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Contribution of *BRCA1* and *BRCA2* Mutations to Breast and Ovarian Cancer in Pakistan

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The population of Pakistan has been reported to have the highest rate of breast cancer of any Asian population (excluding Jews in Israel) and one of the highest rates of ovarian cancer worldwide. To explore the contribution that genetic factors make to these high rates, we have conducted a case-control study of 341 case subjects with breast cancer, 120 case subjects with ovarian cancer, and 200 female control subjects from two major cities of Pakistan (Karachi and Lahore). The prevalence of *BRCA1* or *BRCA2* mutations among case subjects with breast cancer was 6.7% (95% confidence interval [CI] 4.1%–9.4%), and that among case subjects with ovarian cancer was 15.8% (95% CI 9.2%–22.4%). Mutations of the *BRCA1* gene accounted for 84% of the mutations among case subjects with ovarian cancer and 65% of mutations among case subjects with breast cancer. The majority of detected mutations are unique to Pakistan. Five *BRCA1* mutations (2080insA, 3889delAG, 4184del4, 4284delAG, and IVS14-1A→G) and one *BRCA2* mutation (3337C→T) were found in multiple case subjects and represent candidate founder mutations. The penetrance of deleterious mutations in *BRCA1* and *BRCA2* is comparable to that of Western populations. The cumulative risk of cancer to age 85 years in female first-degree relatives of *BRCA1*-mutation–positive case subjects was 48% and was 37% for first-degree relatives of the *BRCA2*-mutation–positive case subjects. A higher proportion of case subjects with breast cancer than of control subjects were the progeny of first-cousin marriages (odds ratio [OR] 2.1; 95% CI 1.4–3.3; $P = .001$). The effects of consanguinity were significant for case subjects with early-onset breast cancer (age <40 years) (OR = 2.7; 95% CI 1.5–4.9; $P = .0008$) and case subjects with ovarian cancer (OR = 2.4; 95% CI 1.4–4.2; $P = .002$). These results suggest that recessively inherited genes may contribute to breast and ovarian cancer risk in Pakistan.

Introduction

Carcinoma of the breast is the most common cancer among women in Pakistan, with incidence rates that resemble those reported in the West (Malik et al. 1992; Bhurgri et al. 2000). Affected women are typically young and often present with advanced disease (Usmani et al. 1996; Ahmed et al. 1997). The age-standardized (world) rate (ASR) of breast cancer in Karachi, Pakistan, is 51.7 per 100,000 per year and is the highest ASR reported for any Asian population, excluding Israel (Parkin et al. 1997; Bhurgri et al. 2000). All other Asian registries report breast cancer rates <40 per 100,000 per year, except for Manila in the Philippines (47.7 per 100,000 per year) (Parkin et al. 1997). The breast cancer rates reported in provinces of neighboring India—ranging from 8.7 per 100,000 per year, in Barshi, Paranda, and Bhum, to 28.2 per 100,000 per year, in Bombay—are considerably lower than those in Pakistan (Parkin et al. 1997).

In North America, age-specific rates of breast cancer double approximately every 10 years until menopause, after which the rates continue to increase slowly. This pattern of rapid premenopausal growth is also seen in Pakistan, but breast cancer risk plateaus after age 45 years. This difference in the slope of the age-specific–incidence curves in Eastern and Western populations indicates that premenopausal breast cancer is relatively more common among Asian populations. Studies on the Pakistani population may further our understanding of the etiology of breast cancer in young Asian women.

Carcinoma of the ovary is the most common cancer of gynecologic origin in Pakistan (Bhurgri et al. 2000). The ASR of ovarian cancer in Karachi, Pakistan (10.2 per 100,000 per year), is comparable to that of Ontario, Canada (10.7 per 100,000 per year) (Parkin et al. 1997; Bhurgri et al. 2000). In contrast, adjusted ovarian cancer rates in India range from 1.2 per 100,000 per year,

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in Barshi, Paranda, and Bhum, to 7.2 per 100,000 per year, in Bombay.

The reasons for the relatively high rates of cancers of the breast and the ovary in Pakistan are not known. Lifestyle and reproductive factors may influence the incidence of breast and ovarian cancer, but the specific factors have not been identified. It is also possible that genetic factors, such as mutations in the breast and ovarian cancer–susceptibility genes *BRCA1* (MIM 113705) and *BRCA2* (MIM 600185), may contribute to a significant proportion of breast and ovarian cancer susceptibility. Pakistan has one of the highest rates of consanguinity in the world (Hashmi 1997). The overall frequency of consanguineous marriages is 60%–76% in Pakistan, and that frequency is 75% among Pakistanis living in the United Kingdom (Darr and Modell 1988; Hashmi 1997; Hussain and Bittles 1998). Inbreeding is known to increase the risk of diseases caused by homozygosity of deleterious recessive genes. Parental consanguinity has been implicated in 60% of mortality and severe morbidity in Pakistani children born in Britain, and autosomal recessive disorders affect 3.7% of all Pakistani children (Powell et al. 1995). An excess of childhood cancers was also reported among children of consanguineous marriages in Britain (Powell et al. 1995). There is little information on the possible role that recessive genes play in adult cancer. One study from Pakistan has described an association between consanguinity and the risk of breast cancer (Shami et al. 1991). Study of the Pakistani population offers the potential to explore the contribution that consanguinity makes to breast and ovarian cancer rates.

To date, no investigations have been undertaken that explore the contribution, to breast and ovarian cancer susceptibility in Pakistan, of dominant and recessive genetic factors. We conducted a hospital-based case-control study of 341 case subjects with breast cancer, 120 case subjects with ovarian cancer, and 200 unaffected female control subjects from two major cities of Pakistan (Karachi and Lahore).

Subjects and Methods

Subjects and Study Design

Women who have had invasive breast cancer or epithelial ovarian cancer diagnosed were ascertained at the National Cancer Institute (NCIK), in Karachi, from August 1998 to August 2000, and at the Jinnah Hospital (JHL), in Lahore, from August 1999 to August 2001. Case and control subjects were interviewed during hospital visits at in- and outpatient clinics. Women were asked for their pregnancy history; for information on cancer treatment, previous surgeries, selected lifestyle factors, and parental marital relationships (i.e., consanguinity); and for detailed information on family history of cancer. Recruitment occurred during a 1-year period at each center. All case subjects with cancer were confirmed histologically. JHL is a government-sponsored hospital, whereas NCIK is a private hospital that offers only oncology services. The case subjects were women with breast or ovarian cancer who were treated, during the study period, by the chief medical oncologist at each of the two hospitals (I.A.M. and Z.A.). Prevalent and incident cases were included. In prevalent cases, diagnosis was made >1 year before the date of interview. Case subjects were unselected for age or family history.

Questionnaire data and blood specimens were collected from 341 case subjects with invasive breast cancer and 120 case subjects with invasive epithelial ovarian cancer. JHL contributed 102 case subjects with breast cancer and 54 case subjects with ovarian cancer, and NCIK contributed 239 case subjects with breast cancer and 66 case subjects with ovarian cancer. The majority of case subjects represented incident cases (i.e., were interviewed within 1 year of diagnosis); case subjects who represented prevalent cases comprised 11% of ovarian cancer cases and 25% of breast cancer cases.

For the evaluation of the potential importance of consanguinity as a risk factor for breast cancer, a control group was required. This study included hospital-based control subjects who were identified at various in- and outpatient clinics in local noncancer hospitals (Karachi) or in the same hospital (Lahore). The control subjects were 200 women with no previous diagnosis of cancer. The majority (89%) of control subjects were ascertained at various in- and outpatient services from local hospitals in Karachi; 22 (11%) control subjects were from outpatient clinics at JHL. The control group was composed of women attending in- and outpatient services for general medical (20%), antenatal (20%), infectious disease (15.5%), gynecologic (noncancer) (15%), maternity (8%), and surgical (5%) departments of the hospitals. Eleven (5.5%) spouses of male patients with cancer were also included as control subjects. Because consanguinity rates are likely to vary by year of birth, by geographic region, by socioeconomic status, and by ethnic group, data on these variables were collected from case and control subjects. Annual income was considered as a surrogate for socioeconomic status.

BRCA1 *and* BRCA2 *Analysis*

Human genomic DNA was isolated from 20 mL of peripheral blood. Blood specimens were sent to the molecular laboratory of the Sunnybrook & Women's College Health Sciences Centre, in Toronto, Canada. A variety of methods were employed to detect the presence of a *BRCA1* or *BRCA2* mutation. Exon 11 of *BRCA1* and exons 10 and 11 of *BRCA2* were screened by pro-

tein-truncation testing (PTT), in all case subjects (see the Genome Database Web site). All mutant bands detected by PTT were confirmed by direct sequencing.

Nine case subjects who had two or more relatives with either breast cancer diagnosed at age <50 years or ovarian cancer and in whom no mutation was found were selected for complete screening by the direct sequencing of all coding regions at Myriad Genetics. Five deleterious mutations were identified for these high-risk patients. All five mutations detected at Myriad Genetics were then analyzed for all cases; these were mutations found in exons 11, 12, and 15 (IVS14) of *BRCA1* and exon 22 of *BRCA2*. Furthermore, we tested all case subjects for mutations in exons 2 and 20 of *BRCA1,* to detect additional mutations (specifically, the 185delAG mutation, which has previously been described in two patients of Pakistani ethnicity with cancer)—exon 20 of *BRCA1* covers another founder mutation (5382insC) in the Jewish population (Bar-Sade et al. 1998; Risch et al. 2001). Analysis of *BRCA1* exons 2, 12, 15 (IVS14), and 20 and *BRCA2* exon 22 was performed by denaturing gradient gel electrophoresis (Fodde and Losekoot 1994). PCR primers were fluorescently labeled, and the PCR fragments were visualized by use of a phosphorimager. In total, we screened *BRCA1* exons 2, 11, 12, 15 (IVS14), and 20 and *BRCA2* exons 10, 11, and 22 in all case subjects. It has been estimated that this testing detects 70%–80% of all germline mutations in the coding regions of these two genes (Peto et al. 1999).

Control subjects were tested for the five recurrent mutations—located in exon 11 of *BRCA2* and exons 11 and 15 (IVS14) of *BRCA1*—observed in case subjects. Exons 2 and 20 of *BRCA1* were also screened in control subjects.

Statistical Analysis

The observed number of cancer cases in first-degree relatives was determined by review of family pedigrees. Kaplan-Meier survival analysis was used for the calculation of the cumulative incidence of cancer in first-degree relatives of *BRCA1*- and *BRCA2*-mutation–positive case subjects. First-degree female relatives were considered to be at risk for cancer from birth until death (or until age at date of interview of the proband). The log-rank test was used to assess the statistical significance of differences in survival curves. The relative risk (RR) of cancer among first-degree relatives of carriers were estimated by use of the Cox proportional hazards model. For nominal data, a χ^2 test was used, and, for comparison of continuous variables, a *t* test was used. The significance level was set at .05, two sided. Statistical analysis was performed using SPSS (version 11) for Windows.

Details on parental consanguinity were available for 97.7% of case subjects. Parental consanguinity was classified into four main types: no consanguinity, first cousins or closer (e.g., double-first-cousin marriages), second cousins, and third or more-distant cousins. Logistic regression was used to generate an estimate of the effect that parental consanguinity has on breast cancer risk. In this analysis, covariates included age, ethnic group, and annual income. Ethnic group was determined by three categories: Punjabi, Sindhi, or other. There were three categories of annual income: low (up to 50,000 Rs [~$900]), high (at least 300,000 Rs [~$5,000]), and

Table 1

Frequency of Mutations in Cases of Breast and Ovarian Cancer by Age at Diagnosis

Patient Groups Based on Type of Cancer and Age (in years)	Total No. of Patients	No. (%) Positive for Mutation in		
		BRCA1	*BRCA2*	Either *BRCA1* or *BRCA2*
Breast cancer:				
20–29	23	1 (4.3)	0 (.0)	1 (4.3)
30–39	136	11 (8.1)	3 (2.2)	14 (10.3)
40–49	137	3 (2.2)	3 (2.2)	6 (4.4)
50–59	29	0 (.0)	0 (.0)	0 (.0)
⩾60	16	0 (.0)	2 (1.3)	2 (1.3)
All ages	341	15 (4.4)	8 (2.3)	23 (6.7)
Ovarian cancer[a]:				
20–29	15	0 (.0)	0 (.0)	0 (.0)
30–39	18	2 (11.1)	0 (.0)	2 (11.1)
40–49	41	6 (14.6)	2 (4.9)	8 (19.5)
50–59	32	5 (15.6)	1 (3.1)	6 (18.8)
⩾60	14	3 (21.4)	0 (.0)	3 (21.4)
All ages	120	16 (13.3)	3 (2.5)	19 (15.8)

[a] Included here, four cases with separate primary cancers of the breast and ovary.

Table 2

Characteristics of *BRCA1-/BRCA2*-Mutation Carriers from Pakistan

Subject (Mutation Carried; Location)[a]	Age at Onset (years)	Ethnicity	Family History[b]				
			BR	<50	⩾50	OV	Other
BR [*n* = 341]:							
BRCA1 mutation:							
47 (IVS14-1G→A)	35.5	Pashtun	1	1	0	0	2
49 (IVS14-1G→A)	39.9	Punjabi	7	3	4	5	4
90 (2080insA; exon 11)	35.5	Pashtun	3	3	0	2	5
133 (2080insA; exon 11)	38.5	Pashtun	3	3	0	2	5
102 (1912T→G; exon 11)	45	Mohajir	0	0	0	0	2
121 (185delAG; exon 2)	47.9	Punjabi	0	0	0	0	1
113 (1616delAAAT; exon 11)	35.6	Mohajir	0	0	0	1	3
204 (4284delAG; exon 12)	39.4	Mohajir	3	3	0	0	5
287 (4284delAG; exon 12)	40.4	Mohajir	2	1	1	0	0
316 (4184del4; exon 11)	35.1	Sindhi	0	0	0	0	0
1128 (4184del4; exon 11)	30.4	Punjabi	1	1	0	0	0
1056 (4184del4; exon 11)	31.4, 32.3[c]	Punjabi	0	0	0	0	0
1019 (1476delG; exon 11)	37	Punjabi	1	1	0	0	1
1071 (2041insA; exon 11)	21	Punjabi	0	0	0	3	1
1119 (3889delAG; exon 11)	38.5	Punjabi	1	1	0	0	0
BRCA2 mutation:							
75 (5302insA; exon 11)	48.4	Punjabi	1	0	1	0	1
83 (9140delA; exon 22)	40.3	Mohajir	1[d]	0	1[d]	0	0
223 (3337C→T; exon 11)	39.2	Memon	1	0	1	0	1
158 (3337C→T; exon 11)	40	Memon	1	0	1	0	3
168 (3913delG; exon 11)	62.1	Sindhi	2	0	2	0	2
1069 (5950delCT; exon 11)	39.7	Punjabi	0	0	0	0	0
1155 (6696delTC; exon 11)	61.4	Punjabi	0	0	0	3	0
130 (2674delG; exon 11)	39.6	Gujrati/Mohajir	0	0	0	0	0
OV [*n* = 116]:							
BRCA1 mutation:							
3 (4184del4; exon 11)	36.5	Punjabi	0	0	0	0	0
5 (2388delG; exon 11)	43.3	Mohajir	0	0	0	0	0
12 (2080insA; exon 11)	45.5	Pashtun	9	8	1	4	5
15 (894delG; exon 11)	54	Mohajir	0	0	0	0	0
16 (IVS14-1G→A)	60.1	Punjabi	8	4	4	4	4
28 (3405C→T; exon 11)	54.6	Mohajir	1	1	0	0	1
1004 (1956delA; exon 11)	46	Punjabi	1	1	0	0	0
1043 (2041insA; exon 11)	36.8	Punjabi	1	1	0	2	1
1063 (185insA; exon 2)	40.4	Punjabi	0	0	0	0	0
1165 (3889delAG; exon 11)	74.2	Punjabi	0	0	0	0	0
286 (1770insT; exon 11)	70	Baloch	0	0	0	0	0
1079 (4627C→A [S1503X]; exon 15)	40.7	Punjabi	1	1	0	0	0
24 (1013delTG; exon 11)	48.4	Mohajir/Aga Khani	0	0	0	0	1
BRCA2 mutation:							
8 (5057delTG; exon 11)	46.8	Parsi	2	0	2	0	1
305 (3179delA; exon 11)	50	Mohajir	0	0	0	0	0

(*continued*)

Table 2 (*continued*)

Subject (Mutation Carried; Location)[a]	Age at Onset of (years)		Ethnicity	Family History[b]				
	OV	BR		BR	<50	⩾50	OV	Other
BR and OV [n = 4][c]								
*BRCA1*mutation:								
1112 (1127delA; exon 11)	45.9	43.8	Punjabi	1	0	1	0	0
1173 (2266delG; exon 11)	52.2	52.5	Punjabi	0	0	0	0	0
1183 (2722C→G; exon 11)	52.8	35	Kashmiri	1	1	0	0	0
BRCA2 mutation:								
1168 (6679insAA; exon 11)	50	46.5	Punjabi	0	0	0	0	0

Subject (Mutation Carried; Location)[a]	Referral Type	Ethnicity	Family History[b]				
			BR	<50	⩾50	OV	Other
Outside study [n= 3]							
BRCA1 mutation:							
220 (2524delTG; exon 11)	Woman at risk	Sindhi	2	2	0	0	0
1185 (3717C→T; exon 11)	OV (after study closure)	Punjabi	1	0	1	0	1
BRCA2 mutation:							
203 (5869delAAAT; exon 11)	Male BR	Sindhi	4	3	1	0	6

Note.— BR = breast cancer; OV = ovarian cancer.

[a] Four-digit study numbers denote case subjects from JHL; candidate founder mutations are underlined.

[b] <50 = No., in each family, of breast cancers diagnosed at age <50 years; ⩾50 = no., in each family, of breast cancers diagnosed at age ⩾50 years.

[c] Bilateral BR.

[d] Male BR.

[e] Ascertained as incident cases of OV.

middle (determined by the intervening range). Estimates of odds ratios (ORs) were also generated for subgroups of patients, by age at diagnosis, ethnic group, place of residence, and income.

The published cancer rates from the Karachi Cancer Registry were also used to compare cancer risk in carriers of mutations in the *BRCA1* and *BRCA2* genes to risk in the general population (Bhurgri et al. 2000). The expected number of cases was calculated from the age-specific incidence rates from the Karachi Cancer Registry. The RR of cancer in first-degree relatives was estimated by comparison of the observed number of cases to the expected figures. The CIs were calculated with the assumption of a Poisson distribution (Breslow and Day 1987).

Results

The present study includes 341 case subjects with invasive breast cancer, 120 case subjects with invasive epithelial ovarian cancer, and 200 unaffected female control subjects. The mean age at diagnosis of breast cancer was 41.3 years (range 21–81 years). The mean age at diagnosis of ovarian cancer was 46.4 years (range 21–79 years). Among the 341 women diagnosed with breast cancer, 23 (6.7%; 95% CI 4.1%–9.4%) mutations were identified, of which 15 were in *BRCA1* (4.4%; 95% CI 2.2%–6.6%) and 8 were in *BRCA2* (2.3%; 95% CI 0.7%–4.0%) (table 1). A greater proportion of women who had breast cancer diagnosed at age <40 years were carriers (9.4%), compared with those who had breast cancer diagnosed at age ⩾40 years (4.4%) (P = .083). The mean ages at diagnoses of breast cancer in carriers and noncarriers were 40.1 years and 41.4 years, respectively (P = .52). The *BRCA1*-mutation–positive breast cancer case subjects had cancer diagnosed, on average, 10 years earlier than did the *BRCA2*-mutation–positive case subjects—at ages 36.8 and 46.4 years, respectively (P = .01).

Among the 120 women diagnosed with ovarian cancer, 19 (15.8%; 95% CI 9.2%–22.4%) mutations were identified, of which 16 were in *BRCA1* (13.3%; 95% CI

7.2%–19.5%) and 3 were in *BRCA2* (2.5%; 95% CI 1.0%–5.3%). Four women with ovarian carcinoma had previously had invasive breast cancer diagnosed, and all four were mutation positive; one *BRCA2* mutation and three *BRCA1* mutations were identified. In contrast to case subjects with breast cancer, a smaller proportion of women who had ovarian cancer diagnosed at age <40 years were carriers, compared with those in whom diagnosis was made at age ⩾40 years (6.1% vs. 19.5%, respectively; $P = .094$). The *BRCA1-/BRCA2*-mutation–positive case subjects with ovarian cancer were, on average, older than the noncarrier cases. The mean ages at diagnoses of ovarian cancer in carriers and noncarriers were 49.9 and 45.8 years, respectively ($P = .19$). The *BRCA1*- and *BRCA2*-mutation–positive case subjects with ovarian cancer had cancer diagnosed at similar ages, 50.1 and 48.9 years, respectively ($P = .86$). Of note, there were no *BRCA1* or *BRCA2* mutations identified among the 15 women in whom diagnosis was made at age <30 years.

In total, 42 *BRCA1* and *BRCA2* mutations were identified among 461 women diagnosed with either breast or ovarian cancer; 31 (74%) mutations were in *BRCA1,* and 11 (26%) were in *BRCA2* (table 2). No *BRCA1* or *BRCA2* mutations were identified for the 200 control women. We observed 21 distinct *BRCA1* mutations and 10 distinct *BRCA2* mutations; 12 of the 21 *BRCA1* mutations (57%) and 8 of the 10 *BRCA2* mutations (80%) were unique to the Pakistani population (see the Breast Cancer Information Core [BIC] Web site). The other mutations have been described in other populations (table 3). One woman with breast cancer diagnosed at age 47 years carried the *BRCA1* 185delAG mutation.

Five *BRCA1* mutations (2080insA, 3889delAG, 4184del4, 4284delAG, and IVS14-1G→A) and one *BRCA2* mutation (3337C→T) were identified in multiple unrelated patients. The two case subjects with the *BRCA1* 2041insA mutation (1043 and 1071) are related by second degree. The *BRCA1* 2080insA mutation was found in two families, both of Pashtun (Pathan) ethnicity. The two families with the *BRCA2* 3337C→T mutation belong to the Memon ethnic group. The two families with the *BRCA1* 4284delAG mutation belong to the Mohajir ethnic group. The *BRCA1* IVS14-1G→A mutation was found in two families, one of Punjabi background and the other of Pashtun ethnicity. Three of the four patients with the *BRCA1* 4184del4 mutation were of Punjabi descent, and the other, who resided in Karachi, identified herself as Sindhi.

Ten percent of the women with breast or ovarian cancer and 3% of the control subjects reported having a first-degree relative in whom a diagnosis of breast cancer had been made. A family history of breast cancer in any relative was considered as a significant risk factor for breast cancer (OR = 4.0; 95% CI 2.2–7.4; $P = 2 \times 10^{-6}$) and for ovarian cancer (OR = 3.6; 95% CI 1.8–7.4; $P = .00046$). More than one-half (56.2%) of the case subjects with a family history of ovarian cancer were found to carry a mutation in either *BRCA1* or *BRCA2*. However, fewer than one-fourth (23.5%) of the case subjects with a family history of breast cancer were carriers. Of the 461 overall case subjects, 34 (7.4%) belonged to families that had a history of both breast and ovarian cancer. In the families with a history of both breast and ovarian cancer, the probability of detecting a *BRCA1* or *BRCA2* mutation rose significantly with the increase in the number of family members with either breast cancer or ovarian cancer (table 4). Of the 42 case subjects who had cancer associated with *BRCA1* or *BRCA2,* 16 (38%) reported no family history of breast cancer in any relative.

The cumulative cancer risk was estimated in the first-degree relatives of *BRCA1-/BRCA2*-mutation–positive case subjects (because subjects 16 and 49 are mother and daughter, this family was included only once for the cumulative risk analysis). A total of 13,950 person-years was accumulated for 368 first-degree relatives in 41 mutation-positive families. Female first-degree relatives had a higher cumulative cancer incidence (46%; to age 85 years) than did male relatives (25%; to age 85 years; $P = .0072$) (fig. 1*A*). Male patients accounted for one-half of all cancers reported in *BRCA2* families and for 22% of cancers in *BRCA1*-mutation–positive families. In *BRCA2*-mutation–positive families, male and female relatives had similar cumulative risks for cancer of any site. Male patients in *BRCA2*-mutation-positive families developed cancers of the breast, head, and neck, and male patients in *BRCA1*-mutation–positive families developed bladder, pancreatic, and testicular cancers, as well as two cases of leukemia. The cumulative lifetime risk of cancer to age 85 years in first-degree relatives of *BRCA2*-mutation–positive case subjects slightly exceeded that for *BRCA1*-mutation–positive case subjects (fig. 1*B*); however, there was a much greater cancer risk before age 50 years in *BRCA1*-mutation–positive relatives than in *BRCA2*-mutation positive relatives ($P = .0047$). In particular, we observed a higher cumulative incidence of cancer among female first-degree relatives of *BRCA1*-mutation–positive case subjects compared to relatives of *BRCA2*-mutation–positive case subjects (RR = 4.1; 95% CI 1.2–14.0; $P = .014$)—the risk difference was pronounced at age <50 years ($P = .0035$) (fig. 1*C*). In female relatives of *BRCA1*-mutation carriers, 10 of 11 (91%) breast cancers and two of four (50%) ovarian cancers occurred at age <50 years. Among women, the cumulative incidence of breast cancer among first-degree relatives of *BRCA1*-mutation carriers was 22%, and that of ovarian cancer was 15%. There were too few cases of breast or ovarian cancer (only one case of

Table 3

***BRCA1* and *BRCA2* Mutations Reported in Multiple Pakistani and Non-Pakistani Patients**

Mutation	No. of Pakistani Patients (Ethnic Population[s])	No. of BIC Entries[a] (Other Ethnic Population[s])
BRCA1:		
4284delAG	2 (Mohajir)	5 (Dutch, German)
4184delTCAA[b]	4 (Punjabi [3], Pashtun [1])	39 (British, French, European)
3889delAG	2 (Punjabi)	5 (Dutch)
2722C→G (S868X)	1 (Kashmiri)	3 (Dutch)
2388delG	1 (Mohajir)	4 (not specified)
2080insA	3 (Pashtun)	1 (not specified)
185insA	1 (Punjabi)	16 (Dutch, Belgian)
185delAG[c]	1 (Punjabi)	490 (Ashkenazi Jewish)
IVS14-1G→A	3 (Punjabi [2], Pashtun [1])	…
BRCA2:		
3337C→T (Q1037X)	2 (Memon)	… (Chinese)
5950delCT	1 (Punjabi)	13 (German)
6696delTC	1 (Punjabi)	2 (Italian)

[a] See the BIC Web site.
[b] Previously reported in Indo-Pakistani families (Neuhausen et al. 1996).
[c] Previously reported in Pakistani patients (Bar-Sade et al. 1998; Risch et al. 2001).

each) among relatives of *BRCA2*-mutation carriers to construct reliable risk estimates. No cancers were reported at age <50 years for relatives of probands who were *BRCA2*-mutation carriers.

Age- and sex-specific cancer rates from the Karachi Cancer Registry were used for comparison of cancer incidence in the first-degree relatives of *BRCA1*- or *BRCA2*-mutation carriers (table 5) (Bhurgri et al. 2000). These estimates confirm that female *BRCA1*- or *BRCA2*-mutation carriers from Pakistan have a greatly increased risk of cancer at age <40 years.

Parental consanguinity of any type was reported among 42.6% of all subjects, including 44.6% and 38% of case and control subjects, respectively ($P = .12$). A higher proportion of case subjects with breast cancer than of control subjects were the progeny of first-cousin marriages (OR = 2.1; 95% CI 1.4–3.3; $P = .001$) (table 6). The effects were significant for early-onset breast cancer (age <40 years) (OR = 2.7; 95% CI 1.5–4.9; $P = .0008$) and for ovarian cancer (OR = 2.4; 95% CI 1.4–4.2; $P = .0017$). More-distant consanguineous marriages were not significantly associated with cancer status (data not shown).

Thirty-nine percent of case and control subjects were of Punjabi ethnicity, and consanguinity is known to be more common among individuals of this ethnic population of Pakistan (Shami et al. 1989; Bittles et al. 1993; Yaqoob et al. 1993). Forty percent of Punjabi case subjects and 21.3% of Punjabi control subjects were the progeny of a first-cousin marriage ($P = .018$). Table 7 compares the proportions of case and control subjects who reported parental consanguinity at the first-cousin level, by age group, ethnicity, residence, and socioeconomic status. Multivariate analysis was performed, to assess the effect that potential confounders, such as age, ethnic group, and socioeconomic status, have on the observed association between consanguinity and cancer risk. The associations were not altered by the inclusion of the potentially confounding variables in this population.

Discussion

In the major Pakistani cities of Karachi and Lahore, breast cancer constitutes up to one-third of all malignant tumors in female patients, and ovarian cancer is the most common cancer of gynecologic origin (Malik et al. 1992; Bhurgri et al. 2000). The newly established Karachi Can-

Table 4

Proportion, by Family History of Cancer, of *BRCA1-/BRCA2*-Mutation–Positive Probands with Breast and Ovarian Cancer

Family Structure[a]	No. Positive for Mutation in *BRCA1*or *BRCA2*/Total (%)
Breast cancer only:	
1 case	6/261 (2.3)
2 cases	8/54 (14.8)
⩾3 cases	3/16 (18.8)
Breast and ovarian cancer (breast):	
1 case	9/25 (36.0)
2 cases	1/3 (33.3)
⩾3 cases	5/6 (83.3)
Ovarian cancer only:	
1 case	10/96 (10.9)
Breast and ovarian cancer (ovary):	
1 case	7/22 (31.8)
2 cases	2/4 (50.0)
⩾3 cases	6/8 (75.0)

[a] The cancer in the proband is included in the number given.

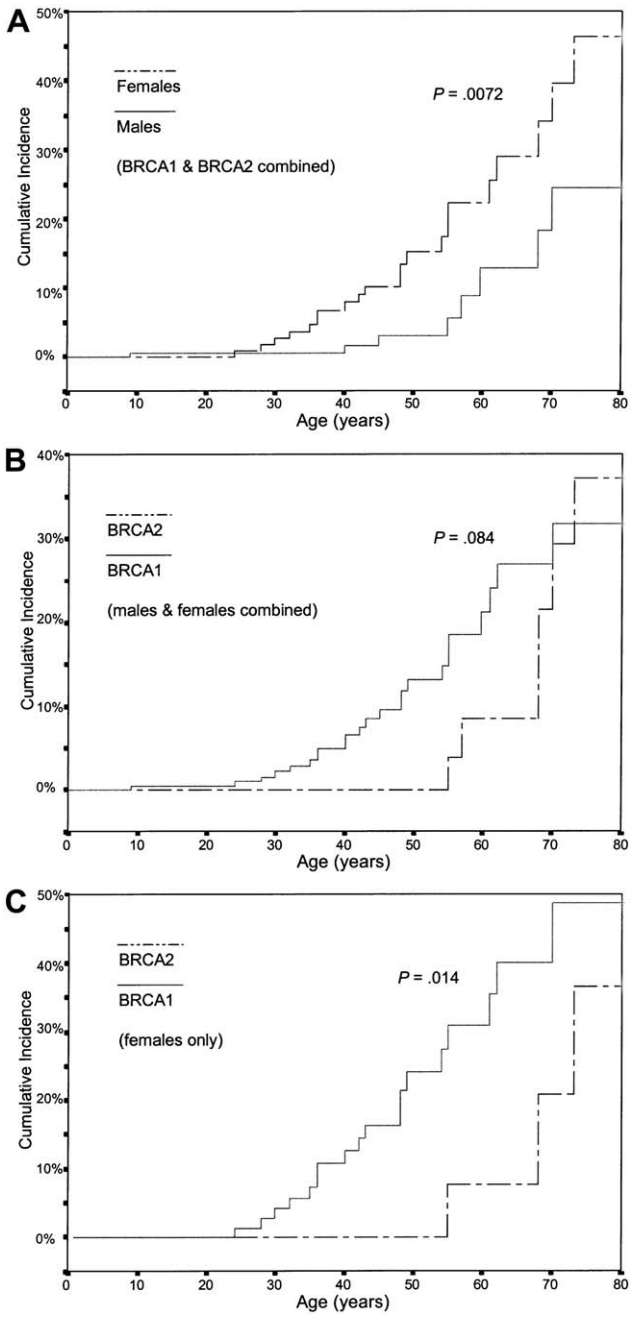

Figure 1 Cumulative incidence of cancer at any site among male (*solid line*) and female (*hatched line*) first-degree relatives of *BRCA1*- and *BRCA2*-mutation carriers (*A*), among first-degree relatives of *BRCA1*-mutation (*solid line*) and *BRCA2*-mutation (*hatched line*) carriers (*B*), and among first-degree relatives of *BRCA1*-mutation (*solid line*) and *BRCA2*-mutation (*hatched line*) carriers (*C*).

cer Registry reported the highest ASR of breast cancer and one of the highest ASRs of ovarian cancer recorded for any Asian population, excluding Israel (Bhurgri et al. 2000). Ours is the first report of the prevalence of *BRCA1* and *BRCA2* mutations in a large series of Pakistani patients with cancer. These patients were drawn from two large medical practices; unfortunately, information was not available on the characteristics of the patients with cancer who attended the hospital but were not included in the study. The patients whom we studied may not represent the greater Pakistani population well in terms of age, ethnic group, and so forth. For example, they were diagnosed at a mean age of 41 years (42.7 years for Karachi patients and 38.2 years for Lahore patients); these ages are less than the average age at onset of breast cancer in Pakistan, and only 5% of these patients had cancer diagnosed at age <60 years.

We identified a total of 42 *BRCA1* and *BRCA2* mutations; under the assumption of 100% sensitivity, this represents mutation prevalences, in Pakistan, of 6.7% for unselected patients with breast cancer and of 15.8% for unselected patients with ovarian cancer. However, the sensitivity of our mutation assay was estimated to be <80%; therefore, the true mutation-prevalence estimates are likely to be close to 8% for breast cancer and 20% for ovarian cancer. If the sensitivity of the assay were <80%, then the mutation prevalence would be even higher. Although they are based on relatively small numbers, these prevalence estimates are among the highest reported to date (Liede and Narod, in press). Mutations in the *BRCA1* gene accounted for 84% of all mutations detected among Pakistani patients with ovarian cancer. The high prevalence of *BRCA1* mutations observed in Pakistani patients with ovarian cancer supports the observation of Risch et al. (2001), who found *BRCA1* mutations in 14% of Indo-Pakistani patients with ovarian cancer who resided in Ontario, Canada. Surprisingly, we did not find that the *BRCA1*-mutation–positive breast cancers were diagnosed at a significantly earlier age than that of the noncarriers (40.1 and 41.4 years, respectively). This situation differs from that of most Western countries and is likely a reflection of the very young average of the Pakistani patients in the series that we studied. Only 5% of the patients in this series were age >60 years; in part, this may be a feature of the selected population under study.

Five *BRCA1* mutations (2080insA, 3889delAG, 4184del4, 4284delAG, and IVS14-1G→A) and one *BRCA2* mutation (3337C→T) were identified in multiple patients and suggest founder effects. Two additional mutation-positive families, ascertained outside the case-control study, are presented in table 2. The majority of mutations described here are unique to Pakistan, but 10 of the 42 mutations have been described in other populations, including four mutations reported for the Dutch population (table 3). The "Jewish" *BRCA1* 185delAG mutation has been described for non-Ashkenazi groups across the Middle East—as well as in Greece, Turkey, and England (Yorkshire)—and in two Indo-Pakistani families (Bar-

Table 5

Cancer Incidence among 368 First-Degree Relatives of 41 *BRCA1-/BRCA2*-Mutation Carriers from Pakistan

Patient Groups Based on Type of Cancer and Age (in years)	Incidence		RR (95% CI)
	Observed	Expected[a]	
Any cancer:			
Male and female patients:			
<40	8	.83	9.7 (4.2–19.0)
⩾40	21	11.6	1.8 (1.1–2.8)
Overall	29	12.4	2.3 (1.6–3.3)
Male patients:			
<40	1	.43	2.3 (0.03–12.9)
⩾40	7	5.2	1.3 (0.54–2.8)
Overall	8	5.7	1.4 (0.61–2.8)
Female patients:			
<40	7	.40	17.6 (7.0–36.2)
⩾40	14	6.4	2.2 (1.2–3.7)
Overall	21	6.8	3.1 (1.9–4.7)
Breast cancer:			
Female patients:			
<40	7	.11	64.6 (25.9–133.1)
⩾40	5	2.3	2.2 (.70–5.1)
Overall	12	2.4	5.0 (2.6–8.7)

[a] Based on data from the Karachi Cancer Registry (Bhurgri et al. 2000).

Sade et al. 1998; Risch et al. 2001). This mutation occurs in an AG repeat region of *BRCA1;* a high mutation rate in this repeat region may explain the different haplotypes that are associated with non-Ashkenazi ethnic groups (Xu et al. 1997; Bar-Sade et al. 1998). The 185delAG mutation was detected in one of the patients with breast cancer who was from Karachi and was of Punjabi descent. Previously, five mutations in *BRCA1* have been reported for Indo-Pakistani families from the United Kingdom and Canada—including 185delAG, 1701del7, 1768delA, 2885delA, and 4184del4 (Neuhausen et al. 1996; Moslehi et al. 1998; Risch et al. 2001).

The *BRCA1* 4184del4 mutation has been reported for families from Britain, France, and elsewhere in Europe, including two Indo-Pakistani families residing in Britain. Haplotype studies determined independent origins for the 4184del4 mutation for the European and Indo-Pakistani families (Neuhausen et al. 1996). The *BRCA1* 4184del4 mutation was the most common mutation described here—detected in four patients and accounting for 13% of all the *BRCA1* mutations in the present study. This mutation was found in three subjects of Punjabi descent and one subject of Sindhi descent. Three mutations—*BRCA1* 3889delAG, *BRCA1* 4284delAG, and *BRCA2* 3337C→T—were specific to the Punjabi, Mohajir, and Memon ethnic groups, respectively. The *BRCA2* 3337C→T mutation has previously been described in three Chinese patients from Hong Kong, although haplotype studies could not establish an ancestral link (Khoo et al. 2000, 2002).

Historically, the region that encompasses Pakistan experienced successive waves of migrations from the northwest, as well as internal migrations across the Indus valley and the Indian subcontinent. In more recent times, the region experienced massive migrations of refugees—from India, after the partition of British India in 1947, and, more recently, from Afghanistan. Pakistani society is composed of many ethnic groups, and many of these are common to the populations of India, Afghanistan, Bangladesh, and Iran. The Pashtun, from the North-West Frontier Province of Pakistan, are estimated at 11 million in number. Approximately the same number of Pashtun live in Afghanistan, where they are the major ethnic group. Two *BRCA1* mutations (2080insA and IVS14-1G→A) were identified among Pashtun case subjects, although only the *BRCA1* 2080insA mutation was found exclusively in Pashtun women. Three *BRCA1* mutations (4184del4, 3889delAG, and IVS14-1G→A) were found among women of Punjabi descent, and one *BRCA1* mutation (4284delAG) was found among women of Mohajir descent.

Nearly one-half of all Pakistanis speak Punjabi. The next most commonly spoken language is Sindhi (12%), followed by the Punjabi variant Siraiki (10%), Pashtu (8%), and Balochi (3%). Although Urdu is the official national language, it is spoken as a native tongue by the <10% of the population who identify themselves as Mohajirs. The ethnic composition of Pakistan corresponds to the linguistic distributions in the population, at least among the largest groups. The subjects whom we studied are representative of the general population

Table 6

Results from Univariate and Multivariate Analyses of Parental Consanguinity

	Parental Consanguinity Results If Parents Are					
		Related		First Cousins or Closer		
					OR (95% CI)	
Patient Group	Not Related, No. (%[a])	No. (%[a])	OR (95% CI), Crude	No. (%[a])	Crude	Adjusted[b]
Control subjects (n = 200)[c]	124 (62.0)	76 (38.0)	…	31 (15.5)	…	…
Case subjects:						
Subjects with breast cancer:						
Age <40 years (n = 159)[d]	72 (48.0)	78 (52.0)	1.56 (.98–2.51)	49 (32.7)	2.72 (1.51–4.88)	2.55 (1.33–4.87)
Age ⩾40 years (n = 182)	110 (61.5)	69 (38.5)	1.31 (.73–2.37)	43 (24.0)	1.64 (.79–3.4)	1.84 (.88–3.85)
Overall (n = 341)	182 (55.3)	147 (44.7)	1.32 (.92–1.89)	92 (28.0)	2.12 (1.35–3.33)	2.24 (1.37–3.66)
Subjects with ovarian cancer (n = 120)	65 (55.6)	52 (44.4)	1.3 (.82–2.07)	36 (30.8)	2.42 (1.4–4.19)	2.41 (1.24–4.67)
BRCA1-/BRCA2-mutation carriers (n = 42)	23 (54.8)	19 (45.2)	1.35 (.69–2.64)	11 (26.2)	1.93 (.88–4.25)	2.04 (.75–5.55)
Noncarriers (n = 419)	224 (55.4)	180 (44.6)	1.31 (.93–1.85)	117 (29.0)	2.22 (1.43–3.45)	2.34 (1.44–3.78)
Overall (n = 461)	248 (55.5)	199 (44.5)	1.32 (.94–1.85)	128 (28.7)	2.19 (1.42–3.39)	2.27 (1.41–3.66)

[a] Percentages were calculated for subjects for whom this information was available.
[b] Adjusted for age, ethnic group, family history (first degree), and socioeconomic status.
[c] Reference group.
[d] Reference group categorized by age groups.

of Pakistan: 39% were Punjabi, 7% were Sindhi, 7% were Pashtun, 4% were Memon, 3% were Gujrati, 3% were Parsi (Zorastrian), and 6% were members of other ethnic groups (Baloch, Kashmiri, etc.). Each ethnic group is primarily concentrated in its home province, with most Mohajirs residing in urban Sindh (Karachi). Since the majority (90%) of control subjects and two-thirds of case subjects were recruited from the Karachi region, we have overrepresentation (31%) of subjects who belong to the Mohajir ethnic group.

Consanguineous marriage is practiced in much of the Muslim world—including North and sub-Saharan Africa, the Middle East, the West, and South Asia—and by migrants from these regions. Approximately 40% of the Pakistani population have practiced consanguinity for ~300 years—typically, inbreeding occurs within thousands of village isolates (Consanguinity and health 1991). Two large studies that have examined adult cancer risk and consanguinity have reported conflicting results. In the first of these studies, Shami et al. (1991) examined consanguinity among 825 outpatients in Islamabad, Pakistan. Parental consanguinity was associated with deafness, mutism, mental retardation, and cancer in adults, and, of the 20 patients with breast cancer, 15 were the progeny of a first-cousin marriage (Shami et al. 1991). Recently, in another study, Denic and Bener (2001) examined consanguinity among 1,445 women who attended primary health care clinics in the United Arab Emirates. A protective effect on breast cancer risk was reported for consanguinity (RR = 0.66; P = .08); however, the effect was statistically significant only for women aged 40–50 years (RR = 0.50; P = .02) (Denic and Bener 2001). In the present study, daughters of parents who were first cousins were at approximately twice the risk of breast and ovarian cancer than were daughters of unrelated parents. Although the control women whom we studied were generally younger, of lower socioeconomic status, and less likely to identify themselves as Punjabi, these factors did not appear to underlie the observed associations for parental consanguinity. We estimate that 20% of cases of

Table 7

Frequency of First-Cousin Marriages in Case and Control Subjects' Parents, Stratified by Subgroup

	No. (%[a]) of		
Subgroup	Case Subjects	Control Subjects	P
Age (in years):			
20–29	16 (42.1)	7 (11.3)	0
30–39	47 (30.5)	13 (18.6)	.04
40–49	46 (26.0)	7 (15.9)	.17
50–59	11 (18.0)	3 (20.0)	1
⩾60	8 (26.7)	1 (11.1)	.65
Ethnicity:			
Punjabi	81 (38.6)	10 (21.3)	.02
Sindhi	7 (25.0)	3 (18.8)	.72
Other	40 (17.9)	18 (13.1)	.24
Province of residence:			
Punjab	63 (40.1)	8 (34.8)	.65
Sindh	64 (21.6)	22 (12.8)	.01
Other	1 (12.5)	1 (20.0)	1
Income:			
Low	52 (39.7)	15 (15.6)	6.5×10^{-5}
Middle	56 (24.3)	11 (14.7)	.08
High	13 (23.2)	5 (18.5)	.78

[a] Percentages were calculated for subjects for whom this information was available.

breast cancer diagnosed in women aged <40 years are attributable to first-cousin marriages.

The major (nongenetic) risk factors for breast and ovarian cancer are reproductive and dietary factors. Pakistani women have a low frequency of these traditional risk factors. The female population, on average, has high levels of fertility, early age at first pregnancy, multiple births, and prolonged breast-feeding. Although the age at first marriage has been gradually rising, women in consanguineous unions marry at earlier ages and are less likely to use modern contraceptive methods than are women from nonconsanguineous marriages (Hussain and Bittles 1999). Generally, Pakistani women do not use exogenous hormones (oral contraception or estrogen-replacement therapy). They generally do not smoke tobacco products, although some women practice chewing pan and pan tobacco. Furthermore, the population of Pakistan is predominantly Muslim (97%), and alcohol consumption is uncommon.

We conclude that genetic factors play a significant role in breast and ovarian cancer incidence in Pakistan. The prevalence and penetrance estimates for Pakistan suggest that dominant *BRCA1* and *BRCA2* mutations are significant contributors to breast and ovarian cancer incidence in this population. The association between parental consanguinity and breast cancer risk suggests that recessive genes may play a role in the etiology of breast cancer in young women. Our data are also pertinent to immigrant women from Pakistan who may seek genetic counseling, for cancer risk, in North America and Europe—as well as to women from the same ethnic groups in countries such as India, Afghanistan, Bangladesh, and Iran.

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Electronic-Database Information

Accession numbers and URLs for data presented herein are as follows:

Breast Cancer Information Core, http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic/ (for information on sequence alterations in *BRCA1* and *BRCA2*)

Genome Database, The, http://www.gdb.org/ (for DNA sequence primers of 13q and 17q loci)

Myriad Genetics, http://www.myriad.com/

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for inherited breast cancer type 1 and ovarian cancer [MIM 113705] and inherited breast cancer type 2 [MIM 600185])

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