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Genetic Heterogeneity in Hereditary Breast Cancer: Role of *BRCA1* and *BRCA2*

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Summary

The common hereditary forms of breast cancer have been largely attributed to the inheritance of mutations in the *BRCA1* or *BRCA2* genes. However, it is not yet clear what proportion of hereditary breast cancer is explained by *BRCA1* and *BRCA2* or by some other unidentified susceptibility gene(s). We describe the proportion of hereditary breast cancer explained by *BRCA1* or *BRCA2* in a sample of North American hereditary breast cancers and assess the evidence for additional susceptibility genes that may confer hereditary breast or ovarian cancer risk. Twenty-three families were identified through two high-risk breast cancer research programs. Genetic analysis was undertaken to establish linkage between the breast or ovarian cancer cases and markers on chromosomes 17q (*BRCA1*) and 13q (*BRCA2*). Mutation analysis in the *BRCA1* and *BRCA2* genes was also undertaken in all families. The pattern of hereditary cancer in 14 (61%) of the 23 families studied was attributed to *BRCA1* by a combination of linkage and mutation analyses. No families were attributed to *BRCA2*. Five families (22%) provided evidence against linkage to both *BRCA1* and *BRCA2*. No *BRCA1* or *BRCA2* mutations were detected in these five families. The *BRCA1* or *BRCA2* status of four families (17%) could not be determined. *BRCA1* and *BRCA2* probably explain the majority of hereditary breast cancer that exists in the North American population. However, one or more additional genes may yet be found that explain some proportion of hereditary breast cancer.

Introduction

Several genes have been identified that play a role in the occurrence of hereditary breast cancer. The recently identified *BRCA1* and *BRCA2* genes appear to account for the majority of hereditary breast cancer in the general U.S. and European populations. Of families with a pattern of breast cancer consistent with hereditary breast cancer, it has been reported that ~50% may be attributed to *BRCA1* (Easton et al. 1993) and 35% may be attributed to *BRCA2* (Wooster et al. 1994). The remaining 15% of hereditary breast cancer could be attributed to a gene or genes that have yet to be identified. The majority of hereditary breast cancer with ovarian cancer is explained by *BRCA1* (Narod et al. 1995). However, it remains unclear whether *BRCA1* or *BRCA2* explains all cases of hereditary breast cancer (Spurr et al. 1995) or additional high-penetrance breast cancer susceptibility genes remain to be identified. A number of rare syndromes, including Li-Fraumeni syndrome, Cowden disease, Muir-Torre syndrome, and Peutz-Jeghers syndrome, account for <1% of all hereditary breast cancer (Hoskins et al. 1995).

The objective of this study is to summarize the experience of two North American hereditary breast cancer populations with respect to the proportion of hereditary breast cancer explained by *BRCA1* or *BRCA2*. Of the families who were ascertained through these clinics between 1991 and 1994, 23 were amenable to genetic linkage analysis. We present an analysis of this sample to estimate the proportion of families that can be explained by *BRCA1* and *BRCA2* and to compare the results of linkage analysis with subsequent mutation analysis in the *BRCA1* and *BRCA2* genes.

Subjects and Methods

Sample Subjects

The families that comprise the sample subjects for the present study were ascertained through female probands as part of hereditary breast cancer research studies at the University of Michigan (UM) and the Dana-Farber Cancer Institute (DFCI) between 1991 and 1994. These probands were either self- or physician-referred because of a family history of breast and/or ovarian cancer. At the time of their accrual to this study, complete family information was obtained from each of these individuals. A record of all cancers that occurred in the family

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was obtained for at least first- and second-degree relatives of the proband. Cancer occurrences were confirmed by obtaining medical records and pathology reports for all available family members whether living or deceased.

The criteria for inclusion in the present sample subset were as follows. Eligible families had at least two breast cancer cases and, in addition, had at least one of the following: one breast cancer diagnosis before age 45 years, one additional ovarian cancer case, or one additional case of bilateral breast cancer. Families included in this study were required to have at least four affected individuals from whom constitutional DNA samples could be obtained (i.e., the availability of archival tumor material only was not sufficient for an individual to be included among these four affected individuals). In addition, at least one sampled family member in each of two generations (affected or unaffected) was required. Peripheral blood samples were drawn on each available adult member of this sample subset after informed consent was obtained. Paraffin-embedded tumor and normal tissue blocks for deceased affected family members were also requested whenever possible. Using these criteria, we identified 23 multigeneration families that were amenable to linkage analysis.

Genetic Markers and Mutation Analysis

The marker loci considered in the linkage analyses are as follows. Four loci were considered in the chromosome 17q linkage analyses presented here: *D17S250* (*mfd15*), *D17S579* (*mfd188*), *D17S588* (*42D6*), and *D17S409* (*LL154*). A summary description of these loci is presented by Easton et al. (1993). Four loci were considered in the chromosome 13q linkage analyses presented here: *D13S289, D13S260, D13S171,* and *D13S267* (summarized by Wooster et al. 1994). The marker allele frequencies used in these analyses have been previously reported in the Genome Data Base (GDB 1995).

The detection of mutations in the *BRCA1* and *BRCA2* genes was accomplished by using a combination of SSCP analysis, allele-specific oligonucleotide hybridization, conformation-sensitive gel electrophoresis, and direct sequencing as reported elsewhere (Ganguly et al. 1993; Castilla et al. 1995; Couch et al. 1996). All mutations were confirmed by direct sequencing of the appropriate PCR product. The individual initially selected for mutation testing was required to have had an early-onset breast or ovarian cancer and be in a direct line of descent from an affected (or "obligate carrier") parent. Direct mutation analysis in the *BRCA1* gene was undertaken initially. Direct mutation analysis in the *BRCA2* gene was undertaken in those families in which no *BRCA1* mutation was identified, regardless of the results of linkage analysis. Additional family members were tested in some families to confirm the presence or absence of mutations. A synopsis of the number of individuals tested in each family is presented in table 1. Individuals were given the opportunity to learn about the results of their genetic tests within the context of an established research protocol that included pre- and posttest genetic counseling.

Statistical Methods

Maximum-likelihood linkage analyses were undertaken to compute both multipoint and two-point LOD scores using genetic markers on chromosomes 17q (*BRCA1*) and 13q (*BRCA2*). Multipoint LOD scores on chromosomes 17q and 13q were based on the multipoint maps *Thra1-BRCA1-D17S579* and *D13S260-BRCA2-D13S267,* respectively. These markers were chosen for the multipoint analysis because they were the most consistently measured marker genotypes measured in this set of families. Two-point LOD scores were computed for all markers separately. Analyses were undertaken for both *BRCA1* and *BRCA2* by using a model of hereditary breast cancer that assumed breast or ovarian cancer was attributable by a single autosomal dominant gene with a disease allele frequency of .0033 (Easton et al. 1993). An age-specific penetrance function was used to relate the probability of having developed breast or ovarian cancer to inheritance of the genetic variant. This function assigned separate age-specific probabilities of developing breast or ovarian cancer as specified by Easton et al. (1993). All likelihood computations were accomplished using the program packages MENDEL (version 2.3; Lange et al. 1987) and LINKAGE (version 5.1; Lathrop et al. 1984).

The statistical power of this sample of families to detect linkage was estimated by using the method of Ploughman and Boehnke (1989) to compute expected maximum LOD scores (ELODs) in view of the available genealogical information in each family on the assumption that only sampled individuals had genotypes available for analysis. These computations assumed that highly informative genetic markers were available and that no untyped loci would exist due to laboratory errors or assay failures. All ELOD values were computed using the programs SLINK (version 2.0; Weeks et al. 1990) and LODSTAT (version 3.0; Ploughman and Boehnke 1989).

Results

Of the 23 families studied here, 12 (52%) were ascertained through UM and 11 (48%) through DFCI. The mean number of individuals sampled per family was 15.6, ranging from 7 to 29 individuals. Twenty-two of the 23 families were Caucasian, and 1 family was African-American. Only breast cancers were observed in 13 families (57%). The remaining families contained

Table 1

Description of Families Used in Genetic Analyses

Family	No. of Relatives Sampled[a]	Total No. Affected[b]	No. of Sampled Affecteds[c]			Mean Age at Cancer Onset[d] (years)	ELOD
			BR	BO	OV		
15	20 (20)	10	8	2	0	41.2	.84
16	12 (1)	4	4	0	0	48.2	.26
17	18 (1)	9	4	0	0	40.2	.86
27	23 (15)	17	7	1	3	34.1	2.24
28	12 (1)	8	5	1	1	40.4	.93
30	12 (1)	5	3	2	0	33.5	1.50
33	8 (1)	10	5	0	0	45.0	.65
34	18 (6)	17	3	4	0	46.9	2.62
36	17 (1)	10	8	0	0	42.7	.91
46	29 (6)	18	4	1	1	44.0	1.41
61	7 (1)	4	4	0	0	38.6	.20
77	18 (1)	14	5	0	0	40.5	1.42
78	8 (1)	8	6	0	0	53.0	1.23
100	11 (6)	6	4	0	1	51.2	.99
129	18 (1)	7	5	0	0	44.2	1.33
130	12 (2)	6	3	1	0	43.4	.62
139	18 (1)	8	5	0	0	41.8	1.30
178	14 (3)	8	5	1	0	38.4	.75
202	11 (2)	24	3	1	0	40.6	2.86
224	13 (1)	11	7	0	0	37.6	.79
230	21 (1)	8	4	0	0	42.3	1.04
383	14 (1)	8	5	0	0	40.0	.83
417	25 (1)	10	5	0	0	39.0	2.07

[a] Number for whom a biosample was available and who were genotyped for linkage analysis. In parentheses is the number of affected subjects who underwent direct mutation testing at *BRCA1* and *BRCA2*.

[b] Total number of family members confirmed to be affected with breast and/or ovarian cancer in collected pedigree (whether sampled or not).

[c] Number sampled with cancer (BR = including unilateral and bilateral breast cancer; BO = breast and ovarian cancer; and OV = ovarian cancer.

[d] Mean age at onset of all individuals in the family affected with breast and/or ovarian cancer (whether sampled or not).

women affected with breast and/or ovarian cancers. No male breast cancers were observed in any family. The mean number of sampled cancers per family was 5.4. The mean age of breast and/or ovarian cancer onset per family ranged from 33.5 to 53.0 years. Twenty-one families (91%) had a mean age at breast or ovarian cancer onset of <50 years. Six families (26%) had a mean age at onset of <40.

The results of linkage analyses using markers on chromosomes 17q and 13q are presented in tables 2 and 3. Nine of the families studied here (39%) provided evidence favoring linkage to chromosome 17q with LOD-score values >1 (table 2). Six these nine families have documented mutations in the *BRCA1* gene. The LOD scores in three additional families ranged from 0.5 to 1.0 and were judged to provide evidence suggestive of linkage to 17q. Two of these three families (202 and 417) have been found to carry *BRCA1* mutations. Two additional families (61 and 100) did not support linkage to chromosome 17q, but *BRCA1* frameshift mutations were later detected in these families. Therefore, the pattern of hereditary breast and ovarian cancer could be attributed to genotypes at the *BRCA1* locus in 14 (61%) of the 23 families. The proportion of *BRCA1* families reported here is consistent with previous reports that suggest approximately half of all hereditary breast or breast/ovarian families can be explained by *BRCA1*.

Linkage analyses for chromosome 13q markers were undertaken for those families that lacked evidence of linkage to the *BRCA1* locus or did not have a documented *BRCA1* mutation at the time of this analysis (table 3). Only one family (61) provided weak evidence favoring linkage to chromosome 13q with a maximum multipoint LOD score of .47 at *D13S260* (table 3). However, a frameshift mutation in *BRCA1* was later detected in this family. Mutations in the *BRCA2* gene were not detected in any of these families. Therefore, there is no evidence that any of the families in our sample can be explained by genotypes at the *BRCA2* locus. This result contrasts with linkage studies of family subsets

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Table 2

Results of Maximum-Likelihood Linkage and Mutation Analysis for *BRCA1*

Family	Mutation[a]	LOD Score	
		Multipoint[b]	Two-Point[c]
15	F	1.62	1.58 (*D17S250*)
16	–	–.22	–.44 (*D17S579*)
17	–	–.98	–1.00 (*D17S579*)
27	F	2.47	2.47 (*D17S579*)
28	–	.85	1.59 (*D17S250*)
30	–	1.68	1.63 (*D17S579*)
33	–	.25	.41 (*D17S579*)
34	N	2.40	1.67 (*D17S579*)
36	–	–1.50	–1.42 (*D17S579*)
46	F	1.67	2.03 (*D17S579*)
61	F	–.33	–.54 (*D17S250*)
77	–	2.09	1.62 (*D17S579*)
78	–	–1.58	–1.72 (*D17S588*)
100	F	–.29	–1.04 (*D17S409*)
129	–	–.19	–.45 (*D17S409*)
130	F	1.18	1.18 (*D17S579*)
139	–	.73	.83 (*D17S579*)
178	M	1.70	1.43 (*D17S579*)
202	F	.63	.71 (*D17S579*)
224	–	–.17	–1.08 (*D17S409*)
230	–	.02	.27 (*D17S409*)
383	–	–1.26	–1.24 (*D17S250*)
417	M	.94	.96 (*D17S579*)

[a] Indicates a frameshift (F), missense (M), nonsense (N), or no (–) mutation in the *BRCA1* gene.

[b] Multipoint LOD scores computed for *Thra1-BRCA1-D17S579*.

[c] "Maximal" value of two-point LOD scores for the indicated marker.

that suggest that *BRCA2* may explain as many as 35% of non-*BRCA1* hereditary breast cancer families (Wooster et al. 1994; Spurr et al. 1995).

In four families (16, 33, 129, and 230), there was insufficient evidence to determine linkage at 17q or 13q (tables 2 and 3). This was due to uninformative marker typings and limited pedigree information. Two of these families (16 and 129) had slightly negative LOD scores at both chromosomes 17q and 13q and did not have *BRCA1* mutations. The other two families (33 and 230) have weakly positive LOD scores at 17q, weakly negative LOD scores at 13q, and no *BRCA1* or *BRCA2* mutations. In five families (17, 36, 78, 224, and 383), there was evidence against linkage at both *BRCA1* and *BRCA2*, as judged by negative LOD scores at both loci (–1.00 to –1.72 at 17q and –0.68 to –1.90 at 13q). No *BRCA1* or *BRCA2* mutations have been detected in these families. The pattern of cancer affection in these families was not consistent with the Li-Fraumeni syndrome or other syndromes involving breast cancer. This result suggests that the pattern of hereditary breast and ovarian cancer in these families may be explained by genes other than *BRCA1* or *BRCA2*. Pedigree drawings of these five families not attributable to *BRCA1* or *BRCA2* are presented in figure 1.

The results of *BRCA1* and *BRCA2* mutation analysis are also indicated in tables 2 and 3 (Castilla et al. [1995] and the Breast Cancer Information Core database at http://www.nchgr.nig.gov/dir/lab_transfer/bic/). Ten *BRCA1* mutations were identified in these 23 families. There were no duplicate mutations detected (i.e., each mutation was unique in this sample). Seven of these mutations (70%) induced frameshifts that were predicted to produce truncated *BRCA1* protein products. This proportion is consistent with that reported elsewhere (Shattuck-Eidens et al. 1995). One mutation was a nonsense mutation. Two mutations were missense variants caused single amino acid changes in the ring-finger region of *BRCA1*. In addition, eight genetic variants (data not shown) were detected that did not result

Table 3

Results of Maximum-Likelihood Linkage and Mutation Analysis for *BRCA2*

Family	Mutation[a]	LOD Score	
		Multipoint[b]	Two-Point[c]
15	–	...[d]	...[d]
16	–	–.12	–.43 (*D13S289*)
17	–	–.54	–.88 (*D13S267*)
27	–	...[d]	...[d]
28	–	...[d]	...[d]
30	–	...[d]	...[d]
33	–	–.28	–.64 (*D13S171*)
34	–	...[d]	...[d]
36	–	–.54	–.96 (*D13S267*)
46	–	...[d]	...[d]
61	–	.47	.46 (*D13S260*)
77	–	...[d]	...[d]
78	–	–.43	–.68 (*D13S267*)
100	–	–1.02	–1.13 (*D13S267*)
129	–	–.11	–.58 (*D13S260*)
130	–	...[d]	...[d]
139	–	...[d]	...[d]
178	–	...[d]	...[d]
202	–	...[d]	...[d]
224	–	–.72	–1.90 (*D13S260*)
230	–	–.42	–.42 (*D13S267*)
383	–	–1.25	–1.55 (*D13S289*)
417	–	–1.16	–1.48 (*D13S260*)

[a] A minus sign (–) indicates no mutation in the *BRCA2* gene was detected.

[b] Multipoint LOD scores computed for *D13S260-BRCA2-D13S267*.

[c] "Maximal" value of two-point LOD scores for the indicated marker.

[d] Inferred as *BRCA1* by linkage and/or mutation analysis.

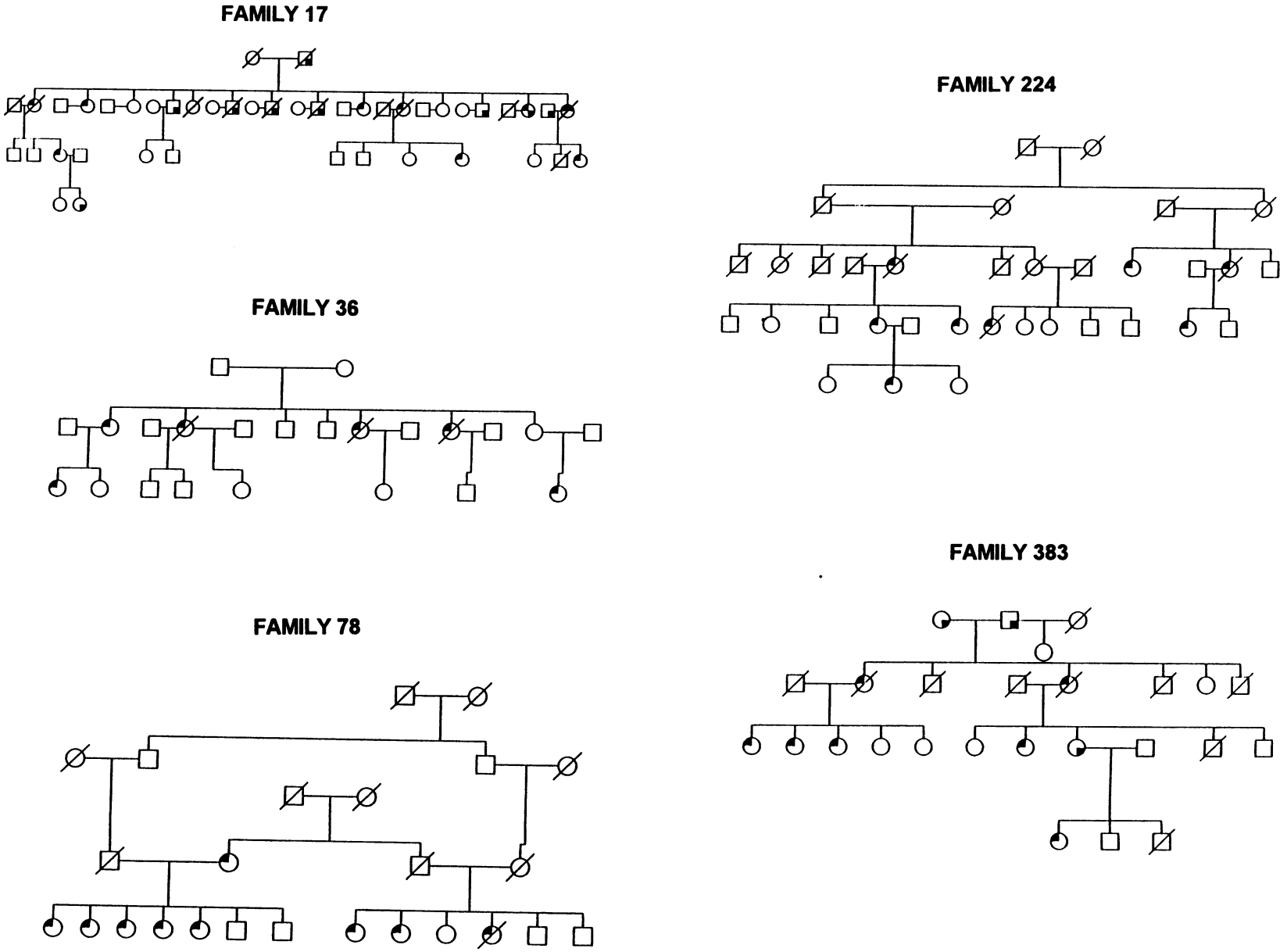

Figure 1 Pedigree drawings of families not attributed to *BRCA1* or *BRCA2*. Affection is designated by shading in upper-left quadrant (breast cancer), upper semicircle (bilateral breast cancer), and lower-right quadrant (cancer diagnosed at other sites).

in changes that affect the predicted amino acid sequence of *BRCA1* and are thought to represent benign polymorphisms. As stated previously, no *BRCA2* mutations were detected in any family.

Finally, we compared the distribution of breast and ovarian cancers in *BRCA1* and non-*BRCA1* families. Among *BRCA1* families, 84 sampled individuals were affected with cancer. Of these, 64 (76%) had breast cancers, 14 (17%) had both breast and ovarian cancers, and 6 (7%) had only ovarian cancers. In contrast, all of the 48 cancer cases in the non-*BRCA1* families were breast cancers. No male breast cancers were present in any family. This result supports previous reports that most hereditary breast and ovarian cancer families can be explained by genetic variability in *BRCA1*.

Discussion

We have described the etiologic spectrum of hereditary breast cancers in a clinic-based sample of families. The finding that occurrence of breast cancer in 22% of our families are not explained by *BRCA1* or *BRCA2* suggests the existence of additional high-penetrance susceptibility genes exist that account for hereditary patterns of breast cancer.

Our results are consistent with previous reports that estimate about half of hereditary breast cancer families, and the majority of families having both breast and ovarian cancers, will be explained by mutations at *BRCA1* (Easton et al. 1993). However, it has been estimated elsewhere that ~35% of hereditary breast cancer families will be explained by genotypes at *BRCA2* (Wooster et al. 1994). On the basis of linkage analyses, it appears that *BRCA2* explains a substantially smaller proportion of families in our sample than has been reported in previous studies (Wooster et al. 1994; Spurr et al. 1995). A number of factors could explain this result. First, our family collection could have been biased toward non-*BRCA2* families. For example, families containing male breast cancer cases were not specifically included in our sampling design. This is the most likely explanation for seeing a lower proportion of *BRCA2* families than might be expected in the general population. Second, *BRCA2* families may have been too small to detect LOD scores of a sufficient magnitude to infer linkage and thus may not have been included in this analysis. Of the families that were included, the average family size in the non-*BRCA1* families was somewhat smaller than in the *BRCA1* families (13.7 individuals vs. 16.3, respectively). However, the inability to detect *BRCA1* or *BRCA2* mutations should not be related to the size of the family collected, since complete mutation screening can be accomplished in a single individual from each family. Finally, it is possible that some of our families that have hereditary patterns of breast cancer may be due to the coincidental occurrence of multiple breast cancers resulting from a non-Mendelian etiology. While additional studies may reveal some of our "unexplained" families to be caused by mutations at *BRCA1* or *BRCA2*, it is very likely that some families with hereditary patterns of breast cancer cannot be explained by *BRCA1* or *BRCA2*.

The conclusion that not all families can be explained by *BRCA1* or *BRCA2* has implications for clinical practice as well as additional genetic analyses of hereditary breast cancer. Our results support previous observations that about half of families with hereditary breast cancer (and the majority of those with hereditary breast and ovarian cancer) will be explained by genotypic variation at *BRCA1*. Some of the remaining families will be explained by *BRCA2*, but there will be additional families that can be explained by neither. Thus, negative *BRCA1* or *BRCA2* test results will not be sufficient to rule out the presence of susceptibility genes that confer a high breast cancer risk in some families. These families may be counseled on the basis of the putative segregation of an autosomal dominant gene (Hoskins et al. 1995). However, until the penetrance of non-*BRCA1*, non-*BRCA2* genes can be defined, risk estimation will remain crude. None of the present sample of families appear to have other (non-*BRCA1* or -*BRCA2*) hereditary cancer syndromes (reviewed by Hoskins et al. 1995). However, these syndromes must also be taken into account in the classification, counseling, and follow-up of members of hereditary breast cancer families. In particular, recent reports of germ-line p53 mutations in hereditary breast and ovarian cancer families (Buller et al. 1993; Jolly et al. 1994) suggest that genetic testing of non-*BRCA1* or -*BRCA2* families for p53 mutations may be appropriate.

Our findings imply that additional breast cancer susceptibility genes that could explain hereditary breast cancer in some families have yet to be identified. These results are consistent with one report of direct *BRCA1* and *BRCA2* mutation analysis, which suggested additional susceptibility genes may explain patterns of hereditary breast cancer in some families (Phelan et al. 1996). Presumably, these additional susceptibility genes will have different population genetic characteristics than those of *BRCA1* or *BRCA2*. First, they will not explain a large proportion of hereditary breast or ovarian cancer in the general population, since the *BRCA1* and *BRCA2* genes account for most such cases (Narod et al. 1995). Second, the collection of families not explained by *BRCA1* or *BRCA2* may have a high degree of genetic heterogeneity. The frequency of hereditary cancers explained by any single additional breast cancer susceptibility locus may be small, and there may be numerous such genes in the population. Stated differently, it is unlikely that the population attributable risk associated with additional hereditary breast cancer susceptibility

genes will be large. Third, the families attributed to non-*BRCA1*, non-*BRCA2* susceptibility genes may have breast but not ovarian cancers. This has implications for the type of families that should be included in the search for additional susceptibility genes. Success in finding genes with these properties may hinge on developing large collections of families, such as the hereditary breast cancer family sample assembled through the European Breast Cancer Linkage Consortium. Our families unexplained by *BRCA1* and *BRCA2* are a part of this larger collection of unlinked families. A number of candidate genes and loci may be considered in this search, including the putative susceptibility locus on chromosome 8q (Sobol et al. 1994) and the recently cloned *ATM* (ataxia telangiectasia) gene (Savitsky et al. 1995).

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