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# Frequency of Breast Cancer Attributable to *BRCA1* in a Population-Based Series of American Women

Beth Newman, PhD; Hua Mu, MD, PhD; Lesley M. Butler; Robert C. Millikan, DVM, PhD; Patricia G. Moorman, PhD; Mary-Claire King, PhD

**Context.**—Previous studies of *BRCA1* mutation prevalence have been based on high-risk groups, yielding estimates that do not reflect the experience of the general population of US patients with breast cancer.

**Objective.**—To determine prevalence of known disease-related mutations and other variants in *BRCA1* and how it differs by race, age at diagnosis, and family history status in a population-based sample of white and black patients with breast cancer unselected for family history.

**Design.**—Case-control study.

**Setting.**—A 24-county area of central and eastern North Carolina.

**Participants.**—Cases were women aged 20 to 74 years diagnosed as having a first invasive breast cancer between May 1993 and June 1996. Controls were frequency matched to cases by 5-year age range and race. The first 211 cases and 188 controls regardless of race and the subsequent 99 cases and 108 controls of African American ancestry are included in this report.

**Main Outcome Measure.**—Germline variants at any site in the coding sequence, splice junctions, 5' untranslated region, or 3' untranslated region of the *BRCA1* gene were analyzed in cases, and selected variants were analyzed in controls. Screening was performed using multiplex single-strand conformation analysis, with all potential variants confirmed using genomic sequencing.

**Results.**—Three of 211 patients with breast cancer had disease-related variants at *BRCA1*, all of which were protein-truncating mutations. After adjustment for sampling probabilities, the proportion of patients with breast cancer with disease-related variants was 3.3% (95% confidence interval, 0%-7.2%) in white women and 0% in black women. Young age at diagnosis alone did not predict *BRCA1* carrier status in this population. In white women, prevalence of inherited mutation was 23% for cases with family history of ovarian cancer, 13% for cases from families with at least 4 cases of breast cancer with or without ovarian cancer, and 33% for cases from families with both breast and ovarian cancer and at least 4 affected relatives. Because these results are based on few families at the highest levels of risk, confidence intervals around these estimates are wide. An additional 5 patients had rare missense mutations or a single amino acid deletion, the biological significance of which is unknown. In black women, a variant in the 3' untranslated region was statistically significantly more common in cases than in controls.

**Conclusions.**—These data suggest that in the general US population, widespread screening of *BRCA1* is not warranted. In contrast, *BRCA1* mutations are sufficiently frequent in families with both breast and ovarian cancer, or at least 4 cases of breast cancer (at any age), that genotyping might be considered. The emerging picture of *BRCA1* population genetics involves complex interactions of family history, age, and genetic ancestry, all of which should be taken into account when considering testing or interpreting results.

COMMERCIAL availability of genetic tests for *BRCA1* and *BRCA2* poses a dilemma. Many professional and advocacy groups currently recommend testing for breast cancer predisposition in the context of research protocols.<sup>1-3</sup> However, when confronted by medical concerns and by the marketplace, clinicians and consumers may find the choice to test difficult to reject. Actual frequencies of inherited *BRCA1* mutations in white and black patients from the general population (ie, not selected for age at diagnosis or family history) may help inform testing decisions for the general population of American women.

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See also pp 922 and 955.

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Most information about inherited genetic susceptibility to breast cancer has come from research on high-risk families, including the basis of proof for *BRCA1*'s existence,<sup>4</sup> its localization by genetic mapping,<sup>4,5</sup> and its cloning.<sup>6</sup> Informativeness of families for genetic analysis is strongly influenced by family size, number of affected relatives, vital status of relatives, and degree of relatedness among relatives with disease. Hence, for earlier research, families with multiple cases of breast cancer in mul-

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Table 1.—Multiplex Single-Strand Conformation Analysis Array Used to Screen the *BRCA1* Gene

Lane	Exon	Size*
1	18	352
	22	297
	9	292
	19	249
2	7	228
	3	339
	21	298
	5	278
3	6	253
	20	226
	24	310
	15	338
4	12	265
	1	315
	10	281
	16B	267
5	2	258
	14	312
	8	291
	16A	275
6	23	255
	13	321
	17	263
	11Ai	472
7	11I2	333
	11K	305
	11J	288
	11D	254
8	11E2	410
	11G	319
	11L	301
	11F	273
9	11P	314
	11C	295
	11E	269
	11H	312
10	11B	296
	11N	253
	11A	309
	11D2	272
11	11B2	209
	11K2	296
	11I	280
	11M	306
12	11H2	286
	11O	289
	11Pi	200
	11A	309

\*Size is measured in base pairs.

multiple generations were needed, thus selection for families with young ages at diagnosis occurred. Now, testing for cancer predisposition due to inherited *BRCA1* mutation is complicated by limited information about frequency of *BRCA1* mutations in the general population. In the human *BRCA1* gene, more than 100 distinct variants have been reported.<sup>7</sup> Of those variants that are disease related, nearly all are frameshift or nonsense mutations leading to truncated proteins.

The purpose of this study was to assess the potential clinical and public health significance of inherited *BRCA1* mutations in white and black women not selected for breast cancer family history or for age at diagnosis. A series of inter-related questions are addressed: What proportion of breast cancer cases in the general population of white and black women carry known disease-related variants at *BRCA1*? How do these frequencies differ by age at diagnosis, race,

Table 2.—Characteristics of 211 Breast Cancer Patients and 188 Controls Screened for Variants at *BRCA1*

Characteristic	Patients With Breast Cancer, No. (%)	Controls, No. (%)
Race		
White	120 (57)	105 (56)
Black	88 (42)	79 (42)
Native American	3 (1)	0 (0)
Asian	0 (0)	2 (1)
Other	0 (0)	2 (1)
Age, y		
20-39	43 (20)	26 (14)
40-49	75 (36)	72 (38)
50-59	32 (15)	40 (21)
60-74	61 (29)	50 (27)
Family history		
Any relative with breast or ovarian cancer	104 (49)	66 (35)
First-degree relative with breast or ovarian cancer	43 (20)	27 (14)
High-risk family,* breast or ovarian cancer	15 (9)	4 (2)
Any relative with ovarian cancer	21 (12)	12 (7)
High-risk family,* both breast and ovarian cancer	4 (4)	1 (1)

\*Defined as 4 or more affected family members including proband (ie, Carolina Breast Cancer Study cases are counted as affected).

or family history? What are the implications of these data for genetic testing?

## METHODS

### Population

The Carolina Breast Cancer Study (CBCS) is a population-based, case-control study of breast cancer in women aged 20 to 74 years from a 24-county area of central and eastern North Carolina.<sup>8</sup> Women with incident, primary, invasive breast cancer were identified from 26 hospitals using a rapid ascertainment mechanism.<sup>9</sup> Potential comparison women, frequency matched on age and race, were identified from the North Carolina Division of Motor Vehicles lists for those younger than 65 years and from the US Health Care Financing Administration lists for those aged 65 to 74 years. Nurses made home visits to conduct interviews and to draw blood. To characterize family history, probands were asked to enumerate first-degree relatives and report history of any cancer, irrespective of site, and age at diagnosis. For more distant relatives, only history of breast and ovarian cancer was queried along with relationship to the proband.

Interviews for phase 1 of the study took place from May 1993 through December 1996, with recruitment of 890 cases and 841 controls. To increase statistical power for subgroups, younger women and black women were oversampled using a modification of randomized recruitment.<sup>10,11</sup> Overall response rates were 77% for cases and 68% for controls, with blood samples obtained for

more than 95% of participants. This report focuses on the first 211 patients with breast cancer and 188 control women, regardless of race, and the subsequent 99 cases and 108 controls of African American ancestry. The expense of fully genotyping *BRCA1* precluded testing the entire series of cases and controls with current technology. The project was approved by the institutional review boards of the University of North Carolina School of Medicine and the University of Washington. In addition, a certificate of confidentiality was obtained from the US Department of Health and Human Services.

### Mutation Analysis

Using DNA extracted from peripheral blood lymphocytes, 211 cases were screened for germline mutations in the *BRCA1* coding sequence, splice junctions and neighboring intronic regions, 5' untranslated region (UTR), and 3' UTR using multiplex single-strand conformation analysis, as described below. In addition, DNA aliquots from all cases were hybridized to allele-specific oligonucleotide probes for 8 frequent mutations in European populations. As a further check on sensitivity of testing, exon 11 was screened using a protein truncation test. All potential rare variants were evaluated by direct genomic sequencing, using bands from the single-strand conformation analysis gel, as well as original DNA, as template. To compare frequencies of polymorphisms and rare variants of unknown clinical significance in cases and controls, the 188 controls were genotyped by the same methods used for that variant in cases. The number of controls differed for some analyses because of restricted amounts of DNA.

**Multiplex Single-Strand Conformation Analysis.**—Forty-seven primer pairs were used to amplify *BRCA1* from genomic DNA using polymerase chain reaction (PCR). Most primers were defined previously.<sup>12,13</sup> New primers were designed for a few regions and are defined using the notation of Table 1 in Friedman et al<sup>12</sup>: exon 5-reverse: 5'-ATG GTT TTA TAG GAA CGC TAT G-3'; exon 6-reverse: 5'-GGT CTT ATC ACC ACG TCA TAG-3'; exon 8-reverse: 5'-TTT GGC AAA ACT ATA AGA TAA GG-3'; exon 9-reverse: 5'-TGC ACA TAC ATC CCT GAA CC-3'; exon 10-reverse: 5'-AGG TCC CAA ATG GTC TTC AG-3'; exon 16A-forward: 5'-AAC AGA GAC CAG AAC TTT GTA ATT C-3'; exon 16A-reverse: 5'-TGC ATT ATA CCC AGC AGT ATC AG-3'; exon 16B-forward: 5'-CCA TCT TCA ACC TCTGCA TTG-3'; exon 16B-reverse: 5'-ACT CTT TCC AGA ATG TTG TTA AGT C-3'; exon 20-forward: 5'-GCC

Table 3.—BRCA1 Variants and Characteristics of Cases in the Carolina Breast Cancer Study

Identification Code	Exon/ Intron	Nucleotide Variant	Amino Acid Change	Race	Age at Diagnosis, y	Family History of Breast or Ovarian Cancer*		Allele Frequency in Control Chromosomes		No. of Citations in Breast Cancer Information Core (BIC) <sup>†</sup>
						No. of First-Degree Relatives	No. of Second- or Third-Degree Relatives	White	Black	
Definitely associated with disease										
NC1	I-5	332(-11) T→G	81 Stop	White	45	1	0	...	...	...
NC2	I-5	332(-11) T→G	81 Stop	White	52	0	1	...	...	...
NC3	E-11	2457 C→T	Gln780 Stop	White	53	3	0	...	...	...
Association with disease unknown										
NC4	E-11	1224 del GAT	delAsp369	White	61	0	1	0/206	0/156	1‡
NC5	E-11	1256 T→G	Ile379Met	Black	67	0	1	0/204	1/152	1
NC6	E-11	3719 G→C	Gln1200His	Black	54	0	0	...	...	0
NC7	E-20	5391 A→G	Arg1758Gly	White	44	0	0	1/172	0/124	0
NC8	E-20	5391 A→G	Arg1758Gly	White	42	0	0	1/172	0/124	0

\*Refers to relatives of the proband (ie, Carolina Breast Cancer Study case not included in definition of family history).

†Ellipses indicate not applicable.

‡Data not yet reported in BIC (R. A. Eeles, written communication, December 1997).

TTA AAT ATG ACG TGT CTG CTC-3'; exon 20-reverse: 5'-TGG AAT ACA GAG TGG TGG GGT G-3'; and exon 24-forward: 5'-AGT CGA TTG ATT AGA GCC TAG-3'. Conditions for PCR amplification and single-strand conformation analysis were as described.<sup>13</sup>

The multiplex single-strand conformation analysis scheme allowed simultaneous analysis of several exons or several regions of exon 11 in the same lane of an electrophoretic gel. The PCR products from the 47 primer pairs were run in 14 gel lanes according to band size and pattern. Within each lane, bands corresponding to different amplified products could be easily distinguished (Table 1). If a band shift was observed, the fragment involved was amplified again, and direct DNA sequencing was performed in both forward and reverse strand directions. In addition, the shifted band was cut out of the gel, suspended in distilled water, and sequenced. Multiplex single-strand conformation analysis detected all variants reported in the study.

**Allele-Specific Oligonucleotide Hybridization.**—The allele-specific oligonucleotides were designed for 8 mutations. Mutations 185delAG, 4184delTCAA, 4446C→T, and 5382insC were genotyped using primers and conditions previously described.<sup>13</sup> For 4 other mutations, primers were designed to distinguish normal and mutant alleles as follows: exon 5: 300 T→G - normal: 5'-CCT TCA CAG TGT CCT TTA-3'; mutant: 5'-CCT TCA CAG GGT CCT TTA-3'; exon 5: 331(+1) G→A - normal: 5'-AAC CAA AAG GTA TAT AAT-3'; mutant: 5'-AAC CAA AAG ATA TAT AAT-3'; exon 6: 332(-11) T→G - normal: 5'-CTC AAA CAA TTT AAT TTC-3'; mutant: 5'-CTC AAA CAA GTT AAT TTC-3'; exon 11: 2457 C→T - normal: 5'-ATG GCA CTC AGG AAA GTA-3'; mutant: 5'-ATG GCA CTT AGG AAA GTA-3'.

Individual dot blots in 96-well formats contained positive and negative controls for each screened mutation. All positive dots were confirmed by directly sequencing both the PCR product from the analysis and an independent aliquot from the original sample. The allele-specific oligonucleotide hybridization independently (and blindly) confirmed the 3 protein-truncating mutations observed.

**Protein Truncation Test.**—The *BRCA1* exon 11 was amplified and then translated in 3 fragments using primers and methods as described.<sup>13</sup> No additional variants were detected by protein truncation test.

**DNA Sequencing.**—Reamplified PCR product of each variant exon or region was analyzed by automated and/or manual direct DNA sequencing of both strands using the original single-strand conformation analysis-PCR primers. Automated sequencing was performed with a DNA sequencer, using dye-labeled dideoxy terminator chemistry (ABI Prism 377, Foster City, Calif: Applied Biosystems of Perkin Elmer). Manual sequencing was conducted with the Sequenase PCR Product Sequencing Kit (Cleveland, Ohio: United States Biochemical) following the manufacturer's instructions.

#### Haplotype Analyses

Primer pairs and conditions for genotyping at markers flanking *BRCA1* were as described.<sup>12</sup> For those CBCS cases with a disease-related *BRCA1* variant, genotypes at these markers were compared with haplotypes from high-risk families with the same variant to assess whether the mutations shared a common origin.

#### Statistical Analyses

Proportions and 95% confidence intervals (CIs) were computed using SAS<sup>14</sup>

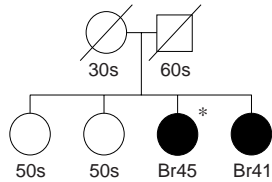
and SUDAAN<sup>15</sup> software. Both cases and controls were sampled using known probabilities based on race and age; weighting was incorporated in statistical analyses to produce parameter estimates that reflect frequencies in the underlying population. Odds ratios (ORs) and CIs were estimated by logistic regression models using PROC GENMOD software,<sup>16</sup> which allowed for age and race adjustments and provided unbiased estimates through inclusion of offset terms derived from the sampling probabilities.

#### RESULTS

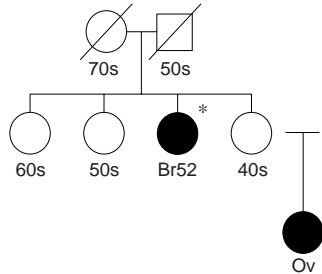
Comparisons of cases and controls with respect to race, age, and family history of breast or ovarian cancer are shown in Table 2. Cases and controls were similar in distribution of age and race, for which they were matched. Cases were more likely than controls to have first-degree or more distant relatives with breast or ovarian cancer and to have multiple affected relatives. Four women reported male relatives with breast cancer, 2 cases and 2 controls. In subsequent tables, results are restricted to women who identified themselves as either white or black, since numbers of women reporting other races were small.

Three of the 211 genotyped cases carried protein-truncating mutations in *BRCA1* (Table 3, Figure). All 3 were white, with breast cancer diagnosis at ages 45, 52, and 53 years. An intron 5 splicing mutation, 332(-11) T→G, was observed in 2 cases (NC1 and NC2). The family of NC1 includes a sister diagnosed as having breast cancer at age 41 years. The family of NC2 includes a niece with ovarian cancer (the age of the niece at diagnosis and which sibling was her parent are not known). This intron 5 mutation destroys an acceptor splice site,

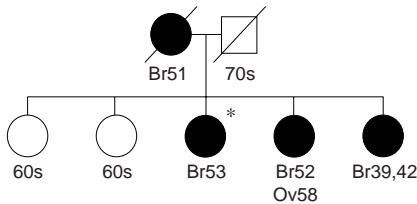
NC1. *BRCA1*: 332 (-11) T→G=81 Stop



NC2. *BRCA1*: 332 (-11) T→G=81 Stop



NC3. *BRCA1*: 2457 C→T=780 Stop



Pedigrees of the 3 North Carolina families with inherited disease-related mutations in *BRCA1*. Circles denote women, squares denote men, and the Carolina Breast Cancer Study case (ie, the proband) is identified with an asterisk. Male siblings and exact ages have been omitted to protect confidentiality. Ages shown indicate age at death for deceased (denoted by slash through the symbol), age at diagnosis for individuals with cancer (denoted by a shaded symbol with Br indicating breast and Ov indicating ovary) or current age for all others. In the family of NC2, the woman with ovarian cancer is a niece of the proband, but we do not know which sibling is her parent.

leading to aberrant messenger RNA splicing and insertion of 59 nucleotides from intron 5 into the messenger RNA. Translation stops at codon 81 of the mutant *BRCA1* sequence.<sup>12</sup> This splice mutation was seen in a prior series of high-risk kindreds (family 82<sup>12</sup>), as well as in 4 other high-risk families of western European ancestry.<sup>17</sup> Genotypes of NC1, NC2, and family 82 at markers flanking *BRCA1* indicate that these families share the same ancestral chromosome 17q21 and hence probably a common origin for the mutation (Table 4). Although not closely related, NC1 and NC2 are from the same North Carolina county, and family 82 is from North Carolina as well.

The single nucleotide substitution in *BRCA1* exon 11, 2457 C→T, is a nonsense mutation causing an immediate stop in translation at codon 780. This mutation was observed in NC3, whose family includes her mother with breast can-

Table 4.—Genotypes at Markers Flanking *BRCA1* in Carolina Breast Cancer Study Cases Compared With Haplotypes Observed in High-Risk Families Segregating the Same Disease-Related Allele\*

Mutations	Genotypes				
	<i>D17S1321</i>	<i>D17S855</i>	<i>D17S1322</i>	<i>D17S1323</i>	<i>D17S1327</i>
Intron 5 splice					
NC1	145, 163	151, 155	... †	151, 151	133, 133
NC2	145, 151	151, 153	...	151, 151	133, 133
Family 82 ‡	145	151	...	151	133
Codon 780 stop					
NC3	149, 153	145, 149	129, 121	157, 151	165, 135
Family 7 ‡	149	145	131	157	165

\*Genotypes are denoted as the sizes (in base pairs) of alleles.

†Ellipses indicate marker not tested.

‡Haplotype of chromosome with the disease-related allele, determined from linkage in extended family.

cer, a sister with bilateral breast cancer diagnosed at a young age, another sister with breast and ovarian cancer, and a brother with bladder cancer. This mutation was found previously in an American kindred of Dutch ancestry with 7 cases of breast cancer in 3 generations (family 7<sup>13</sup>). The same mutation was subsequently seen in 3 families in the Netherlands, 1 in Germany, and 4 in the United States.<sup>7,18</sup> Genotypes of NC3 and family 7 at markers flanking *BRCA1* suggest a founder effect with a recent mutation at marker D17S1322 (Table 4).

Three missense mutations and one 3-base pair deletion were observed in 5 patients (Table 3). The consequences of the variants for disease risk are unknown. Substitutions of histidine for glutamine in exon 11 at codon 1200 in 1 black case (NC6) and of glycine for arginine in exon 20 in 2 white cases (NC7, NC8) have not been reported elsewhere. The exon 20 variant was observed once in 148 controls in the CBCS. These cases (NC6 through NC8) reported no relatives with breast or ovarian cancer. The substitution of methionine for isoleucine at codon 379 was observed in 1 black case (NC5) and 1 black control from this series and was reported once elsewhere.<sup>7</sup> One case, NC5, reported a paternal aunt with breast cancer and a father and brother with prostate cancer. The 3-base pair deletion in exon 11, leading to deletion of an aspartic acid, was observed in 1 white case (NC4) in this series, who reported a paternal aunt with ovarian cancer. This same deletion also was seen in a breast cancer patient of English ancestry (R. A. Eeles, written communication, December 1997).

In our study area of North Carolina, prevalence of inherited disease-related *BRCA1* variants was 3.3% in white cases, 0% in black cases, and 2.6% for the population of breast cancer patients as a whole (Table 5). All estimates were adjusted for sampling probabilities. In white women, inherited *BRCA1* predisposition was responsible for 6.6% of cases with any relative with breast or ovarian cancer and 22.8% with any rela-

tive with ovarian cancer. As expected, inherited mutations were more frequent in high-risk families. Again in white women, 13.4% of cases from high-risk families with either breast or ovarian cancer and 33% of cases from high-risk families with both breast and ovarian cancer had a *BRCA1* mutation. Breast cancer cases diagnosed before age 50 years were no more likely to have inherited *BRCA1* disease-related variants than cases diagnosed after age 50 years. Weighted mean ages at diagnosis were 51.5 years for women with disease-related variants, 55.8 years for women with other rare missense or in-frame deletion mutations, and 55.7 years for all other breast cancer cases.

Variants in introns 8, 16, 18, 22 and at coding nucleotides 710, 1186, 2430, 2731, 3232, 3238, 3667, 4158, 4801, and 4956 were observed in the cases. All of these are considered polymorphisms and have assigned PM identification numbers.<sup>7</sup> Five of the variants (including those in intron 8, exon 11 at nucleotides 2430, 3232, 3667, and intron 16) are inherited as a unit because they are in linkage disequilibrium. Four novel variants (in exons 11, 12, 16 and intron 12) were detected in black women, all of which are silent (no amino acid change) or occur in noncoding regions (Table 6). A fifth variant in exon 3 also was observed in 1 black case, reported previously.<sup>7</sup> One missense mutation, 3537 A→G in exon 11, was observed at low frequency in black cases and controls.

The single nucleotide substitution C→G at position 36 in the 3' UTR was more common in black cases than in black controls: 18 of 86 cases and 5 of 74 controls had genotypes CG or GG. The rarer G allele also was observed in 1 white and 1 Native American case and 1 white control. In this series of black women, the age-adjusted OR for breast cancer and the G allele was 3.5 (95% CI, 1.2-10.0). To provide an independent test of association, the subsequent 99 cases and 108 controls in the CBCS who identified themselves as black were genotyped with an age-adjusted OR for breast can-

cer and the G allele of 1.7 (95% CI, 0.5-6.3). Combined, the overall, age-adjusted OR was 2.8 (95% CI, 1.3-6.2). In black women, the weighted mean age at diagnosis was 54.5 years for women with the rare G allele and 52.5 years for those homozygous for the C allele. The 3' UTR site is in partial linkage disequilibrium with a variant in intron 22. The association of the intron 22 variant with breast cancer was not statistically significant.

## COMMENT

In white women in this North Carolina population, the prevalence of known, disease-related *BRCA1* mutations was 3.3%. In black women in the study described herein, there were no *BRCA1* mutations of the types known to be related to disease, although such mutations have been identified in black families selected for high risk.<sup>19,20</sup> Hence,

Table 5.—Prevalence of Definite Disease-Related Alleles at *BRCA1*, Overall and by Race, Age, and Family History, in White and Black Women With Breast Cancer

Characteristics	Prevalence Adjusted for Sampling Probabilities, % (95% CI)*
Black women	0 (0)
Aged 20-49 y at diagnosis	0 (0)
Aged 50-74 y at diagnosis	0 (0)
White women	3.3 (0-7.2)
Aged 20-49 y at diagnosis	1.4 (0-4.1)
Aged 50-74 y at diagnosis	4.3 (0-9.7)
Any relative with breast or ovarian cancer	6.6 (0-13.8)
High-risk family,† breast or ovarian cancer	13.4 . . . ‡
Any relative with ovarian cancer	22.8 (0.5-45.0)
High-risk family,† both breast and ovarian cancer	33.3 . . . ‡
All women	2.6 (0-5.5)

\*Parentheses around confidence intervals (CIs) indicate that the lower bound approaches but does not include 0.

†Defined as 4 or more affected family members including proband (ie, Carolina Breast Cancer Study cases are counted as affected).

‡SE equals 0, so a 95% CI cannot be calculated.

*BRCA1* mutations are rare among incident (ie, newly diagnosed) patients with breast cancer not selected for family history or age at diagnosis. These are the vast majority of patients encountered in a primary care setting.

The low prevalence of 1 in 30 cases attributable to *BRCA1* in white patients is consistent with statistical projections from other population-based series of the proportion of breast cancer due to all susceptibility genes combined.<sup>21,22</sup> Similar results were obtained in statistical analyses of families of ovarian cancer patients, although this approach also potentially includes susceptibility genes other than *BRCA1*.<sup>23,24</sup> As expected, *BRCA1* mutation prevalence in high-risk patient series is considerably higher. The proportion of breast cancer families attributable to inherited *BRCA1* mutations was 60% to 75% for those reporting 3 or more cases of breast or ovarian cancer,<sup>25-27</sup> and 45% for families with 3 or more cases of breast cancer in absence of ovarian cancer.<sup>28</sup> In breast cancer patients attending referral clinics (often because of family history and/or young age at diagnosis), 11% to 16% inherited disease-related variants.<sup>29-32</sup> In contrast, 7.5% of surviving breast cancer patients from western Washington with very early onset (<35 years) were *BRCA1* mutation carriers<sup>33</sup> and a population-based study of incident cases of breast cancer before the age of 40 years in Australian women found a prevalence of 3.6% (95% CI, 0.3% to 12.6%) for protein truncation mutations in *BRCA1* (J. L. Hopper, written communication, December 1997).

The North Carolina population contributed 1 in-frame deletion and several missense mutations to a class of variants whose biological meaning is unclear. Screening large series of well-matched controls is necessary, but not sufficient, for clarifying the role of these variants in breast cancer because the variants are rare in both cases and controls.<sup>34</sup> Devel-

opment of functional assays may help determine their biological activity and thereby clarify their clinical significance.

In black women in this series, a relatively common variant in the 3' UTR was almost 3 times more common in cases than controls, suggesting that this putative polymorphism may confer a moderately increased risk of breast cancer. Although located in a noncoding region, critical sequences in the 3' UTR do influence stability of messenger RNA and therefore phenotype.<sup>35</sup> In the future, using cultured cells from women with the 3' UTR variant and those with wild-type sequence, it will be possible to test experimentally for differences in stability of the wild-type vs variant *BRCA1* message.

Who is most likely to carry a *BRCA1* variant that influences disease risk? First, ovarian cancer is an important marker of inherited risk: 23% of white patients with breast cancer who had a family history of ovarian cancer had an inherited mutation in *BRCA1*. Second, families with at least 4 cases of breast or ovarian cancer, regardless of age, frequently reflect inherited susceptibility: 13% of cases from high-risk families with breast or ovarian cancer and 33% of cases from high-risk families with both breast and ovarian cancer had inherited *BRCA1* mutations. Because these results are based on few families at the highest levels of risk, any fluctuation in number of families with *BRCA1* mutations would make considerable difference in the point estimates. Evidence that prostate cancer risk also is influenced by *BRCA1*<sup>36-38</sup> suggests that it may be worthwhile to include this cancer in future definitions of family history to identify high-risk individuals.

None of the 43 women in the CBCS whose breast cancers were diagnosed when they were younger than age 40 years carried disease-related *BRCA1* variants. A recent report of *BRCA1* testing in women from high-risk families

Table 6. Allele Frequencies of *BRCA1* Variants Presumed to Be Polymorphisms in White and Black Cases and Controls

Exon/Intron	Nucleotide Variant	Amino Acid Change	Allele Frequencies*				No. of Citations in Breast Cancer Information Core <sup>7</sup>
			Cases		Controls		
			White (n = 240)†	Black (n = 176)†	White (n = 210)†	Black (n = 156)†	
E-3	233 G→A	Silent	1.0/0.0	0.99/0.01	. . . ‡	. . .	3
E-11	2933 A→G	Silent	1.0/0.0	0.99/0.01	. . .	. . .	0
E-11	3537 A→G	Ser1140Gly	1.0/0.0	0.98/0.02	1.0/0.0	0.98/0.02	3
E-12	4232 G→A	Silent	1.0/0.0	0.99/0.01	. . .	. . .	0
I-12	4302(+12)delGT	Noncoding	1.0/0.0	0.99/0.01	. . .	. . .	0
E-16	4932 T→C	Silent	1.0/0.0	0.99/0.01	. . .	. . .	0
I-20	5396(+59) ins12bp	Noncoding	0.99/0.01	0.99/0.01	0.99/0.01	1.0/0.0	15
3' UTR	5711(+36) C→G	Noncoding	0.99/0.01	0.89/0.11	0.99/0.01	0.97/0.03	2

\*The 2 numbers appearing in the body of the table represent the frequency of the more common, wild-type allele (to the left of the slash) and the frequency of the less common, variant allele (to the right of the slash).

†Sample sizes shown are number of chromosomes.

‡Ellipses indicate data not applicable.

also noted the absence of mutations among probands younger than 30 years.<sup>32</sup> Similarly, the majority of younger patients with breast cancer studied in various other series have not tested positive for *BRCA1* mutations.<sup>29-31,33</sup> Hence, young age at diagnosis in itself does not identify a group of breast cancer patients likely to have inherited susceptibility at *BRCA1*. However, younger average age at diagnosis in family members generally is a better predictor.<sup>31,32</sup> This occurs because a history of younger ages at diagnosis in family members (including the proband) provides more information than does the age at diagnosis of the proband herself.

Three previous studies of *BRCA1* found higher prevalences of disease-related variants in their very early-onset breast cancer patients.<sup>29,31,33</sup> Differences between prevalences of *BRCA1* mutations in young patients in those populations compared with the North Carolina population could be due to differences in allele frequencies or to sampling variability (CIs frequently overlap). Alternatively, unrecognized selection for patients who were not only young, but also from high-risk families, may account for higher estimates in the other studies. Possible explanations cannot be distinguished because the previous studies did not include older women to evaluate overall mutation frequency in those series or the relative distribution of variants by age. The well-established link between *BRCA1* and early-onset breast cancer frequently overlooks the fact that nearly all families inheriting *BRCA1* or *BRCA2* mutations include women whose conditions were diagnosed at a wide range of ages.<sup>28,39</sup>

Interpretation of risk in the context of predictive genetic testing poses clinical dilemmas. First, negative test results (ie, no detected mutation in *BRCA1* or *BRCA2*) can have any of several meanings. There may be no inherited predisposition to the disease. Alternatively, a proportion of mutations are large deletions or genomically complex alterations that will be missed by approaches based solely on genomic DNA.<sup>12,40,41</sup> Although in our series of high-risk American families,<sup>12,13</sup> mutations detectable only by analysis of complementary DNA constituted approximately 5% to 10% of *BRCA1* mutations, such mutations are far more common in Holland,<sup>41</sup> and hence potentially in other populations where founder effects have major influence. Also, not all inherited breast cancer can be attributed to *BRCA1* or *BRCA2*,<sup>25,26,42</sup> and the other susceptibility genes have not yet been identified. Hence, unless a specific mutation has been identified in other family member(s), a negative test result does not

provide complete information. Second, positive test results cannot yet be interpreted precisely because risk of cancer associated with mutation (ie, penetrance) is not yet well characterized for *BRCA1* and *BRCA2*. Resolution of the question of risk associated with *BRCA1* in the general population awaits analysis of women at risk, not selected from high-risk families, but for whom individual genetic information has been obtained. No such analysis has been completed yet, although several such analyses are in progress.

In women with inherited *BRCA1* disease-associated mutations, options for preventing breast or ovarian cancer remain limited. Minimally, more frequent clinical screening by physical breast examination is recommended. The benefits of mammographic screening in the general population of women younger than 50 years remains controversial,<sup>43,44</sup> but what about its usefulness in a high-risk population? Since the sensitivity of mammography differs among women,<sup>45</sup> it seems most prudent to decide when to begin mammographic screening on an individualized basis. Prophylactic mastectomy and oophorectomy are clearly far more drastic alternatives, for which some data on effectiveness have recently appeared. In women electing prophylactic mastectomy (primarily due to family history or to prior nonmalignant breast conditions), the reduction in breast cancer was 90% of that expected over the succeeding 17 years.<sup>46</sup> Alternatives for women with inherited mutations that fall between the extremes of screening and surgery, such as preventive use of tamoxifen, are under investigation.<sup>47</sup>

What should the policy for predictive genetic testing be? The social and legal issues surrounding genetic testing remain more challenging than the technical ones.<sup>48-53</sup> However, the ongoing debate may become moot as genetic testing becomes increasingly integrated in clinical practice. Potential clinical benefits of genetic testing may be optimized under certain conditions. Testing women from the general population for the entire *BRCA1* and *BRCA2* sequences is of questionable value, because a large number of women would be tested, expensively, to detect few mutations, and the negative results cannot be definitive, except in the context of a family with a known disease-related mutation. For populations with relatively common founder mutations, selected screening of breast and ovarian cancer patients may be reasonable. For other American women, the guidelines suggested by the results of this study may be realistic: family history of both breast and ovarian cancer or 4 or more cases of breast cancer (at any age) in the family. In either

event, clinical decision making is substantially benefited by the involvement of specialists familiar with the subtleties and uncertainties of genetic testing, including how to communicate effectively results that may be inconclusive.

Coverage for medical follow-up for women with *BRCA1* or *BRCA2* mutations is an integral part of any rational health care system. Currently, these costs are spread across the entire population, usually in association with treatment of end-stage disease. However, when possible, consequences of these predispositions will be best treated with preventive care. Shifting the financial burden to individuals as this becomes a prospect seems shortsighted. In the end, we all are predisposed to some condition(s) as the consequence of our genotypes, albeit most of the predisposing genes have not yet been identified.

In summary, prevalences of *BRCA1* mutations among breast cancer patients in this population-based study (3.3% in white and 0% in black cases) may be generalizable to large portions of the United States. In North Carolina, most white residents trace their ancestry primarily to northern and western Europe (US Census Bureau data, unpublished findings, 1990). Less than 2% of North Carolina residents are of Jewish ancestry.<sup>54</sup> Because there are few mutation carriers in this North Carolina population, fluctuations in this number could make considerable difference in the point estimate. However, the upper limit of the CI for white patients (7.2%) excludes the higher estimates of prevalence observed in other studies from clinical settings (11%-16%).<sup>29-32</sup> This suggests, for most of the United States, that widespread screening of breast cancer patients (or the general population) for *BRCA1* is not warranted.

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