Variation of Breast Cancer Risk Among BRCA1/2 Carriers

Colin B. Begg, PhD
Robert W. Haile, DrPH
Ake Borg, PhD
Kathleen E. Malone, PhD
Patrick Concannon, PhD
Duncan C. Thomas, PhD
Leslie Bernstein, PhD
Jørgen H. Olsen, MD, DMSc
Charles F. Lynch, MD, PhD
Hoda Anton-Culver, PhD
Marinela Capanu, PhD
Xiaolin Liang, MD, MA
Amanda J. Hummer, MS
Cami Sima, MD, MS
Jonine L. Bernstein, PhD

Context The risk of breast cancer in BRCA1 and BRCA2 mutation carriers has been examined in many studies, but relatively little attention has been paid to the degree to which the risk may vary among carriers.

Objectives To determine the extent to which risks for BRCA1 and BRCA2 carriers vary with respect to observable and unobservable characteristics.

Design, Setting, and Participants Probands were identified from a population-based, case-control study (Women’s Environmental Cancer and Radiation Epidemiology [WECARE]) of asynchronous contralateral breast cancer conducted during the period of January 2000 to July 2004. Participants previously diagnosed with contralateral breast cancer or unilateral breast cancer were genotyped for mutations in BRCA1 and BRCA2. All participants had their initial breast cancer diagnosed during the period of January 1985 to December 2000, before the age of 55 years.

Main Outcome Measure Incidence of breast cancer in first-degree female relatives of the probands was examined and compared on the basis of proband characteristics and on the basis of variation between families.

Results Among the 1394 participants with unilateral breast cancer, 73 (5.2%) were identified as carriers of deleterious mutations (42 with BRCA1 and 31 with BRCA2). Among the 704 participants with contralateral breast cancer, 108 (15.3%) were identified as carriers of deleterious mutations (67 with BRCA1 and 41 with BRCA2). Among relatives of carriers, risk was significantly associated with younger age at diagnosis ($P=0.04$), and there was a trend toward higher risk for relatives of contralateral breast cancer vs unilateral breast cancer participants (odds ratio, 1.4 [95% confidence interval, 0.8-2.4]; $P=0.28$). In addition, there were significant differences in risk between carrier families after adjusting for these observed characteristics.

Conclusion There exists broad variation in breast cancer risk among carriers of BRCA1 and BRCA2 mutations.

JAMA. 2008;299(2):194-201 www.jama.com

THE MAGNITUDE OF THE RISK OF breast cancer in carriers of mutations in BRCA1 or BRCA2 is critical for guiding decisions concerning cancer prevention options. Many previous studies have reported on the cumulative risk to various ages (penetrance) of breast cancer in carriers. The recent literature primarily has involved studies of breast cancer incidence in the relatives of probands identified without consideration of family history. This literature has included studies of self-selected volunteers, but there appears to be some degree of consensus that the most reliable approach is to use population-based ascertainment. Most of this literature has been focused on the magnitude of the risk, with relatively little attention being paid to the degree by which risk may vary among carriers.

Population-based studies to date have used incident cases from existing case-control investigations as probands. Estimates of risk based on studies of incidence in populations are less likely to be subject to the bias that occurs when cases are selected on the basis of observable characteristics.

Author Affiliations: Department of Epidemiology and Biostatistics, Memorial Sloan-Kettering Cancer Center, New York, New York (Drs Begg, Capanu, Liang, Sima, and J. Bernstein and Ms Hummer); Department of Preventive Medicine, University of Southern California, Los Angeles (Drs Haile, Thomas, Langholz, and L. Bernstein); Department of Oncology, University Hospital, Lund, Sweden (Dr Borg); Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, Washington (Dr Malone); Department of Biochemistry and Molecular Genetics, University of Virginia, Charlottesville (Dr Concannon); Institute of Cancer Epidemiology, Danish Cancer Society, Copenhagen, Denmark (Dr Olsen); Department of Epidemiology, University of Iowa, Iowa City (Dr Lynch); and Department of Medicine, University of California, Irvine (Dr Anton-Culver). Corresponding Author: Colin B. Begg, PhD, Memorial Sloan-Kettering Cancer Center, 307 E 63rd St, New York, NY 10021 (beggc@mskcc.org).
incident cases are inevitably inflated if there exists risk variation among carriers caused by additional, possibly unknown, genetic variants that influence risk.\textsuperscript{8-11} The thesis that substantial variation in the risk of breast cancer exists due to unknown genetic factors has become well established,\textsuperscript{12} and this has been supported by statistical modeling of disease aggregation,\textsuperscript{13} and theoretical models to explain the aggregation.\textsuperscript{14} However, although there is little doubt that other genes influence the risk of breast cancer, such as relatively rare mutations in \textit{TP53,} \textsuperscript{15} and possibly \textit{CHEK2}\textsuperscript{16} and \textit{ATM},\textsuperscript{17} as well as common low penetrance variants in genes that remain unidentified at this point, there is little direct evidence that variation in risk exists among \textit{BRCA1} and \textit{BRCA2} mutation carriers specifically.

In this article, we report the results of an investigation that provides direct evidence about risk variation among carriers. We use the information on lifetime risk of breast cancer in first-degree relatives of breast cancer patients (probands) who were identified as \textit{BRCA1} or \textit{BRCA2} carriers in the Women’s Environmental Cancer and Radiation Epidemiology (WECARE) Study,\textsuperscript{18} a population-based case-control study. The WECARE Study is novel in that it involved recruitment of cases of asynchronous contralateral breast cancer and matched controls who had experienced a prior unilateral breast cancer. If there is no appreciable risk variation among \textit{BRCA1} or \textit{BRCA2} mutation carriers, then we would expect the risk estimates in relatives of carriers with contralateral breast cancer to be similar to the estimates from relatives of carriers with unilateral breast cancer. Likewise, evidence of risk variation may be demonstrated by significant differences in risk in groups of carrier families distinguished by any factors that are plausibly associated with risk, such as age at diagnosis of the proband.

**METHODS**

The WECARE Study is a population-based, cancer registry–based, nested case-control study of contralateral breast cancer. Participant recruitment was completed in 2004. The design has been described in detail in a previous article,\textsuperscript{18} but the essential features are as follows. Participants were identified and interviewed through 5 population-based cancer registries, 4 in the United States (covering Iowa, the Orange County and San Diego regions of California, Los Angeles County, California, and 3 counties in the Seattle, Washington area) and 1 covering all of Denmark.

All participants had a diagnosis of a first invasive breast cancer between January 1985 and December 2000. The cancer had to have occurred prior to the age of 55 years without evidence of spread beyond the regional lymph nodes at diagnosis. Individuals also had only breast cancer prior to the second primary cancer diagnosis for participants with contralateral breast cancer and to the corresponding matching date for unilateral breast cancer controls. Participants also had to be alive at the time of contact, able to complete the interview, and provide a blood sample. Participants were eligible if they had an in situ or invasive diagnosis of a contralateral breast cancer at least 1 year after the first primary breast cancer diagnosis, and if they resided in the same reporting area for both diagnoses.

Control participants were selected randomly from the pool of available breast cancer patients in the cohort after matching individually on the basis of year of birth (5-year strata), year of diagnosis (4-year strata), registry, and race. Controls also were counter-matched in a ratio of 2 to each contralateral breast cancer case on the basis of whether or not they had received radiotherapy treatment as recorded in the cancer registry.\textsuperscript{19} The study was reviewed and approved by local institutional review boards at each of these registry sites, and all biological samples and data were obtained after the participants provided informed consent.

Recruitment took place during the period from January 2000 to July 2004. A total of 998 women with contralateral breast cancer were eligible and were approached for inclusion in the study, and 708 of these women (71%) agreed to participate. Of the 2112 women who were selected as potential unilateral breast cancer participants, 1399 (66%) agreed to participate. The nonparticipants were similar to the participants with respect to age and calendar year of diagnosis and radiotherapy treatment of the initial primary breast cancer. Successful genotyping was accomplished in 704 participants with contralateral breast cancer and 1394 participants with unilateral breast cancer, and these 2098 individuals represent the probands for the analyses in this article.

All participants were interviewed by telephone using a structured questionnaire. They were questioned about the breast cancer incidence in each of their first- and second-degree relatives. For each relative, the interviewer ascertained the age at diagnosis of breast cancer, the vital status, and the dates of death (if relevant) of the relatives. For the purposes of this article, only the information on female first-degree relatives was used to restrict the analysis to relatives for which the data are most likely to have high accuracy.\textsuperscript{20,21}

**Mutation Screening**

Coding and flanking intronic regions were screened for mutations or polymorphic variants by denaturing high-performance liquid chromatography (DHPLC). \textit{BRCA1} (GenBank No. U14680) was covered by 30 PCR amplicons, while 41 amplicons were used for \textit{BRCA2} (GenBank No. NM_000059). The majority of fragments were run at more than 1 DHPLC elution temperature condition for increased sensitivity. A few fragments were screened by direct sequencing because of complex melting profiles unsuitable for DHPLC or because of the presence of multiple common variants and combinations thereof that made interpretation of chromatograms difficult. With the exception of the prevalent polymorphic variants (occurring in ≥10% of samples) with distinguishable chromatograms, all vari-
ant DHPLC results (extra, shoulder, widened, or shifted peaks) were fol-
lowed up by direct sequencing of the appropriate amplicons.

Three laboratories performed the screening using fixed sets of primers and
DHPLC protocols. Consistency in screening between and within laboratories was
ensured via a laboratory quality-control plan including (1) blinded screening of
an initial set of 21 positive controls by all laboratories; (2) initial screening of the
same randomly selected 21 samples by all laboratories; (3) rescreening by 1 lab-
atory of a randomly selected 10% sample of all cases screened each at the partici-
pating laboratories; and (4) blinded re-
screening of a random 10% sample of each
laboratory’s own sample by that same
laboratory.22

The analyses are focused exclu-
sively on those sequence variants that are
considered to have a deleterious
effect based on current evidence. Spec-
cifically, the following sequence vari-
ant categories were classified as dele-
terious: (1) changes known or predicted
to truncate protein production includ-
ing all frameshift and nonsense vari-
ants with the exception of BRCA2
K3326X and other variants located 3’
thereof; (2) splice site mutations oc-
curring within 2 base pair of an intron/
exon boundary or shown to result in ab-
erant splicing; and (3) missense
changes that have been demonstrated
to have a deleterious effect on, for ex-
ample, the function of the
BRCA1 finger and BRCT domains. The classi-
fication of missense changes of un-
known clinical significance is an on-
going challenge in the field and we
recognize that a small portion of the nu-
merous missense changes identified and
scored as unclassified variants may ac-
tually be deleterious. Our approach to
classifying mutations as deleterious is
comparable with that used in the cli-
nican care sector and it is compatible with
classifications used by the Breast
Cancer Information Core (http://research
.nhgri.nih.gov/projects/bic/). No at-
tempt was made to screen for larger
genomic deletions or duplications.

Thus, some deleterious mutations may
have escaped detection due to techni-
cal reasons or location in a region not
covered by the current methodologi-

Statistical Analysis
All data analyses involve the inci-
dence rates of breast cancer in the iden-
tified first-degree biological relatives of
the probands (parents, full siblings, and
children). These rates exclude the pro-
band, although the analyses involve
subgroups defined by characteristics of the
proband. Person-years at risk of
breast cancer were determined for each
relative up to the age at diagnosis of
breast cancer, if diagnosed, age at death,
or current age at the time the proband
was interviewed.

To examine risk variation among car-
rriers on the basis of characteristics of the
proband and to construct formal statis-
tical tests for its presence, we con-
ducted Poisson regression analyses of the
incidences of breast cancer in family
members of carrier probands. In these
analyses, the periods at risk were grouped
into 10-year age intervals and stratified
on the basis of the relationship of the rela-
tive to the proband (mother, sister,
daughter). Various characteristics of the
proband, such as contralateral breast can-
cer vs unilateral breast cancer status, age
at diagnosis, and geographic site of re-
cruitment (United States vs Denmark),
also were included as covariates.

The analyses also adjusted for the lo-
cation of the individual mutation on the
BRCA1 or BRCA2 genes. Mutations on
BRCA1 were grouped into 3 regions:
nucleotides 1 to 2400 (47 probands),
nucleotides 2401 to 4184 (36 pro-
bands), and nucleotides 4185 and above
(26 probands). The BRCA2 mutations
were classified as within the ovarian
cancer cluster region (22 probands with
nucleotides 3059-6629) or not (50 pro-
bands). These classifications are con-
sistent with the meta-analysis by An-
toniou et al.23 To account for residual
variation in risk between carriers in
these analyses, a random effect was in-
cluded for each family in which the ran-
dom effects were assumed to conform to
a normal distribution. In this method,
each family is assumed to have a dis-
tinct risk. The estimated variance of
these random effects was then evalu-
ated for departure from 0 to test for the
presence of unexplained risk varia-
tion. This analysis was performed using
Stata software version 7.0 (StataCorp,
College Station, Texas).

The cumulative incidences of breast
cancer to various ages in relatives of car-
rriers and in relatives of noncarriers were
calculated using the Kaplan-Meier
method. The penetrance (imputed cu-
mulative risk in a defined population
of mutation carriers) was calculated by
the kin-cohort method proposed by
Chatterjee and Wacholder.24 In our
analyses, the penetrance was calcu-
lated in several populations defined by
the observed risk factors. Conceptu-
ally, the method calculates the pen-
etrance as double the rate observed in
the first-degree relatives of carriers (be-
cause approximately half of these will
be carriers), with an adjustment for the
baseline incidence rate in relatives of
noncarrier probands.

As an approximate benchmark for
evaluating the estimated penetrance
curves, a population cumulative inci-
dence curve was constructed to reflect
the population incidence of breast can-
cer. Reported age-specific rates from the
Surveillance, Epidemiology, and End
Results registries were used for this pur-
pose, weighted to account for the cal-
endar periods in which the individual
relatives were at risk for breast cancer.
For calendar periods prior to 1975, we
have used the 1975 rates, which are the
earliest rates reported in the Surveil-
ance, Epidemiology, and End Results
registries. Statistical tests were consid-
ered to be significant if the P value was
less than .05.

RESULTS
Mutation screening of all coding exons
and flanking intronic regions of
BRCA1 and BRCA2 resulted in the
identification of 470 unique sequence
variants, among which a total of 113
unique deleterious mutations were
identified. 57 located in BRCA1 and 56
in BRCA2. Of the 113 unique deleteri-
ous mutations, 73 consisted of small frameshift deletions or insertions predicted to cause protein truncation, 26 were nonsense mutations, and 7 were splice-site mutations. Seven missense mutations were defined as deleterious, including C44S and C61G in the BRCA1 RING domain, R1699W, A1708E, G1738E and M1775R in the BRCA1 BRCT domains, as well as M11, disrupting the translation initiation codon of BRCA2.

Among the 1394 unilateral breast cancer participants, 73 (5.2%) were carriers of deleterious mutations (42 with BRCA1 and 31 with BRCA2) (TABLE 1). Among the 704 participants with contralateral breast cancer, 108 (15.3%) were carriers of deleterious mutations (67 with BRCA1 and 41 with BRCA2). Data were reported for 598 first-degree female relatives of these 181 carrier probands (350 in first-degree female relatives of the 103 carrier probands, although this comparison is not statistically significant (odds ratio [OR], 1.4; 95% confidence interval [CI], 0.8-2.4; P=.28). The magnitudes of the trends are replicated broadly in the separate analyses of BRCA1 and BRCA2 carriers. For the analysis involving BRCA1, there is no evidence that risk is affected by location of the mutation on the gene (P=.99), while for BRCA2, significantly higher breast cancer risks are evident for mutations outside of the ovarian cancer cluster region (P=.03). For BRCA1, sisters (P=.07) and daughters (P=.03) appear to be at a higher risk than mothers. There is no apparent difference in overall risk for BRCA1 vs BRCA2 mutations (OR, 1.1 [95% CI, 0.6-1.8]; data not shown). There is strong evidence of residual between-family variation in risk, even after adjusting for contralateral breast cancer vs unilateral breast cancer status, proband age at diagnosis, and mutation location. This is evidenced by the statistically significant tests of residual between-family variation in all three analyses (P = .004 overall, P = .04 for BRCA1, and P = .03 for BRCA2).

The estimated cumulative risks of breast cancer are displayed in the FIGURE. The curves indicate that relatives of either BRCA1 or BRCA2 mutation carriers have a substantially greater risk than relatives of noncarriers, and

### Table 1. Participant Characteristics (Probands)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Unilateral (n = 1394)</th>
<th>Contralateral (n = 704)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD) [range], y</td>
<td>45.0 (6.2) [23-55]</td>
<td>45.0 (6.3) [24-55]</td>
</tr>
<tr>
<td>Carrier status, No. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRCA1</td>
<td>42 (3.0)</td>
<td>67 (9.5)</td>
</tr>
<tr>
<td>BRCA2</td>
<td>31 (2.2)</td>
<td>41 (5.8)</td>
</tr>
<tr>
<td>Negative status for BRCA carrier, No. (%)</td>
<td>1321 (94.8)</td>
<td>596 (84.7)</td>
</tr>
<tr>
<td>Race/ethnicity, No. of participants/No. of carriers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>1283/64</td>
<td>645/94</td>
</tr>
<tr>
<td>Hispanic white</td>
<td>48/4</td>
<td>24/7</td>
</tr>
<tr>
<td>Black</td>
<td>39/4</td>
<td>21/3</td>
</tr>
<tr>
<td>Asian</td>
<td>22/0</td>
<td>13/4</td>
</tr>
<tr>
<td>Other</td>
<td>2/1</td>
<td>1/0</td>
</tr>
</tbody>
</table>

### Table 2. Aggregation of Breast Cancer in Families of WECARE Study Probands

<table>
<thead>
<tr>
<th>Requirement</th>
<th>3</th>
<th>2</th>
<th>1</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>All probands</td>
<td>10 (0.5)</td>
<td>73 (4)</td>
<td>449 (21)</td>
<td>1565 (75)</td>
</tr>
<tr>
<td>BRCA1 carriers</td>
<td>1 (1)</td>
<td>14 (13)</td>
<td>30 (28)</td>
<td>64 (58)</td>
</tr>
<tr>
<td>BRCA2 carriers</td>
<td>2 (3)</td>
<td>5 (8)</td>
<td>23 (31)</td>
<td>41 (58)</td>
</tr>
<tr>
<td>Unilateral breast cancer probands</td>
<td>2 (1)</td>
<td>39 (3)</td>
<td>260 (19)</td>
<td>1093 (78)</td>
</tr>
<tr>
<td>BRCA1 carriers</td>
<td>0</td>
<td>4 (10)</td>
<td>13 (31)</td>
<td>25 (60)</td>
</tr>
<tr>
<td>BRCA2 carriers</td>
<td>0</td>
<td>2 (6)</td>
<td>10 (32)</td>
<td>19 (61)</td>
</tr>
<tr>
<td>Contralateral breast cancer probands</td>
<td>8 (1)</td>
<td>34 (5)</td>
<td>189 (27)</td>
<td>472 (67)</td>
</tr>
<tr>
<td>BRCA1 carriers</td>
<td>1 (2)</td>
<td>10 (15)</td>
<td>17 (26)</td>
<td>39 (58)</td>
</tr>
<tr>
<td>BRCA2 carriers</td>
<td>2 (5)</td>
<td>3 (10)</td>
<td>13 (30)</td>
<td>22 (65)</td>
</tr>
</tbody>
</table>

Abbreviation: WECARE, Women’s Environmental Cancer and Radiation Epidemiology.
that relatives of case (contralateral breast cancer) probands have higher risk than relatives of control (unilateral breast cancer) probands, regardless of carrier status. Both of these differences are statistically significant in a Poisson regression analysis, similar in structure to Table 3, but which includes the families of all noncarrier and carrier probands (BRCA1 vs noncarriers: OR, 2.4 [95% CI, 1.7-3.5]; BRCA2 vs noncarriers: OR, 2.6 [95% CI, 1.7-4.0]; contralateral breast cancer vs unilateral breast cancer: OR, 1.7 [95% CI, 1.4-2.0]).

Penetrance estimates in mutation carriers are imputed from these curves (Table 4). From relatives of unilateral breast cancer probands, the penetrance is estimated to be 20% by age 50 years, increasing to 40% by age 70 years, and 50% by age 80 years. The corresponding penetrance estimates from relatives of contralateral breast cancer probands are 32% by age 50 years, 51% by age 70 years, and 57% by age 80 years. Table 4 also displays the penetrance estimates obtained separately from relatives of carriers diagnosed in distinctive age ranges.

The quantitative impact on the penetrance of the observed between-family residual risk variation can be interpreted as follows. Table 4 shows that the average risk to age 70 years in a first-degree relative of a unilateral breast cancer proband is 40%. Our random-effects analysis demonstrates that the actual risks in individual carrier families may be much higher or much lower than this average value. In fact, assuming a constant risk of breast cancer from age 30 years to age 70 years in carriers, our random-effects variance of 0.90 (Table 3) implies that carriers in carrier families at the upper 95th percentile have risks similar to the population risk of breast cancer.

**COMMENT**

Our study is one of the largest individual population-based family studies to date to address the breast cancer risks in BRCA1 and BRCA2 carriers, comprising 181 carrier probands, with a total of 103 breast cancers reported in the 598 first-degree female relatives of these probands. We examined variation of risk between carrier families by determining whether distinct risk profiles can be identified when carrier families are sorted by observed characteristics of the probands. We observed a statistically significant trend of increasing risk with decreasing age at diagnosis of the proband (P = .04). Furthermore, there is strong evidence of residual variation in risk between carrier families due to unobserved risk fac-

**Table 3. Relative Risks of Breast Cancer for Female Relatives of BRCA1 and BRCA2 Mutation Carriers**

<table>
<thead>
<tr>
<th>Factora</th>
<th>Relatives of All Carrier Probandsb</th>
<th>Relatives of BRCA1 Probandsb</th>
<th>Relatives of BRCA2 Probandsb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Person-Years</td>
<td>OR (95% CI)</td>
<td>Person-Years</td>
</tr>
<tr>
<td>Breast cancer proband</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unilateral</td>
<td>35 12,027 1 [Reference]</td>
<td>0.28 21 6516 1 [Reference]</td>
<td>.43 14 5511 1 [Reference]</td>
</tr>
<tr>
<td>Contralateral</td>
<td>68 16,021 1.4 (0.8-2.4)</td>
<td>40 9661 1.3 (0.7-2.7)</td>
<td>28 6360 1.4 (0.6-3.4)</td>
</tr>
<tr>
<td>Age group at diagnosis, y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>45-54</td>
<td>28 9407 1 [Reference]</td>
<td>35 8806 1.9 (0.8-4.7)</td>
<td>18 6010 1 [Reference]</td>
</tr>
<tr>
<td>35-44</td>
<td>53 13,703 1.8 (0.9-3.3)</td>
<td>35 896 1.9 (0.8-2.5)</td>
<td>25 18 4987 1.5 (0.6-3.9)</td>
</tr>
<tr>
<td>&lt;35</td>
<td>22 4938 2.2 (1.0-4.7)</td>
<td>16 3974 1.9 (0.7-5.2)</td>
<td>6 964 3.6 (1.0-13.3)</td>
</tr>
<tr>
<td>Relationship</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mothers</td>
<td>52 11,778 1 [Reference]</td>
<td>27 7094 1 [Reference]</td>
<td>25 4884 1 [Reference]</td>
</tr>
<tr>
<td>Sisters</td>
<td>47 12,015 1.5 (1.0-2.4)</td>
<td>30 6824 1.7 (1.0-3.2)</td>
<td>17 5091 1.1 (0.5-2.4)</td>
</tr>
<tr>
<td>Daughters</td>
<td>4 4255 1.5 (0.5-4.9)</td>
<td>4 2159 4.6 (1.3-16.0)</td>
<td>0 2096 NA</td>
</tr>
<tr>
<td>Country</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>United States</td>
<td>80 21,048 1 [Reference]</td>
<td>51 12,841 1 [Reference]</td>
<td>29 8207 1 [Reference]</td>
</tr>
<tr>
<td>Denmark</td>
<td>23 7,009 0.7 (0.4-1.3)</td>
<td>10 3336 0.6 (0.3-1.5)</td>
<td>13 3664 0.8 (0.3-1.9)</td>
</tr>
<tr>
<td>BRCA1 range</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2401-1418</td>
<td>20 5488 1.0 (0.5-2.1)</td>
<td>20 5488 1.0 (0.5-2.1)</td>
<td>20 5488 1.0 (0.5-2.1)</td>
</tr>
<tr>
<td>&gt;1415</td>
<td>14 3746 1.0 (0.4-2.1)</td>
<td>14 3746 1.1 (0.5-2.5)</td>
<td>14 3746 1.1 (0.5-2.5)</td>
</tr>
<tr>
<td>BRCA2 range</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3059-6629</td>
<td>6 3331 1 [Reference]</td>
<td>6 3331 1 [Reference]</td>
<td>6 3331 1 [Reference]</td>
</tr>
<tr>
<td>&lt;3059, &gt;6629</td>
<td>36 8540 3.2 (1.1-9.4)</td>
<td>36 8540 3.2 (1.1-11.5)</td>
<td>36 8540 3.2 (1.1-11.5)</td>
</tr>
<tr>
<td>Random-effects variance</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; NA, data not estimable; OR, odds ratio.

Note: All factors are characteristics of the probands, with the exception of mothers, sisters, and daughters, which are characteristics of the relatives in the analyses.

The frequencies and person-years of follow-up refer to the occurrences of breast cancer, and years at risk in the first-degree relatives of the 181 carrier probands in the study. All P-values reported are likelihood ratio tests that examine the significance of the indicated factor in affecting risk, after adjusting for the other listed factors in a multivariate model. For diagnosis age and BRCA1 mutation location, linear trend tests were used.

Estimated variance of the between-family random effects. The magnitude of the estimated variance represents the variance of the distinct relative risks estimated for each of the families on a logarithmic scale.
tors on the basis of a statistically significant random-effects variance, even after accounting for observable proband characteristics ($P = .004$). We observe that risks in relatives of contralateral breast cancer probands are higher than risks in relatives of unilateral breast cancer probands ($P < .001$), although this comparison is not statistically significant when conducted solely in the carrier families ($P = .28$).

These trends are consistent with the hypothesis that risks to $BRCA1$ or $BRCA2$ mutation carriers vary substantially due to the presence of additional unknown risk factors for breast cancer, which are more prevalent in the families of women diagnosed at a younger age, and in the families of women with contralateral breast cancer. These unknown factors, which could include variants in candidate genes such as ATM or CHEK2 or other unknown genes, may ultimately explain the strong familial clustering in the families exhibiting multiple cases of breast cancer, the preponderance of which are not linked to either $BRCA1$ or $BRCA2$ mutations.

Our results complement recent studies that examined risk variation in carriers on the basis of factors such as parity, age at first live birth, breastfeeding, and mammographic density. Although the results from those studies are not fully consistent, they suggest that the relative risks conferred by these risk factors in carriers may be similar to the relative risks in noncarriers. In a recent study, Chen and Parmigiani have examined between-study heterogeneity of $BRCA1$ and $BRCA2$ risks, but we emphasize that our analyses address between-family risk variation. Our results underscore the conclusion that there is no single risk associated with $BRCA1$ or $BRCA2$ carrier status. On the contrary, risks for carriers vary substantially based on observable factors, such as the characteristics of the affected relatives (proband in our case) examined in this study, host factors such as the preceding ones, and other undetermined factors.

An alternative explanation for the observed risk variation is the possibility that individual variants in the $BRCA1$ and $BRCA2$ genes lead to substantially different breast cancer risks. In our study, we adjusted for potential within-gene effects of this nature by classifying the variants broadly using their position on the gene. We observed no trend for the location of $BRCA1$ mutations, but mutations in $BRCA2$ outside the ovarian cancer clus-

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**Table 4.** Estimates of Risk (Penetrance) of Breast Cancer for $BRCA1/2$ Carriers Classified by Proband Characteristics

<table>
<thead>
<tr>
<th>Proband Characteristics</th>
<th>Estimated Cumulative Risk (95% CI), %, by Age, y</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50</td>
</tr>
<tr>
<td>Breast cancer proband</td>
<td></td>
</tr>
<tr>
<td>Unilateral</td>
<td>20 (11-32)</td>
</tr>
<tr>
<td>Contralateral</td>
<td>32 (20-44)</td>
</tr>
<tr>
<td>$BRCA1$ proband</td>
<td></td>
</tr>
<tr>
<td>Unilateral</td>
<td>30 (16-46)</td>
</tr>
<tr>
<td>Contralateral</td>
<td>38 (23-54)</td>
</tr>
<tr>
<td>$BRCA2$ proband</td>
<td></td>
</tr>
<tr>
<td>Unilateral</td>
<td>9 (2-22)</td>
</tr>
<tr>
<td>Contralateral</td>
<td>22 (6-40)</td>
</tr>
<tr>
<td>Proband age group, y</td>
<td></td>
</tr>
<tr>
<td>&lt;35</td>
<td>34 (16-66)</td>
</tr>
<tr>
<td>35-44</td>
<td>32 (20-44)</td>
</tr>
<tr>
<td>≥45</td>
<td>14 (5-24)</td>
</tr>
</tbody>
</table>

Abbreviation: CI, confidence interval.
ter region were shown to have substantially elevated breast cancer risk compared with mutations within it. However, our sensitivity for exploring variations at the level of the individual mutation is low due to the low frequencies of occurrence of individual variants.

Regardless of whether risk variation within BRCA1 and BRCA2 contributes meaningfully to the overall risk variation observed, it seems likely that other genetic factors play a major role. This conclusion is supported by a recent detailed review of studies that addressed this issue.\textsuperscript{31,32} Also, recently published genome-wide association studies suggest elevated breast cancer risk at several candidate loci.\textsuperscript{33,34} Furthermore, the fact that the preponderance of familial clustering occurs in families in which the proband is not a BRCA1 or BRCA2 carrier, a phenomenon discussed in depth in an earlier investigation by Cui and Hopper,\textsuperscript{35} also points to the existence of unexplained risk variation in the entire sample. It is also possible that some of the risk variation is due to environmental or lifestyle factors that aggregate in families, such as age at first birth, and that also act as modifiers of risk in carriers, although a genetic explanation is more plausible.\textsuperscript{36}

The overall penetrance estimates for BRCA1 or BRCA2 mutation carriers from our study are consistent with the literature on this topic from other population-based studies, but are at the low end of a very broad range that has been reported. The most comprehensive study of this type is the pooled analysis of 22 studies by Antoniou et al.\textsuperscript{37} These authors derive a risk to age 70 years of 65% in BRCA1 carriers and 45% in BRCA2 carriers. Their analysis included hospital-based as well as population-based studies, many of which used probands with early onset breast cancer (similar to our study), and some with ovarian cancer or male breast cancer probands.

Although our study is population-based, the sampling of probands was unusual, and this could affect the results through unforeseen selection effects. Probands were selected if they were alive and eligible during the recruitment period between 2000 and 2004, if they had a diagnosis of breast cancer from 1985 onward (2 diagnoses for contralateral breast cancer probands), and if they had no prior cancer other than breast cancer. This corresponds to a single ascertainment family-based design,\textsuperscript{38} and it could lead to overestimates of risk if families were inadvertently ascertained twice. We attempted to compare, in an algorithmic fashion, the family information of all pairs of probands, but this search revealed only 1 pair of sisters among the probands, confirmed on follow-up. Thus, we believe that double counting of members is not a concern requiring statistical adjustment.\textsuperscript{39}

The ascertainment of probands who have survived sufficiently long to be eligible for the study could lead to a selection bias if some of the heritable factors affecting cancer risk also affect prognosis, but we have no way to test this assumption. Our recruitment of women with a relatively young age at diagnosis is likely to have led to generally higher risk estimates from their relatives than would be expected in a study involving women of unrestricted age at diagnosis. Interestingly, we observed a significantly higher risk in sisters of probands than in mothers. There is no obvious explanation for this finding, although it is consistent with a previous meta-analysis.\textsuperscript{40} This trend also was observed when we analyzed noncarrier probands (data not shown). Finally, our analysis is based on first-degree relatives, and so information on risk factors of these relatives is unavailable, except for age.

Our results imply that the risk of breast cancer in carriers who might be identified at random in the population without evidence of familial breast cancer may be even lower than the 40% at age 70 years that we estimate from families of probands with unilateral breast cancer. It is reasonable to infer that the reduction in risk from the estimates in contralateral breast cancer probands to the estimates in unilateral breast cancer probands may be mirrored in a corresponding reduction if we were able to measure risk in carriers identified from unselected population disease-free controls. Although population-based screening for these mutations is not recommended at this time, it is possible that in the future, as technology advances and genotyping costs are reduced, widespread genetic screening for important risk factors for breast cancer and other diseases may become routine, and will likely serve as the foundation for tailored risk reduction interventions. For this reason, accurate estimation of the risks conferred in the population and identification of important sources of variation in these risks constitute important scientific goals with significant implications for the clinical management of female carriers of BRCA1 or BRCA2 mutations.

Author Contributions: Drs Begg and Sima had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Begg, Haile, Borg, L. Bernstein, Olsen, Anton-Culver, J. Bernstein.

Acquisition of data: Borg, Malone, Concannon, Langholz, L. Bernstein, Olsen, Lynch, Anton-Culver, Liang, J. Bernstein.

Analysis and interpretation of data: Begg, Haile, Borg, Thomas, L. Bernstein, Capanu, Liang, Hummer, Sima, J. Bernstein.

Drafting of the manuscript: Begg, Hummer, Sima.

Critical revision of the manuscript for important intellectual content: Haile, Borg, Malone, Concannon, Thomas, Langholz, L. Bernstein, Olsen, Lynch, Capanu, Liang, J. Bernstein.

Statistical analysis: Begg, Thomas, Langholz, Capanu, Hummer, Sima.

Obtained funding: Haile, Malone, Concannon, L. Bernstein, Olsen, J. Bernstein.

Administrative, technical, or material support: Haile, Borg, L. Bernstein, Lynch, Liang, J. Bernstein.

Study supervision: Begg, J. Bernstein.

Financial Disclosures: None reported.

Funding/Support: The study was supported by awards CA097397, CA083178, and CA098438 from the National Cancer Institute.

Role of the Sponsor: The National Cancer Institute had no role in the design and conduct of the study, the collection, management, analysis, and interpretation of the data, or in the preparation, review, and approval of the manuscript, other than with respect to the review of the grant application at the initiation of the project.

WECARE Study Collaborative Group: Coordinating Centers: Memorial Sloan-Kettering Cancer Center (New York, New York): Jonine L. Bernstein, PhD (WECARE Study primary investigator), Xiaolin Liang, MD, MS (informatics specialist), Abigail Wolitzer, MSPH (project director); National Cancer Institute (Bethesda, Md): Daniela Seminara, PhD, MPH (program officer). Laboratories: University of Virginia (Charlottesville): Patrick Concannon, PhD (primary investigator), Sharon Teraoka, PhD (laboratory director), Eric R. Olson (laboratory manager); University of Southern Californ-
REFERENCES


