior Probability of Detecting a *BRCA1* Mutation in Screened Families (Couch et al. 1997)

Family No.	Predicted Probability (%)	Ethnicity[a]	Genomic Rearrangement[b]
30	96.6	W	+
28	92.4	W	−
771	88.5	W	+
685	85.3	AJ	−
236	78.5	AJ	−
807	77.1	AJ	−
440	75.4	W	+
863	67.9	AJ	−
1,112	67.9	AJ	−
933	65.9	W	−
166	57.1	W	+
435	57.1	W	−
96	55.0	W	−
62	54.9	W	−
299	54.9	W	−
1,062	47.8	AJ	−
497	45.7	AJ	−
199	43.5	W	−
592	43.5	W	−
457	34.7	AJ	−
85	32.7	W	−
487	32.7	W	−
531	32.7	W	+
107	23.4	W	−
142	23.4	W	−
192	23.4	W	−
230	23.4	W	−
686	23.4	W	−
792	23.4	W	−
1,053	23.4	W	−
165	16.2	W	−
206	16.2	W	−
232	16.2	W	−
360	16.2	W	−
372	16.2	W	−
545	16.2	W	−
651	16.2	W	−
217	10.8	W	−
340	7.1	W	−
341	7.1	W	−
445	7.1	W	−
536	1.3	W	−

[a] AJ = Ashkenazi Jewish; W = white.

[b] A plus sign (+) denotes a positive result; a minus sign (−) denotes a negative result.

mutations. Mutations may be missed by Southern blotting if the aberrant banding pattern is not discernible by separation on an agarose gel. To date, the smallest rearrangement in *BRCA1* detected to date by Southern blotting is 1 kb (Puget et al. 1997), and smaller rearrangements certainly may occur. In addition, it is possible that, although a portion of genomic rearrangements are large enough to be detected by Southern blot analysis, they cannot be identified by use of this technique because of the nature of the rearrangement. An example is the *BRCA1* exon 13 duplication. The first family identified with this mutation was initially tested by Southern blot analysis and was thought to be negative for genomic rearrangements. Subsequent use of PCR-based screening of the mutant cDNA led to the discovery and mapping of the duplication (Puget et al. 1999*a*). A recently described technique (Yan et al. 2000) for converting cells to haploidy for genetic analysis may be a useful tool for mutation detection in some families, because it isolates the mutant allele from the wild type so that the absence of a PCR product is not obscured by a wild-type product.

The answer to the question of which families are most likely to require Southern blotting after a negative *BRCA1/2* coding-region analysis may be derived from a description of the families with rearrangements. The sample set used for this study consisted of families seeking genetic analysis and is representative of the families with breast/ovarian cancer who we continue to see in our risk evaluation clinic, with respect to numbers of affected individuals and the range of age at diagnosis, suggesting that it is a valid source from which to begin formulation of clinical recommendations. The five families with rearrangements had a range of 1–10 breast cancers; three of the five families had two or more cases of ovarian cancer, and four of the five families had at least one individual with multiple primary breast/ovarian cancer. Overall, in the present study, 4 (30%) of the 13 families who had a case of multiple primary breast/ovarian cancer had a detectable genomic rearrangement. Families with two or more cases of ovarian cancer and/or at least one case of multiple primary breast/ovarian cancer are relatively uncommon, even in a referral clinic, and these features are strong predictors for the presence of *BRCA1* coding-region mutations. The families without rearrangements had a range of 1–8 cases of breast cancer; 26/37 families had only one case of ovarian cancer, and 9/37 families had an individual with both breast cancer and ovarian cancer (table 3). These data support the hypothesis that the higher the prior probability of finding a *BRCA1* mutation in a given family, the more likely that Southern blot analysis will provide clinically relevant information.

Indeed, in this study, the prior probabilities of having a *BRCA1* mutation were between 33% and 97% (mean 70%) for the five families with genomic rearrangements (table 4) (Couch et al. 1997). In the 37 families without detectable genomic rearrangements, the mean prior probability of having a *BRCA1* mutation was 37% (range 7%–92%). The mean prior probability in families with genomic rearrangements corresponds either to a non-Ashkenazi Jewish family with at least one case of multiple primary breast/ovarian cancer and an average age at diagnosis of 35–39 years or to an Ashkenazi

Table 5

Comparison of *BRCA1* and *BRCA2* Mutation Frequencies in Families with Breast and Ovarian Cancer

No. of Ovarian Cases for Ascertainment and Source	No. (%) of Families					Geographic Source
	Positive for		Negative for	With *BRCA1* Genomic		
	BRCA1	*BRCA2*	*BRCA1/2*	Rearrangements	Total	
>1:						
Ford et al. (1998)	76 (80.9%)	13 (13.8%)	5 (5.3%)	Not tested	94	North America and western Europe
Moslehi et al. (2000)	25 (50.0%)	8 (16%)	17 (34.0%)	Not tested	50	North America and Israel
Levy-Lahad et al. (1997)[a]	7 (36.8%)	5 (26.4%)	7 (36.8%)	Not tested	19	Israel
O. Sinilnikova and S. Mazoyer (unpublished data)	29 (60.4%)	3 (6.3%)	11 (22.9%)	5 (10.4%)	48	United Kingdom
Martin et al. (unpublished data)	55 (51.9%)	7 (6.6%)	39 (36.8%)[b]	5 (4.7%)	106	United States
≥2:						
Gayther et al. (1999)	32 (51.6%)	6 (9.6%)	24 (38.8%)	Not tested	62	United States

[a] This study only measured the frequency of Ashkenazi Jewish founder mutations.

[b] The families that tested negative for *BRCA1* and *BRCA2* mutations in the study by Martin et al. (unpublished data) were the families that were tested for *BRCA1* genomic rearrangements in the present study (see the Families, Material, and Methods section).

Jewish family with at least one breast cancer, one ovarian cancer, and a mean age at diagnosis of <50 years. In the families without rearrangements, the mean prior probability (37%) of having a *BRCA1* mutation corresponds either to a non-Ashkenazi Jewish family with at least one case of breast cancer, one case of ovarian cancer, and a mean age at diagnosis of <50 years or to an Ashkenazi Jewish family with at least one case of breast cancer, one case of ovarian cancer, and a mean age at diagnosis of >60 years. Whereas the present study is limited by a small sample size and was not designed to provide a comprehensive survey of the frequency and type of *BRCA1* rearrangements, the data do suggest that, in families with a high likelihood of having *BRCA1* mutations, genomic rearrangements represent a source of false-negative test results. Again, bearing in mind the small sample set, false-negative results from PCR-based methodologies may be as high as 30% in American families with at least one case of multiple primary breast/ovarian cancer. Taken together, these data suggest that testing for genomic rearrangements might be considered in families with a prior probability of ⩾30% of having a *BRCA1* mutation.

On the basis of the data in the present study, comparison of the relative frequency of *BRCA1* and *BRCA2* coding-region mutations and genomic rearrangements in similar populations suggests that the frequency of *BRCA1* genomic rearrangements in families with breast/ovarian cancer is equal to or greater than the frequency of *BRCA2* coding-region mutations (table 5). In support of this observation, data from a study (O. Sinilnikova and S. Mazoyer, unpublished data) of 48 breast and ovarian cancer families with one or more cases of ovarian cancer show *BRCA1* genomic rearrangements in 10% of families, whereas *BRCA2* coding and splice-site mutations were detected in 6%. A third study provides additional data on the frequency of *BRCA2* coding-region and splice-site mutations in families in the United Kingdom who have two or more cases of ovarian cancer and a history of breast cancer; mutations were found in 10% of these families (Gayther et al. 1999). Although a genomic-rearrangement screen has not yet been performed on the cohort from the United Kingdom, the *BRCA2* mutation frequency is consistent with results of previous studies of non-Ashkenazi Jewish families. Thus, data from several sources suggest that analysis for *BRCA1* genomic rearrangements in families with breast/ovarian cancer may yield as many mutations as does *BRCA2* coding-region screening in non-Ashkenazi Jewish families with breast/ovarian cancer, a current standard of practice.

In summary, this study provides evidence that genomic rearrangements are a source of false-negative test results in a clinically relevant population of American families with breast/ovarian cancer. When the mutation frequency from coding-region analysis as well as genomic-rearrangement screening in *BRCA1* are combined, they approach but do not fully account for linkage-based estimates of the proportion of families with breast/ovarian cancer caused by germline mutations in *BRCA1*. To maximize sensitivity, genomic-rearrangement analyses, including Southern blotting, PTT, and other analyses, may need to be incorporated into mutation testing for families with breast/ovarian cancer who have high predicted probabilities of having a *BRCA1* mutations. A reasonable guide may be to consider Southern blot analysis for those families who have a predicted probability of ⩾30% of having a *BRCA1* mutation and who are found to be negative for *BRCA1* and *BRCA2* mutations by use of PCR-based methods

but to recognize that additional studies must be performed to validate this suggestion.

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Electronic-Database Information

Accession numbers and the URL for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for *BRCA1* [MIM 113705], for *BRCA2* [MIM 600185])

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