Letters to the Editor

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Founder *BRCA1/2* Mutations among Male Patients with Breast Cancer in Israel

To the Editor:

Germline mutations in the *BRCA1* (MIM 113705) and *BRCA2* (MIM 600185) genes are associated with a high risk of breast and ovarian cancer in women (Ford et al. 1998). The risk of male breast cancer is higher among carriers of *BRCA2* mutations compared with carriers of *BRCA1* mutations, although the absolute risk to male carriers is not well characterized (Wooster et al. 1995; Ford et al. 1998). Studies of *BRCA2* in small population- and clinic-based series of male breast cancer patients from the United States and the United Kingdom have found carrier frequencies of 4%–14% (Couch et al. 1996; Friedman et al. 1997; Mavraki et al. 1997). Carrier frequencies for specific founder *BRCA2* germline mutations have been higher: 21%–40% (Thorlacius et al. 1996; Haraldsson et al. 1998; Csokay et al. 1999). The number of cases analyzed has ranged from 18 to 54, however, and the confidence limits on the carrier frequencies are very large. Among predominantly Ashkenazi Jewish populations, three founder mutations in the *BRCA1/2* genes are present in >2% of all individuals, and they may account for ~80% of all the mutations in these genes (Struewing et al. 1997; Frank et al. 1998). This allows the relatively efficient analysis of larger numbers of cases.

All male patients with breast cancer (n = 165) diagnosed in five Israeli hospitals during 1980–97 were reviewed for inclusion in this study. Jewish patients were characterized as Ashkenazi or non-Ashkenazi, on the basis of either the recorded place of birth in the Israeli Population Registry or, if they were born in Israel, their father's recorded place of birth. Non-Ashkenazi Jews were born in Turkey, Syria, Iraq, Iran, Afghanistan, Morocco, or Libya. Israeli-born non-Jews were designated as Arab, Christian, or Druze. Samples were analyzed anonymously. Among the 122 histologically verified cases for whom adequate pathological material could be obtained, 1 was excluded, because analysis from multiple specimens differed with respect to mutation status and marker genotypes. Eighty-nine cases were Ashkenazi, 21 were non-Ashkenazi, and 14 were Arabs.

Unstained 5-μm paraffin sections were scraped from slides and were digested in 100 μl of buffer containing 10 mM Tris (pH 8.0), 50 mM KCl, 10 mM EDTA, 2 mM $MgCl_2$, 1% Tween-20 and 10 μg of Proteinase K at 57°C overnight. The samples were then heated to 100°C for 5 min and then 1–5 μl were used as template in the PCR reactions. A 20-μl multiplex PCR reaction was used to amplify the three segments containing the founder mutations. Each reaction contained 1 × PCR Buffer, 2.5 mM $MgCl_2$, 100 μM each dNTP, 15% sucrose, and 0.75 U *Taq* Gold (PE Biosystems). Primers included 300 nM 2F103 (6FAM-tcgcgttgaagaagtacaaaatgtc), 300 nM 2R102 (caaattaatacactcttgtgctgacttac), 200 nM 20F101 (HEX-gtcaatggaagaaaccaccaaggtc), 200 nM 20R101 (tgcaaaggggagtggaatacacagt), 200 nM 11F101 (TET-tagggaagcttcataagtcagt ctca), and 200 nM 11R101 (cttgcgttttgtaatgaagcatct). Cycling consisted of 94°C for 12 min, followed by 10 cycles of 92°C for 10 s, annealing at 68°C for 10 s, and 72°C for 20 s, with the annealing temperature being decreased 1.5°C per cycle, followed by 30 cycles of 92°C for 15 s, 55°C for 15 s, and 72°C for 30 s, followed by 72°C for 10 min. The PCR products were diluted 1:149 with water and then were diluted 1:6 with loading cocktail, according to the manufacturers' recommendations, and were electrophoresed on an ABI 310 capillary electrophoresis machine by use of GENESCAN software (PE Biosystems). Known mutant, wild-type, and no-DNA controls were run with each PCR reaction. Mutants were identified by visual inspection of the electropherograms, with the small-insertion or -deletion mutations resulting in an extra peak in the pattern. Primers to amplify the chromosome 13 markers D13S260, D13S1695, D13S1698, and D13S1701 were redesigned to result in shorter amplicons. All carriers of the 6174delT mutation and a reference family with this mutation were analyzed for these four markers, on the ABI 310 (primer sequences are available, on request, from the corresponding author.)

The results of mutation testing are shown in table 1. Of the 19 mutation carriers, 17 were Ashkenazi and 2 were non-Ashkenazi Jews. Carrier frequencies were not statistically different between the two ethnic groups (P = .7). None of the Arab men was a carrier, and the

Table 1

Israeli Male-Breast-Cancer Mutation Testing Results

Population	No. Tested	No. (%) Positive for Mutation [95% CLs]			
		BRCA1		*BRCA2*	
		185delAG	5382insC	6174delT	Total
Jews:					
All	110	4 (3.6) [1, 9]	0	15 (13.6) [8, 21]	19 (17.3) [11, 26]
Ashkenazi	89	4 (4.5) [1, 11]	0	13 (14.6) [8, 24]	17 (19.1) [12, 29]
Sephardic	21	0	0	2 (9.5) [1, 30]	2 (9.5) [1, 30]
Arabs	14	0	0	0	0

5382insC mutation was not detected in any patients. The mean ages at diagnosis of all 19 mutation carriers (64 years) and of the *BRCA2* 6174delT mutation carriers (66 years) were not significantly different than that of noncarriers (68 years). Reliable genotypes could not be obtained for all the markers for all carriers, but 14 of 14 carriers successfully genotyped at D13S1698 shared the same allele as was seen in the reference family; 12 of 12 carriers successfully genotyped at D13S260 shared the correct allele; 7 of 8 carriers successfully genotyped at D13S1701 shared the correct allele; and 13 of 15 carriers successfully genotyped at D13S1695 shared the correct allele.

These results suggest that ⩾17% of Jewish men diagnosed with breast cancer in Israel carry a mutation in *BRCA1* or *BRCA2*, since only the three common founder mutations were screened. This study was based on the analysis of a small amount of pathological tissue, and the proportion of the tissue that was tumor was unknown. Our methods may have led both to false negatives, caused by the complete loss of the relevant genes in a tumor—although this might be expected to result in preferential loss of the wild-type and retention of the mutant allele—and false positives, caused by contamination of the specimens during processing and handling. Our observed carrier frequency is somewhat higher than those in most series studied in the United States and the United Kingdom but is lower than frequencies observed in several other populations with common founder mutations. Unlike that in female carriers, who have an earlier age at diagnosis of breast cancer, the age at diagnosis among male carriers was not significantly lower than among noncarriers. This may reflect the fact that most men carried a *BRCA2* mutation, because the age at diagnosis for female breast cancer appears to be later for *BRCA2* carriers than for *BRCA1* carriers (Ford et al. 1998).

The *BRCA1* 185delAG founder mutation has been observed in both Ashkenazi and non-Ashkenazi Jewish women with breast or ovarian cancer (Bar-Sade et al. 1998). We observed the 6174delT mutation in two non-Ashkenazi Jewish men with breast cancer, and this is the first published observation of this mutation in this population (Neuhausen et al. 1998). Our characterization of Jewish men as Ashkenazi or non-Ashkenazi was based on the place of birth as recorded in the Israeli Population Registry. Since ethnic origin was not determined in person or by biological testing, some misclassification could have occurred. If verified in other studies, however, this finding would suggest that this mutation is considerably older than previously estimated (Neuhausen et al. 1998). Genotyping of four microsatellite markers near *BRCA2* was consistent with their sharing the same haplotype as was seen in Ashkenazi carriers of this mutation. Although the number of cases was small, we did not observe an earlier age at onset among mutation carriers, nor did we detect any of the three mutations in Arab men with breast cancer.

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Electronic-Database Information

Accession numbers and the URL for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www

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References

Bar-Sade RB, Kruglikova A, Modan B, Gak E, Hirsh-Yechezkel G, Theodor L, Novikov I, et al (1998) The 185delAG BRCA1 mutation originated before the dispersion of Jews in the diaspora and is not limited to Ashkenazim. Hum Mol Genet 7:801–805

Couch FJ, Farid LM, DeShano ML, Tavtigian SV, Calzone K, Campeau L, Peng Y, et al (1996) BRCA2 germline mutations in male breast cancer cases and breast cancer families. Nat Genet 13:123–125

Csokay B, Udvarhelyi N, Sulyok Z, Besznyak I, Ramus S, Ponder B, Olah E (1999) High frequency of germ-line BRCA2 mutations among Hungarian male breast cancer patients without family history. Cancer Res 59:995–998

Ford D, Easton DF, Stratton M, Narod S, Goldgar D, Devilee P, Bishop DT, et al (1998) Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families. Am J Hum Genet 62:676–689

Frank TS, Gumpper KL, Manley SA, Ward BE (1998) The proportion of atypical protein-truncating mutations in BRCA1 and BRCA2 in women of Ashkenazi ancestry. Abstract 402 from the 21st Annual San Antonio Breast Cancer Symposium, December 12–15

Friedman LS, Gayther SA, Kurosaki T, Gordon D, Noble B, Casey G, Ponder BA, et al (1997) Mutation analysis of BRCA1 and BRCA2 in a male breast cancer population. Am J Hum Genet 60:313–319

Haraldsson K, Loman N, Zhang QX, Johannsson O, Olsson H, Borg A (1998) BRCA2 germ-line mutations are frequent in male breast cancer patients without a family history of the disease. Cancer Res 58:1367–1371

Mavraki E, Gray IC, Bishop DT, Spurr NK (1997) Germline BRCA2 mutations in men with breast cancer. Br J Cancer 76:1428–1431

Neuhausen SL, Godwin AK, Gershoni-Baruch R, Schubert E, Garber J, Stoppa-Lyonnet D, Olah E, et al (1998) Haplotype and phenotype analysis of nine recurrent BRCA2 mutations in 111 families: results of an international study. Am J Hum Genet 62:1381–1388

Struewing JP, Hartge P, Wacholder S, Baker SM, Berlin M, McAdams M, Timmerman MM, et al (1997) The risk of cancer associated with specific mutations of BRCA1 and BRCA2 among Ashkenazi Jews. N Engl J Med 336: 1401–1408

Thorlacius S, Olafsdottir G, Tryggvadottir L, Neuhausen S, Jonasson JG, Tavtigian SV, Tulinius H, et al (1996) A single BRCA2 mutation in male and female breast cancer families from Iceland with varied cancer phenotypes. Nat Genet 13: 117–119

Wooster R, Bignell G, Lancaster J, Swift S, Seal S, Mangion J, Collins N, et al (1995) Identification of the breast cancer susceptibility gene BRCA2. Nature 378:789–792

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