Clinical Characteristics of Individuals With Germline Mutations in *BRCA1* and *BRCA2*: Analysis of 10,000 Individuals

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Purpose: To assess the characteristics that correlate best with the presence of mutations in *BRCA1* and *BRCA2* in individuals tested in a clinical setting.

Patients and Methods: The results of 10,000 consecutive gene sequence analyses performed to identify mutations anywhere in the *BRCA1* and *BRCA2* genes (7,461 analyses) or for three specific Ashkenazi Jewish founder mutations (2,539 analyses) were correlated with personal and family history of cancer, ancestry, invasive versus noninvasive breast neoplasia, and sex.

Results: Mutations were identified in 1,720 (17.2%) of the 10,000 individuals tested, including 968 (20%) of 4,843 women with breast cancer and 281 (34%) of 824 with ovarian cancer, and the prevalence of mutations was correlated with specific features of the personal and family histories of the individuals tested. Mutations were as prevalent in high-risk women of African (25 [19%] of 133) and other non-Ashkenazi ancestries as those of European ancestry (712 [16%] of 4379) and were significantly less prevalent in women diagnosed before 50 years of age with ductal carcinoma in situ than with invasive breast cancer (13% v 24%, *P* = .0007). Of the 74 mutations identified in individuals of Ashkenazi ancestry through full sequence analysis of both *BRCA1* and *BRCA2*, 16 (21.6%) were nonfounder mutations, including seven in *BRCA1* and nine in *BRCA2*. Twenty-one (28%) of 76 men with breast cancer carried mutations, of which more than one third occurred in *BRCA1*.

Conclusion: Specific features of personal and family history can be used to assess the likelihood of identifying a mutation in *BRCA1* or *BRCA2* in individuals tested in a clinical setting.

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THE PREVIOUS DECADE HAS seen the identification of several genes responsible for autosomal-dominant transmission of greatly increased risk of specific cancers. Some hereditary cancer syndromes, such as familial adenomatous polyposis and multiple endocrine neoplasia type 2, have a sufficiently distinctive phenotype and were often recognized on the basis of their clinical presentation even before the responsible genes were identified. Other hereditary cancer syndromes that lacked such pathognomonic features were not clearly defined as such before their genetic characterization. Although it was certainly recognized that some families demonstrated an apparently autosomal dominant pattern of breast and ovarian cancer, for example, the clinical characterization of the syndrome of inherited risk of breast and ovarian cancer (hereditary breast and ovarian cancer [HBOC]) predominantly resulted from the ability to analyze the *BRCA1* and *BRCA2* genes for the mutations responsible for this syndrome. The large extended families in whom these genes were originally discovered, with multiple individuals who had developed breast cancer at an early age (often in conjunction with ovarian cancer), were quickly found not to be typical of most families in which such mutations actually occur.[1] In fact, it is increasingly recognized that "families with an obvious cancer syndrome are likely to represent only a small fraction of individuals with inherited predisposition to cancer."[2]

Although clinical evaluation of the *BRCA1* and *BRCA2* genes has been available since 1996, there has been no clear consensus on what specific personal and family history features should prompt consideration of hereditary cancer risk assessment. Other unresolved issues include the spectrum and prevalence of *BRCA1* and *BRCA2* mutations in specific populations (including individuals of Ashkenazi, African, and other ancestries), whether ductal carcinoma in situ (DCIS) of the breast should be considered as equivalent to invasive breast cancer in clinical risk assessment, and the role of *BRCA1* as well as *BRCA2* mutations in male breast cancer.

Since 1996, our United States–based laboratory has performed sequence analysis of *BRCA1* and *BRCA2* for the

purposes of clinical patient care, distinct from testing done in support of specific research protocols, for individuals believed on the basis of their personal and family history to be at risk of HBOC. In the absence of a specific mutation already identified in a family, such testing typically consists of sequence analysis of over 17,500 base pairs of the protein-coding and adjacent noncoding regions of the *BRCA1* and *BRCA2* genes or sequence analysis for three specific founder mutations prevalent in Ashkenazi Jewish individuals. This retrospective study of 10,000 consecutive tests performed in a clinical setting is designed to correlate the presence of mutations with the personal and family histories of the individuals being tested to characterize the spectrum of deleterious mutations in *BRCA1* and *BRCA2* and the clinical features that should prompt consideration of testing for hereditary risk of breast and ovarian cancer.

PATIENTS AND METHODS

Scope of Analysis

A total of 10,000 consecutive individuals analyzed over a 3-year period were included, consisting of 7,461 analyzed for the coding sequences of *BRCA1* and *BRCA2* and 2,539 analyzed only for three specific founder mutations prevalent in individuals of Ashkenazi Jewish ancestry,[3,4] as described below. (Results from individuals who were tested for the complete sequences of *BRCA1* and *BRCA2* after a negative test result for the three Ashkenazi-prevalent mutations were collated with the *BRCA1-BRCA2* group, not the three-mutation analysis group.) Excluded were samples not otherwise analyzed for the entirety of both *BRCA1* or *BRCA2*, analyses performed in the context of any specific research protocol, and samples where it was known that the individual was being tested for a specific mutation already identified in a family member.

Acquisition of Clinical Information

Corresponding clinical information about the proband and his or her family was obtained through a routine requisition that accompanied each test sample. The requisition allows the submitting health care provider to specify the ancestry of the proband, the family history (including breast, ovarian, and other cancers, age of diagnosis, and relationship to patient), whether the proband had not been diagnosed with cancer, or whether there was a history of breast, ovarian, or other cancers, including the age of diagnosis of each. In addition, the version of the requisition form in use for most (but not all) of the period of this study distinguished between ductal carcinoma in situ and invasive breast cancer in the proband. For the purposes of this study, a sample was excluded from consideration in a specific data analysis if the information being collated was not provided on the requisition. For example, a proband was considered to have had no family history of cancer only when the corresponding portion of the accompanying requisition specified "none" rather than if this section was simply left blank.

Characteristics of Sample Set

The 10,000 individuals analyzed included 9,090 women, 263 men, and 647 individuals who did not specify their sex. Of the individuals tested, 9,106 provided a birth date, and the median age of testing in this group was 49 years (range, 6 to 97 years; although the policy of the laboratory is to refrain from testing individuals under age 18 years, rare specific exceptions have been made for extraordinary circumstances in accordance with professional society guidelines[5]). Of the individuals included in this study, 5,503 indicated a personal history of breast or ovarian cancer, including 4,679 with breast cancer (of whom 76 were men), 584 with ovarian cancer, and 240 with both. Of the individuals with breast cancer, 4,663 indicated a median age of diagnosis of 44 years (range, 6 to 96 years). Of the individuals with ovarian cancer, 779 indicated a median age of diagnosis of 53 years (range, 10 to 87 years).

The largest proportion of individuals in this cohort specified their place of ancestry as Northern/Western Europe (4,073 [41%]). Other ancestries of the individuals tested (in descending order) were Ashkenazi (3,022 [30%]), Central/Eastern European (1,041 [10%]), Latin American/Caribbean (229 [2.3%]), Native American (218 [2.2%]), African (163 [1.6%]), Asian (112 [1.1%]), and Near Eastern/Middle Eastern (91 [0.9%]). A total of 1,775 individuals (18%) did not specify ancestry. These proportions add up to more than 100%, because some individuals designated multiple ancestries. All individuals who underwent full sequence analysis of the entire *BRCA1* and *BRCA2* genes and who did not specifically indicate Ashkenazi ancestry on the clinical requisition were considered to be non-Ashkenazi for the purposes of this analysis.

Analysis of BRCA1 and BRCA2

All analyses of *BRCA1*, *BRCA2*, and the three prevalent mutations in Ashkenazi individuals were performed by direct gene sequencing, as previously described.[1] In brief, patient samples were each assigned a unique bar code for robotic specimen tracking. Most samples were received as 7 mL of anticoagulated blood, from which DNA was extracted and purified from leukocytes isolated from each sample. Aliquots of patient DNA were each subjected to polymerase chain reaction (PCR) amplification (35 reactions for *BRCA1*, 47 reactions for *BRCA2*, and three reactions for analysis of three Ashkenazi founder mutations). The amplified products were each directly sequenced in forward and reverse directions using fluorescent dye–labeled sequencing primers. Chromatographic tracings of each amplicon were analyzed by proprietary sequence analysis software followed by visual inspection and confirmation, assisted by comparison of the proband sequence to a consensus wild-type sequence constructed for each gene. Multiple amplicons were reviewed in parallel by a trained staff in such a way that the identification of one mutation did not influence the review of the remaining sequence data. Each genetic variant (exclusive of nonreportable polymorphisms as below) was independently confirmed by repeated analysis, including PCR amplification of the indicated gene regions and sequence determination.

Naming and Interpretation of Sequence Analysis Results

All mutations and genetic variants were named according to the convention of Beaudet and Tsui.[6] Nucleotide numbering starts at the first transcribed base of *BRCA1* and *BRCA2* according to GenBank entries U14680 and U43746, respectively. (Under these conventions, two mutations commonly referred to as 185delAG and 5382insC are named 187delAG and 5385insC, respectively.) All variants were interpreted according to the following criteria. If more than one variant was observed in a single analysis, the overall interpretation was that of the most clinically significant mutation observed.

Positive for a deleterious mutation. Mutations were interpreted as positive for a deleterious mutation if they prematurely terminate

(truncate) the protein product of *BRCA1* at least 10 amino acids from the C-terminus or the protein product of *BRCA2* at least 110 amino acids from the C-terminus, based on documentation of deleterious mutations in *BRCA1* and *BRCA2*. In addition, specific missense mutations and noncoding intervening sequence mutations were interpreted as deleterious on the basis of data derived from linkage analysis of high-risk families, functional assays, biochemical evidence, or demonstration of abnormal mRNA transcript processing. A few mutations for which the available evidence indicated a reasonable presumption (but not proof) that the mutation was deleterious were reported as suspected deleterious, and such results were grouped with those reported as positive in this study.

Genetic variant of uncertain significance. This group includes missense mutations and mutations that occur in analyzed intronic regions whose clinical significance has not yet been determined, chain-terminating mutations that truncate *BRCA1* and *BRCA2* distal to amino acid positions 1853 and 3308, respectively, and mutations that eliminate the normal stop codons for these proteins.

No deleterious mutation detected. Such results include samples identical to a consensus wild-type sequence as well as samples in which genetic variants of no substantial clinical consequence were identified. For the purposes of a result intended for patient care, nonreportable variants include nontruncating alterations observed at an allele frequency of greater than 1% of a suitable control population (providing that no data suggest clinical significance), variants for which published data demonstrate absence of substantial clinical significance, variants in the protein-coding region that neither alter the amino acid sequence nor are predicted to significantly affect exon splicing, and variations in the analyzed noncoding portions of the gene that have been demonstrated to have no deleterious effect on the length or stability of the mRNA transcript. Rare genetic variants for which available evidence strongly indicates but does not yet prove that the variant did not contribute substantially to cancer risk were reported as genetic variant, favor polymorphism and were considered to be negative for the purpose of this study. Statistical analyses, consisting of tests of association and binomial probabilities, were performed using *S*-PLUS 6.0 for UNIX (MathSoft, Inc, Seattle, WA).

RESULTS

Overall, 1,731 deleterious mutations in *BRCA1* and *BRCA2* were identified in the 10,000 individuals tested. A total of 1,720 individuals (17.2%) carried mutations, including 11 individuals (all of Ashkenazi ancestry) who carried two mutations, one in each gene. Of 9,746 individuals who provided information about their ancestry, mutations were identified in 1,054 (15.7%) of 6,724 non-Ashkenazi individuals and 617 (20.4%) of 3,022 Ashkenazi individuals.

A total of 1,129 deleterious mutations (15.1%) were identified through 7,461 full-sequence analyses of *BRCA1* and *BRCA2*; 689 (61%) of the mutations identified occurred in *BRCA1* and 440 (39%) in *BRCA2*. In all, 424 different deleterious mutations were seen (212 each in *BRCA1* and *BRCA2*), of which 256 (60%) were frameshift mutations, 106 (25%) were nonsense, nine (2.1%) were missense, and 53 (12.5%) occurred in the analyzed regions of the noncoding introns. In both *BRCA1* and *BRCA2*, mutations in coding regions were distributed evenly along the lengths of

each gene in approximate proportion to the relative size of the exons. For example, 113 (53%) and 92 (43%) of the distinct mutations occurred in the largest exons (exon 11) of each *BRCA1* and *BRCA2*.

In addition, full sequence analysis identified one or more variants of uncertain clinical significance in the absence of deleterious mutations in 970 (13%) of these 7,461 individuals. An additional 505 individuals, or 6.8% of those who had undergone full sequence analysis of *BRCA1* and *BRCA2*, had genetic variants that were at one time classified as of uncertain significance but that were subsequently characterized through ongoing investigation as polymorphisms or, far less commonly, as deleterious.

The 2,539 analyses performed for the three Ashkenazi founder mutations revealed a total of 602 mutations (23.7%), consisting of 320 observations (53%) of the *BRCA1* mutation 187delAG (also known as 185delAG, as described in the Patients and Methods section), 94 observations (16%) of the *BRCA1* mutation 5385insC (also known as 5382insC), and 188 observations (31%) of the *BRCA2* mutation 6174delT.

Of the 4,843 women with breast cancer, 968 (20%) carried deleterious mutations, including 628 in *BRCA1* and 346 in *BRCA2* (six women had two mutations, one each in *BRCA1* and *BRCA2*). The median age of diagnosis of the first breast cancer was 40 years (range, 21 to 75 years) for women with mutations in *BRCA1* and 41 years (range, 24 to 72 years) for women with mutations in *BRCA2*. Of the 824 women with ovarian cancer, 281 (34%) carried deleterious mutations, of which 199 were in *BRCA1* and 82 were in *BRCA2*. The median age of diagnosis of ovarian cancer was 49 years (range, 32 to 78 years) for *BRCA1*-mutation carriers and 55 years (range, 27 to 79 years) for *BRCA2*-mutation carriers. Deleterious mutations were identified in 350 (10.6%) of 3,311 women who specifically indicated that they did not have breast or ovarian cancer.

Because of the contribution of age of onset of breast cancer and family history of cancer toward the decision to be tested in a clinical setting, these data presumably do not reflect the overall population prevalence of mutations in women with a diagnosis of breast or ovarian cancer. The prevalence of mutations in *BRCA1* and *BRCA2* was therefore correlated with the personal and family history of the individual tested (Table 1). Because of the higher prevalence of mutations in *BRCA1* and *BRCA2* associated with Ashkenazi ancestry,[3,4] these data were analyzed separately for Ashkenazi and non-Ashkenazi individuals. For the purposes of this correlation, the designation "family history" includes at least one first- or second-degree relative, and the designation "no breast cancer less than 50" does not

Table 1. Prevalence of Mutations in *BRCA1* and *BRCA2* Correlated With Personal and Family History of Cancer in 4,716 Non-Ashkenazi Individuals

Proband	Family History											
	No Breast Cancer < 50 Years of Age or Ovarian Cancer in Anyone		Breast Cancer < 50 Years of Age in One Relative; No Ovarian Cancer in Anyone		Breast Cancer < 50 Years of Age in > One Relative; No Ovarian Cancer in Anyone		Ovarian Cancer at Any Age in One Relative; No Breast Cancer < 50 Years of Age in Anyone		Ovarian Cancer in > One Relative; No Breast Cancer < 50 Years of Age in Anyone		Breast Cancer < 50 Years of Age and Ovarian Cancer at Any Age	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
No breast cancer or ovarian cancer at any age	9/229	3.9	19/434	4.4	46/419	11	6/153	3.9	10/117	8.5	58/354	16.4
Breast cancer ≥ 50 years of age	4/172	2.3	22/197	11.2	12/118	10.2	3/69	4.3	1/18	5.6	19/87	21.8
Breast cancer < 50 years of age	55/579	9.5	89/484	18.4	117/322	36.3	34/194	17.5	7/42	16.7	126/267	47.2
Ovarian cancer at any age, no breast cancer	5/77	6.5	14/41	34.1	11/26	42.3	23/83	27.7	12/28	42.9	38/71	53.5
Breast cancer ≥ 50 years of age and ovarian cancer at any age	5/27	18.5	1/9	11	4/11	36.4	1/6	17	1/3	33	3/6	50
Breast cancer < 50 years of age and ovarian cancer at any age	5/25	20	7/14	50	4/5	80	5/9	56	2/2	100	13/18	72.2

NOTE. Family history includes at least one first- or second-degree relative and excludes proband.

exclude the possibility of relatives diagnosed with breast cancer at a later age.

Of the 6,724 non-Ashkenazi individuals tested, 4,716 provided sufficient information to allow their personal and family history of cancer to be correlated with the presence of a deleterious mutation (Table 1). In general, the highest prevalence of mutations was seen when a proband's personal and family history included women with ovarian cancer at any age as well as women diagnosed with breast cancer before 50 years of age. Mutations were also observed in a substantial proportion of individuals from families where at least two women had been diagnosed with early-onset breast cancer, even in the absence of a family history of ovarian cancer. For example, mutations were identified in 89 (18%) of 484 women with breast cancer diagnosed before age 50 years who indicated even a single relative with early-onset breast cancer. As shown in Table 2, the prevalence of mutations generally increased with earlier age

of onset of breast cancer. In contrast, there was no consistent increase in the prevalence of mutations seen among women whose ovarian cancer was diagnosed before age 50 years compared with those diagnosed at a later age.

Of 3,022 individuals who indicated Ashkenazi ancestry, 2,233 provided sufficient information about their personal and family history of cancer for correlation with test results. Of these, 1,656 were tested for three specific founder mutations only and 577 underwent full analysis of *BRCA1* and *BRCA2*. As expected, a higher prevalence of mutations in the Ashkenazi group correlated with a family history of early-onset breast cancer or ovarian cancer (Table 3) as well as with decreasing age of onset of breast cancer in the proband (Table 4). Even in the absence of any family history of early-onset breast cancer or ovarian cancer at any age, however, deleterious mutations were identified in 32 (12.9%) of 248 Ashkenazi women with breast cancer diagnosed before age 50 years, 15 (25.9%) of 58 Ashkenazi

Table 2. Prevalence of Mutations in *BRCA1* and *BRCA2* in Non-Ashkenazi Individuals Correlated With Age of Diagnosis of Cancer and Family History

	Family History											
	No Breast Cancer < 50 Years of Age or Ovarian Cancer in Anyone		Breast Cancer < 50 Years of Age in One Relative; No Ovarian Cancer in Anyone		Breast Cancer < 50 Years of Age in > One Relative; No Ovarian Cancer in Anyone		Ovarian Cancer at Any Age in One Relative; No Breast Cancer < 50 Years of Age in Anyone		Ovarian Cancer in > One Relative; No Breast Cancer < 50 Years of Age in Anyone		Breast Cancer < 50 Years of Age and Ovarian Cancer at Any Age	
Proband	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Breast cancer ≥ 50 years of age	4/172	2.3	22/197	11.2	12/118	10.2	3/69	4.3	1/18	5.6	19/87	21.8
Breast cancer 40-49 years of age	16/284	5.6	31/289	10.7	41/172	23.8	15/115	13	3/25	12	55/141	39
Breast cancer < 40 years of age	39/295	13.2	58/195	29.7	76/150	50.7	19/79	24.1	4/17	23.5	71/126	56.3
Ovarian cancer ≥ 50 years of age, no breast cancer	3/45	6.7	8/25	32	6/10	60	9/52	17	6/15	40	19/35	54.3
Ovarian cancer < 50 years of age, no breast cancer	2/29	6.9	6/15	40	5/15	33.3	12/28	42.9	6/13	46.2	19/34	55.9

NOTE. Family history includes at least one first- or second-degree relative and excludes proband.

women with ovarian cancer, and 12 (52%) of 23 Ashkenazi women with both breast and ovarian cancer. Mutations were generally more prevalent in the Ashkenazi than non-Ashkenazi group, except for women diagnosed with either breast cancer after the age of 50 years or with ovarian cancer at any age who indicated a single relative with breast cancer diagnosed before age 50 years.

A total of 737 individuals of Ashkenazi ancestry underwent full sequence analysis of *BRCA1* and *BRCA2*, including 322 individuals who were first tested (and found to be negative) for the three founder mutations prevalent in this population. Nonfounder mutations accounted for 16 (21.6%) of the 74 deleterious mutations identified in Ashkenazi individuals who underwent full sequence analysis, of which seven occurred in *BRCA1* and nine in *BRCA2*. Of these 16 individuals with nonfounder mutations, five indicated European as well as Ashkenazi ancestry and the other 11 indicated only Ashkenazi ancestry. The *BRCA2* mutation 4075delGT was present in two Ashkenazi individuals and had been seen previously, as had six other mutations in this group, but the remaining eight (50%) represented unique mutations not seen in other families to date. Of note, the *BRCA1* mutation 188del11, previously reported as a putative fourth founder mutation,[7] was not identified. Nonfounder mutations were identified in six (4.7%) of 129 Ashkenazi women with both a personal and family history of breast cancer diagnosed before age 50 years or ovarian

cancer at any age, compared with an overall mutation prevalence of 539 (31.3%) of 1721 in non-Ashkenazi individuals with a comparable personal and family history.

Of the 322 Ashkenazi individuals who underwent full-sequence analysis only after negative results from a three-mutation test, six (1.9%) carried a nonfounder deleterious mutation, including five (2.5%) of 203 who had indicated a personal history of breast or ovarian cancer and one (0.9%) of 110 who had indicated that they did not have cancer at the time of testing, a difference that was not significant ($P > .05$, χ^2). (Nine individuals did not specify whether they had cancer.) A personal or family history of ovarian cancer was indicated in all but one of the women who had a positive full sequence result after a negative three-mutation analysis. Full sequence analysis in Ashkenazi individuals also demonstrated several recurrent missense mutations, such as the *BRCA1* variant M1008I and the *BRCA2* variants P655R and I2285V, which were each seen at a prevalence of greater than 5% of this at-risk population. Clinical observations support the likelihood that these are population variants of little clinical consequence, although additional analysis will be necessary to confirm this.

The prevalence of deleterious mutations was analyzed according to other specified ancestries. For this purpose, only individuals who specified a single ancestry were included. Compared with a mutation prevalence of 712 (16%) of 4,379 individuals who specified only European

Table 3. Prevalence of Mutations in *BRCA1* and *BRCA2* Correlated With Personal and Family History of Cancer in 2,233 Ashkenazi Individuals

| | Family History | | | | | | | | | | | |
| Proband | No Breast Cancer < 50 Years of Age or Ovarian Cancer in Anyone | | Breast Cancer < 50 Years of Age in One Relative; No Ovarian Cancer in Anyone | | Breast Cancer < 50 Years of Age in > One Relative; No Ovarian Cancer in Anyone | | Ovarian Cancer at Any Age in One Relative; No Breast Cancer < 50 Years of Age in Anyone | | Ovarian Cancer in > One Relative; No Breast Cancer < 50 Years of Age in Anyone | | Breast Cancer < 50 Years of Age and Ovarian Cancer at Any Age | |
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
No breast cancer or ovarian cancer at any age	12/242	5	38/307	12.4	34/188	18.1	29/158	18.4	18/70	25.7	65/211	30.8
Breast cancer ≥ 50 years of age	7/153	4.6	9/86	10.5	5/29	17.2	3/26	11.5	4/8	50	8/24	33.3
Breast cancer < 50 years of age	32/248	12.9	35/144	24.3	29/66	43.9	25/53	47.2	6/10	60	32/57	56.1
Ovarian cancer at any age, no breast cancer	15/58	25.9	1/13	7.7	5/6	83	7/17	41.2	6/8	75	12/14	85.7
Breast cancer ≥ 50 years of age and ovarian cancer at any age	4/13	30.8	0/1	0	1/1	100	1/2	50	None tested		1/2	50
Breast cancer < 50 years of age and ovarian cancer at any age	8/10	80	4/5	80	1/2	50	0/1	0	None tested		None tested	

NOTE. Family history includes at least one first- or second-degree relative and excludes proband.

ancestry, deleterious mutations were identified in 576 (21%) of 2,768 individuals who indicated only Ashkenazi ancestry, 25 (19%) of 133 individuals who indicated only African ancestry, 31 (18%) of 177 individuals who specified only Latin American/Caribbean ancestry, 15 (14%) of 104 individuals who indicated only Native American ancestry, 11 (12%) of 91 individuals who indicated only Asian ancestry, and six (9%) of 69 individuals who indicated only Near Eastern/Middle Eastern ancestry. There was no indication of frequently recurring founder mutations in any of these populations; for example, of the 25 mutations seen among individuals of African ancestry, only one mutation was seen as often as three times. Deleterious mutations were identified in 18 (24%) of 64 women of African ancestry who had been diagnosed with ovarian or early-onset breast cancer, comparable with the prevalence of mutations (503 [23%] of 2,170) among such

individuals who indicated only European ancestry. In fact, the prevalence of mutations did not differ significantly between European ancestry and any other except for Ashkenazi Jewish ($P < .0001$, χ^2).

The requisition form that accompanied most (but not all) of the samples analyzed in this study specifically distinguished between invasive breast cancer and DCIS, an epithelial abnormality of the breast often regarded as a preinvasive cancer of the breast. Excluding women who also had been diagnosed with ovarian cancer, the prevalence of mutations was significantly lower in women with DCIS diagnosed before age 50 years (26 [13%] of 199) than in women with invasive breast cancer diagnosed before age 50 years (587 [24%] of 2,466) ($P = .0007$, χ^2). Analysis of these data in non-Ashkenazi and Ashkenazi women matched for family history indicated that the prevalence of mutations in each category was generally lower in women

Table 4. Prevalence of Mutations in *BRCA1* and *BRCA2* in Ashkenazi Individuals Correlated With Age of Diagnosis of Cancer and Family History

	Family History											
	No Breast Cancer < 50 Years of Age or Ovarian Cancer in Anyone		Breast Cancer < 50 Years of Age in One Relative; No Ovarian Cancer in Anyone		Breast Cancer < 50 Years of Age in > One Relative; No Ovarian Cancer in Anyone		Ovarian Cancer at Any Age in One Relative; No Breast Cancer < 50 Years of Age in Anyone		Ovarian Cancer in > One Relative; No Breast Cancer < 50 Years of Age in Anyone		Breast Cancer < 50 Years of Age and Ovarian Cancer at Any Age	
Proband	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Breast cancer ≥ 50 years of age	7/153	4.6	9/86	10.5	5/29	17.2	3/26	11.5	4/8	50	8/24	33.3
Breast cancer 40-49 years of age	15/161	9.3	21/100	21	11/40	27.5	17/41	41.5	4/7	57	20/41	48.8
Breast cancer < 40 years of age	17/87	19.5	14/44	31.8	18/26	69.2	8/12	66.7	2/3	67	12/16	75
Ovarian cancer ≥ 50 years of age, no breast cancer	7/30	23.3	1/6	17	3/3	100	5/9	56	5/5	100	3/5	60
Ovarian cancer < 50 years of age, no breast cancer	7/22	31.8	0/7	0	2/3	67	2/8	25	1/3	33	8/8	100

NOTE. Family history includes at least one first- or second-degree relative and excludes proband.

diagnosed with DCIS than with invasive breast cancer (Tables 5 and 6).

Finally, the prevalence of mutations was analyzed for 76 males who indicated a diagnosis of breast cancer (median age, 59 years), of whom 57 were analyzed for *BRCA1* and *BRCA2* and 19 for the three Ashkenazi founder mutations only. Deleterious mutations were identified overall in 21 (28%) of 76 of the males with breast cancer, with eight mutations occurring in *BRCA1* and 14 in *BRCA2* (one Ashkenazi individual had one mutation in each gene). The

median age of diagnosis was 52 years for males with *BRCA1* mutations and 59 years for males with *BRCA2* mutations, compared with 59 years for males without mutations.

Mutations were more prevalent in men with a family history of breast or ovarian cancer at any age (16 [36%] of 44) compared with those without (two [13%] of 16), although this difference did not achieve significance (*P* = .1121, Fisher's exact test). Mutations were more prevalent in male breast cancer patients of Ashkenazi ancestry (11

Table 5. Comparison of Prevalence of Mutations in *BRCA1* and *BRCA2* in Non-Ashkenazi Individuals With DCIS Versus Breast Cancer Diagnosed Before Age 50 Years

	Family History											
	No Breast Cancer < 50 Years of Age or Ovarian Cancer in Anyone		Breast Cancer < 50 Years of Age in One Relative; No Ovarian Cancer in Anyone		Breast Cancer < 50 Years of Age in > One Relative; No Ovarian Cancer in Anyone		Ovarian Cancer at Any Age in One Relative; No Breast Cancer < 50 Years of Age in Anyone		Ovarian Cancer in > One Relative; No Breast Cancer < 50 Years of Age in Anyone		Breast Cancer < 50 Years of Age and Ovarian Cancer at Any Age	
Proband	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
DCIS < 50 years of age	3/36	8.3	6/50	12	3/18	16.7	3/12	25	0/5	0	6/19	31.6
Breast cancer < 50 years of age	55/579	9.5	89/484	18.4	117/322	36.3	34/194	17.5	7/42	16.7	126/267	47.2

NOTE. Family history includes at least one first- or second-degree relative and excludes proband.

Table 6. Comparison of Prevalence of Mutations in *BRCA1* and *BRCA2* in Ashkenazi Individuals With DCIS Versus Breast Cancer Diagnosed Before Age 50 Years

	Family History											
	No Breast Cancer < 50 Years of Age or Ovarian Cancer in Anyone		Breast Cancer < 50 Years of Age in One Relative; No Ovarian Cancer in Anyone		Breast Cancer < 50 Years of Age in > One Relative; No Ovarian Cancer in Anyone		Ovarian Cancer at Any Age in One Relative; No Breast Cancer < 50 Years of Age in Anyone		Ovarian Cancer in > One Relative; No Breast Cancer < 50 Years of Age in Anyone		Breast Cancer < 50 Years of Age and Ovarian Cancer at Any Age	
Proband	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
DCIS < 50 years of age	0/30	0	2/17	11.8	0/4	0	1/3	33	1/1	100	1/4	25
Breast cancer < 50 years of age	32/248	12.9	35/144	24.3	29/66	43.9	25/53	47.2	6/10	60	32/57	56.1

NOTE. Family history includes at least one first- or second-degree relative and excludes proband.

[39%] of 28) compared with those of non-Ashkenazi ancestry (10 [21%] of 48), although this difference was also not significant in this small sample set (*P* = .1117, Fisher's exact test). Of note is that one of the mutations identified in an Ashkenazi man with breast cancer was a unique mutation that was only identified through full sequence analysis of *BRCA1* and *BRCA2* (as above).

DISCUSSION

This compilation of the results of genetic testing for HBOC in 10,000 individuals provides an estimate of the prevalence of deleterious *BRCA1* and *BRCA2* mutations in a population selected and tested for the purposes of medical care. Because the personal and family histories of the individuals tested were ascertained from a routine test requisition form, there are likely inaccuracies resulting from errors in patient recall as well as in the documentation by the health care provider. This may explain in part the surprising observation of mutations in 3.9% of cancer-free non-Ashkenazi individuals who reported no family history of ovarian cancer or early-onset breast cancer (ie, diagnosed before age 50 years). Alternatively, this finding may result not from inaccuracies in the personal and family history information but from individuals who were prompted to seek testing by a strong family history of breast cancer after age 50 years. For this reason, the prevalence of mutations in such a group tested in a clinical setting is presumably much greater than what would be observed in a comparable group selected at random from the general population.

Despite the limitations inherent in using a clinical test requisition form to obtain personal and family history, the findings of this study are likely to be predictive of results seen in a comparable clinical setting, where personal and family history information is primarily provided verbally by individuals interested in their own risk of cancer. Furthermore, a comparison of these results with data obtained through a more carefully controlled research study indicates that the family histories of breast cancer as obtained from the test requisition form were generally accurate. For example, the 18.4% prevalence of mutations in non-Ashkenazi women with breast cancer diagnosed before age 50 years who report only one relative with early-onset breast cancer (Table 1) is comparable with the 20% prevalence observed in a controlled research protocol in which all family histories were carefully obtained and confirmed.[1]

A family history of breast cancer is typically more accurate than that of ovarian cancer,[8] however, and observations in this study specifically indicate the possibility that ovarian cancer is overreported in the family history. For example, in the non-Ashkenazi population, the prevalence of mutations in women with ovarian cancer who reported exactly one relative with breast cancer diagnosed before age 50 years was 34%; in the corresponding situation of a woman with early-onset breast cancer who reported exactly one relative with ovarian cancer, however, the prevalence of mutations was only 17.5% (Table 1). (Alternatively, this apparent discrepancy could reflect genetic heterogeneity of breast cancer among the women tested.) Even accounting for its limitations, a reported family history of ovarian cancer was associated with a greatly increased prevalence of mutations compared with the general population *BRCA1* and *BRCA2* mutation prevalence of less than 0.2%.[9]

Although frequently referred to as breast cancer genes, *BRCA1* and *BRCA2* mutations were actually more prevalent among women with a diagnosis of ovarian cancer. This is consistent with growing evidence regarding the contribution of mutations in these genes to the overall incidence of this disease. A recent population series of women with ovarian

cancer estimated the prevalence of mutations associated with that diagnosis to be approximately 12%[10] in contrast to approximately 5% for breast cancer.[11,12] Although the age of onset of hereditary ovarian cancer is somewhat younger than that of the general population, a diagnosis after age 50 years does not reduce the likelihood that a woman's ovarian cancer was attributable to a mutation in BRCA1 or BRCA2. This is consistent with data indicating that hereditary ovarian cancer is often diagnosed after age 50 years.[12,13] Because of the generally high prevalence of mutations associated with this disease, it has been suggested that genetic testing should be considered for women who have been diagnosed with invasive ovarian cancer, regardless of family history.[10,14]

The prevalence of mutations in women with ovarian cancer or breast cancer diagnosed before age 50 years was especially high in women of Ashkenazi ancestry, even in the absence of a family history of the disease, as has been observed in previous studies.[14,15] This is attributable primarily to three specific founder mutations whose prevalence among Ashkenazi Jewish individuals has been well documented.[3,15] The observation of 16 other mutations in individuals who identified themselves as Ashkenazi indicates that the absence of the three most prevalent mutations does not necessarily rule out the possibility of HBOC in this group. The increased prevalence of mutations among Ashkenazi Jewish individuals should not obscure the fact that most of the individuals in this study who carried BRCA1 and BRCA2 mutations, and indeed by far most of North Americans with such mutations, are not of Ashkenazi ancestry.

A consideration of the Ashkenazi founder mutations led to an additional observation. Given the mutation frequencies of BRCA1 and BRCA2 in these samples, several individuals would have been expected to carry two mutations in the same gene. In fact, each of the 11 Ashkenazi individuals who had two deleterious mutations had one mutation each in BRCA1 and BRCA2, and none were found to carry two deleterious mutations in the same gene. The absence of such individuals is statistically significant (binomial test $P = 2.3 \times 10^{-9}$). In association with animal studies that have shown that deleterious mutations in each copy of BRCA2 are lethal in embryogenesis,[16] it seems unlikely that an individual can carry mutations in both alleles of BRCA1 or BRCA2. Furthermore, the number of individuals with two different Ashkenazi founder mutations is significantly lower than the expected equilibrium proportion (binomial test $P = 2.5 \times 10^{-5}$ for 10 of 2,539 individuals tested for the three founder mutations), indicating that there may also be some selection against mutations in each BRCA1 and BRCA2 as well.

The results of analysis of individuals of other ancestries did not indicate significant differences in mutation prevalence between those who indicated European ancestry and those of other ancestries, with the exception of Ashkenazi Jewish descent. Although some previous studies have reported that BRCA1 and BRCA2 mutations are less prevalent in African-American individuals than other groups,[17,18] our findings as well as those of others[19] indicate that this is not the case. Although this study represents the largest reported series of African-American individuals tested to date, only a small proportion of the individuals tested overall were of African ancestry (1.6% of the total, or 163 individuals). The reasons for which individuals of African ancestry were substantially underrepresented in this population of individuals tested clinically for BRCA1 and BRCA2 mutations are not clear but raise the question of access to hereditary risk assessment in this population.

The prevalence of mutations in women with DCIS was significantly lower than in women with invasive breast cancer. There are several possible explanations for this. One is in the diagnosis of DCIS itself, which is often subjective if not unreliable, so that a single lesion diagnosed as DCIS by one expert may be interpreted as benign hyperplasia by another.[20] Another explanation is that DCIS may represent not a true preinvasive cancer but rather a spectrum of intraductal abnormalities of heterogeneous malignant potential. Indeed, an earlier analysis of the pedigrees of women with mutations in BRCA1 did not reveal the increased number of diagnoses of DCIS that would have been expected if this lesion were equivalent to early breast cancer.[21] It should be noted that although the prevalence of mutations associated with DCIS was lower than in women with invasive breast cancer, it was nonetheless higher than in women without a diagnosis of breast neoplasia at all, suggesting that some but not all DCIS may represent incipient breast cancer. Alternatively, it may be necessary to compare the prevalence of mutations in women with DCIS diagnosed 10 years younger than women with invasive cancer to allow for the time it would take for the former to progress to the latter. Indeed, there was no significant difference in the prevalence of mutations between women with DCIS diagnosed before age 40 years (nine [26.4%] of 34) compared with those with invasive cancer diagnosed before age 50 years (373 [28.5%] of 1,309). Ascertainment bias may confound this comparison, however, because a diagnosis of DCIS before age 40 years can only be made through mammography at an early age, and women who undergo mammography before age 40 years are likely to do so because of a family history of early breast cancer. Clearly, additional investigation is necessary to delineate the relationship between DCIS and mutations in BRCA1 and BRCA2. Nonetheless, these data indicate that for the purpose of hereditary risk assessment, a history of DCIS should be distinguished from that of invasive breast cancer at a comparable age. Moreover, the lower mutation prevalence observed in DCIS compared

with invasive breast cancer has implications for interpreting the results of previous estimates of the prevalence of mutations in *BRCA1* and *BRCA2*, because estimates that grouped DCIS with invasive breast cancer likely underestimated the prevalence of mutations in the latter.[22]

This study includes the largest series of men with breast cancer analyzed for *BRCA1* and *BRCA2* to date. The increased risk of male breast cancer associated with mutations in *BRCA2* has been well described.[23,24] Less widely recognized is that mutations in *BRCA1* also increase this risk, albeit not as much.[12] We identified *BRCA1* and *BRCA2* mutations in 28% of men with breast cancer, of which a substantial proportion (eight [36%] of 22) occurred in *BRCA1*. Mutations were especially associated with a positive family history of breast or ovarian cancer, consistent with a previous analysis of male breast cancer,[25] although that study reported a lower prevalence of mutations in this group overall and did not identify mutations in *BRCA1*. This may reflect differences between the populations studied but more likely results from differences in the sensitivities of detection methods used in that study (single-strand conformation polymorphism and protein-truncation test) compared with the full sequence analysis used in the present study.[26]

It should be emphasized that these data likely underestimate the true prevalence of clinically significant abnormalities in *BRCA1* and *BRCA2* that occur in a clinical setting. This is not only because some of the genetic variants reported as being of uncertain significance are likely to be subsequently characterized as deleterious but because analysis of the sequence of the protein-coding regions and adjacent noncoding regions does not detect large rearrangements of the genes. Such rearrangements predominantly represent recombinations of the *Alu* sequences located in the noncoding introns. These result in large inversions, duplications, and deletions that may involve one or more entire exons. By eliminating the PCR-priming sites that flank the affected exon or by introducing such large insertions between priming sites that PCR amplification cannot occur, such rearrangements become invisible to standard sequence analysis strategies. Such chromosomal rearrangements may account for as many as 15% of abnormalities in *BRCA1* and *BRCA2*.[27] Several *BRCA1* rearrangements are recurrent,[28-30] allowing for their detection by an analysis designed specifically for that purpose. Such an assay is presently being validated for inclusion in the *BRCA1* and *BRCA2* clinical test described in this study. In contrast, however, there is as yet no robust method to screen for rare family-specific chromosomal rearrangements in a clinical laboratory setting, although there is ongoing research in this area.[31]

In summary, this large series of individuals tested for *BRCA1* and *BRCA2* mutations through a clinical laboratory provides the basis for characterizing the phenotype of HBOC. It is of interest that of the 4,716 non-Ashkenazi individuals who provided their family history information (Table 1), 1,693 (36%) reported a family history of only a single first-degree or second-degree relative with early-onset breast cancer or ovarian cancer and, of those, 13% (224 of 1,693) carried deleterious mutations, indicating that a family history as reported by an individual who carries a mutation in *BRCA1* or *BRCA2* may be neither dramatic nor obvious. Indeed, careful evaluation of paternal as well as maternal family history is required, especially in women diagnosed with breast cancer before age 50 years or ovarian cancer at any age, to enable the appropriate identification and counseling of individuals at risk for carrying mutations in *BRCA1* and *BRCA2*. The data presented here, correlating such mutations with features of a personal and family history as documented in a clinical setting, will hopefully assist the health care professional to perform this evaluation.

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