

Associations of High-Grade Prostate Cancer with *BRCA1* and *BRCA2* Founder Mutations

Illir Agalliu,¹ Robert Gern,¹ Suzanne Leanza,² and Robert D. Burk^{1,2,3}

Abstract **Purpose:** Protein-truncating mutations in *BRCA1* and in particular *BRCA2* genes have been associated with prostate cancer. However, there is still uncertainty about the magnitude of association particularly with Gleason score, and family history of prostate, breast, and ovary cancers. **Experimental Design:** To further examine associations between three founder mutations located in *BRCA1* (185delAG, 5382insC) or *BRCA2* (6174delT) genes and prostate cancer, we conducted a study of 979 prostate cancer cases and 1,251 controls among Ashkenazi Jewish men. Detailed information was obtained on prostate cancer pathology, age at diagnosis, and family history of all cancers. Odds ratios (OR) and 95% confidence intervals (CIs) were estimated using logistic regression models. **Results:** Prostate cancer risk was increased (OR, 1.9; 95% CI 0.9-4.1) for *BRCA2* mutation carriers but not for *BRCA1* mutation carriers. *BRCA2* mutation carriers had an OR of 3.2 (95% CI, 1.4-7.3) for Gleason score of 7 to 10, but no association was observed for Gleason score of <7. Carriers of *BRCA1*-185delAG mutation also had an OR of 3.5 (95% CI, 1.2-10.3) for Gleason score of ≥ 7 tumors; however, the association of either *BRCA1*-185delAG or 5382insC mutation was not statistically significant. Associations between founder mutations and prostate cancer were stronger in men with no first-degree family history of breast and/or ovarian cancers but were unaffected by family history of prostate cancer. **Conclusion:** These results indicate that the *BRCA2* founder mutation confers a 3-fold elevated risk of high-grade prostate cancer. Although *BRCA1* mutations were not associated with prostate cancer, the *BRCA1*-185delAG was associated with high Gleason score tumors. These findings should be carefully considered in genetic counseling and/or evaluating therapeutic options.

Prostate cancer is the most commonly diagnosed solid tumor and the second leading cause of cancer deaths among men in the United States (1). Although the etiology of prostate cancer is poorly understood, a large twin study has estimated that 42% of disease variation can be attributed to genetic factors and 58% to environmental/lifestyle factors (2). Moreover, one of the strongest risk factors for this disease is a family history of prostate cancer; having a first-degree relative diagnosed with prostate cancer is associated with a 2- to 3-fold elevation in the

relative risk (RR; refs. 3, 4), and both early age at diagnosis and multiple affected family members are strong predictors of risk in relatives. Taken together, these results suggest a strong inherited component to disease risk. Nevertheless, attempts to identify "high-risk" genes with a dominant mode of inheritance, such as *APC* for colon cancer (5) or *VHL* for renal cancer (6), have been unsuccessful for prostate cancer (7). Recent genome-wide association studies of prostate cancer have identified a number of loci and genetic variants that individually contribute to a small increase in prostate cancer risk (8–13) but still do not explain the familial risk of this cancer (14).

Of the known cancer susceptibility genes, *BRCA1* and *BRCA2* in particular, have been candidate genes of interest in prostate cancer etiology. Studies of families segregating *BRCA2* mutations (15–20), kin-cohort studies of cancer incidence among relatives of population-based breast or ovarian cancer cases (21, 22), as well as studies of populations who harbor founder *BRCA1* or *BRCA2* mutations (23–25) suggest that men who carry a disease-associated *BRCA2* allele have an increased relative risk (RR) of prostate cancer (2- to 5-fold elevation). These findings suggest that protein-truncating *BRCA2* mutations may play a role in prostate cancer susceptibility. Unlike *BRCA2* mutations, mutations in the *BRCA1* gene have been inconsistently associated with prostate cancer risk. Several epidemiologic studies that have examined the risk of prostate cancer among *BRCA1* mutation carriers have reported small, but not statistically significant, or null results (16, 25, 26). However, one of the challenges of epidemiologic studies that

Authors' Affiliations: Departments of ¹Epidemiology and Population Health, ²Pediatrics (Genetics), and ³Microbiology and Immunology, and Obstetrics, Gynecology and Women's Health, Albert Einstein College of Medicine, Bronx, New York

Received 7/14/08; revised 10/24/08; accepted 10/26/08.

Grant support: U.S. Army (PC001076) and in part by a Translational-Medicine Grant from the Albert Einstein College of Medicine.

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Note: Current address for R.W. Gern; Office of Epidemiology, Evaluation, and Health Information; Division of Public Health; Georgia Department of Human Resources; 2 Peachtree Street, Northwest; Suite 14-464; Atlanta, GA 30303.

Requests for reprints: Robert D. Burk, Department of Pediatrics (Genetics), Albert Einstein College of Medicine, 1300 Morris Park Avenue, Ullmann Building, Room 515, Bronx, NY 10461. Phone: 718-430-3720; Fax: 718-430-8975; E-mail: burk@aecom.yu.edu.

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doi:10.1158/1078-0432.CCR-08-1822

Translational Relevance

Protein-truncating *BRCA2* mutations have been associated with prostate cancer. However, there is still uncertainty about the magnitude of association particularly with Gleason score and the clinical relevance. We conducted a study of 979 prostate cancer cases and 1,251 controls among Ashkenazi Jewish men to examine associations between three founder mutations located on *BRCA1* (185delAG, 5382insC) or *BRCA2* (6174delT) genes and prostate cancer. *BRCA2* mutation carriers had an increased risk of prostate cancer (OR, 1.9; 95% CI, 0.9–4.1) compared with noncarriers, but no associations were observed for the *BRCA1* mutation carriers taken together. In addition, *BRCA2* mutation carriers had an OR of 3.2 (95% CI, 1.4–7.3) of high Gleason score, but no increased risk was observed for lower grade cancer. Results indicate that protein-truncating mutations in the *BRCA2* gene increase susceptibility to high-grade prostate cancer.

examine the role of disease-associated mutations of *BRCA1* or *BRCA2* to the susceptibility of prostate cancer is the fact that the prevalence of such mutations in the general population is rare.

One ethnic population that has been used to examine the associations between prostate cancer and mutations in *BRCA1* or *BRCA2* are the Ashkenazim because ~2.0% of this population carries at least one of 3 founder mutations (*BRCA1*-185delAG and 5382insC, and *BRCA2*-6174delT) in these 2 genes. A number of studies have examined the role of these mutations in relation to prostate cancer risk in the Ashkenazi Jewish population (24, 25, 27–30). Results of these studies have been inconsistent largely due to small sample sizes or lack of covariate information. For instance, some studies reported associations between *BRCA1* or *BRCA2* mutations and prostate cancer (24, 25, 30), whereas others have reported no association (27–29). To further examine the associations between the three founder mutations (185delAG, 5382insC, and 6174delT) located on *BRCA1* or *BRCA2* genes and risk of prostate cancer, we conducted a large case-control study of prostate cancer among Ashkenazi Jewish men. In addition, we were interested in exploring whether associations vary by age at diagnosis, by family history of prostate cancer and other cancers, as well as by clinical features of prostate cancer. This information is clinically relevant because *BRCA1* and *BRCA2* genetic testing is becoming more routine in medical practice.

Materials and Methods

Study population. Prostate cancer cases and controls were recruited from the Ashkenazi Jewish community through letters and advertisements. To be eligible for the study, the advertisement stated that men should be of Ashkenazi descent and over age 50 y, although a few men (1%) under age 50 y were included. From 1998 through 2005, 3,817 individuals contacted the study coordinator to participate in the study and were mailed informed consent forms, questionnaires, and materials to obtain a DNA sample. Informed consent and study materials were received from 2,486 (65.1%) individuals. Participants lived throughout the United States and the majority of men were from either New York (44.7%), Florida (20.9%), California (6.5%), or New Jersey (6.5%). Of the 2,486 participants, there were 1,014 self-reported cases of prostate

cancer, 1,270 men without a history of prostate cancer (controls), and 60 affected and 142 unaffected relatives of cases who only provided a DNA sample. Of these, 979 prostate cancer cases and 1,251 controls satisfied the criteria of having both parents of Ashkenazic descent, completed a self-administered questionnaire, provided a DNA sample, and were included in this analysis. Cases were diagnosed with adenocarcinoma of the prostate between 1978 and 2005 (mean and median year of diagnosis was 1996). Clinical information on Gleason score, and extent of disease based on tumor invasiveness, tumor present at resection margins, prostate capsule invasion, seminal vesicle involvement and lymph node involvement was obtained from pathology reports of prostate biopsy or from surgery for men who underwent radical prostatectomy; records were available on 902 (92%) of the 979 cases. All eligible participants completed a 40-page questionnaire, and donated a DNA sample (i.e., buccal cells and/or a blood sample). The 40-page questionnaire included detailed questions regarding personal, demographic and lifestyle factors, medical history, family history of prostate cancer and other cancers, information on prostate cancer screening, and information about prostate cancer diagnosis. The study protocols and materials were approved by the Institutional Review Board of the Albert Einstein College of Medicine, and written informed consent was obtained from all study participants. Men were informed and consented that results of genetic testing would not be available to them at the end of the study.

DNA isolation. Participants were sent self-contained kits of blood collection materials for obtaining a blood sample at their next health care appointment, a buccal cell collection kit consisting of 4 to 6 Dacron swabs, and/or a mouthwash collection kit consisting of a 44-mL bottle of Scope brand mouthwash, and two 25-mL screw top collection tubes. All kits included detailed instructions about collection of specimens and prepaid return addressed mailers. Participants returned by postal services their buccal swabs, oral mouthwash samples, and/or EDTA anticoagulated whole blood samples for genetic studies.

Genomic DNA was isolated from 3 mL of blood using the Puregene DNA Isolation kit (Gentra Systems) according to the manufacturer's Whole Blood protocol. Genomic DNA was isolated from buccal swabs using the Puregene DNA Isolation kit (Gentra Systems) following the manufacturer's DNA Purification of Buccal Brushes in One Tube protocol. The mouthwash samples were transferred from their original collection tubes to 50-mL centrifuge tubes. The cells were pelleted by centrifugation, the supernatant was decanted, and the cells were then rinsed with 1.5 mL TE buffer and repelleted. To each sample, 1.5 mL of cell lysis buffer [10 mmol/L Tris/HCl (pH 8.0), 10 mmol/L EDTA, 0.1 mol/L NaCl, 2% SDS pH 8.0] and 75 μ L (20 mg/mL) Proteinase K (Roche) was added. The samples were vortexed vigorously and then incubated overnight at 55°C. The samples were treated with RNase A (Gentra Systems), followed by Phenol/Chloroform extraction using a Phase Lock Gel Tube protocol (Eppendorf). The DNA was precipitated in Isopropanol and washed with 70% Ethanol. The pellets were air dried and resuspended in 200 μ L of TE buffer. The DNA content of the extracted genomic materials was quantified using the PicoGreen dsDNA quantitation kit (Molecular Probes) in a microplate format using a Perkin-Elmer HTS7000 BioAssay Reader and stored at -20°C.

Genotyping assays. DNA fragments containing the *BRCA1* 185delAG, 5382insC, and *BRCA2* 6174delT were PCR amplified using a modified multiplex PCR as described by Fodor et al. (31), and detected by oligonucleotide hybridization as previously described (32). Briefly, PCR products were blotted onto Biotodyne B membrane filters (pore size, 0.45 μ m; Pall Biotodyne) using a Robbins Hydra 96 microdispenser. The filters were treated with 3% hydrogen peroxide (Sigma) at room temperature for 15 min, then washed in 0.1x saline-sodium phosphate-EDTA [1x saline-sodium phosphate-EDTA is 180 mmol/L NaCl, and 10 mmol/L Na₂HPO₄, and 1 mmol/L EDTA pH 7.4] and 0.5% sodium Dodecyl sulfate solution at 65°C for 30 min; thereafter, the filters were hybridized individually with each of the biotin labeled probes at 51°C overnight (probe sequences: *BRCA1*-185 wild-type, 5'-AGAAAATCTTAGAGTGTCCCA-3'; *BRCA1*-185delAG,

5'-AGAAATCTTAGTGCCCA-3'; *BRCA1*-5382 wild-type, 5'-AGAGAA-TCCCAGGACA-3'; *BRCA1*-5382insC, 5'-AGAGAAATCCCCAGGACA-3'; *BRCA2*-6174 wild-type, 5'-GCAAGTGGAATCT-3'; *BRCA2*-6174delT, 5'-GCAAGGGAAATCT-3'). The filters were treated with streptavidin and enhanced chemiluminescence (GE Biosciences). All results were interpreted by two experienced investigators. Initially, samples containing either *BRCA1* 185delAG, or 5382insC or *BRCA2*-6174delT were sequenced to confirm genotyping results. Positive controls for all three mutations were included on all dot blot membranes. Ten percent of samples were repeated with complete concordance.

Statistical analysis. The distribution of founder mutations in *BRCA1* (185delAG, 5382insC) and *BRCA2* (6174delT) genes was assessed separately for cases and controls, and deviation of genotype frequencies from Hardy-Weinberg Equilibrium among controls was assessed by a χ^2 test. Unconditional logistic regression was used to examine associations between these three founder mutations in *BRCA1* and *BRCA2* and prostate cancer and to compute odds ratios (OR) and 95% confidence intervals (CI; ref. 33). First, we examined association between prostate cancer and each mutation in each gene separately, and then we compared risk among any mutation carriers versus noncarriers. The associations between these mutations and prostate cancer were adjusted for age at diagnosis (cases) and age at study participation (controls). Additional adjustments for first-degree family history of prostate cancer and prostate-specific antigen (PSA) test or digital rectal examination (DRE) screening did not substantially change the risk estimates; thus the final models were adjusted only for age. In addition, the association between founder mutations in *BRCA1* and *BRCA2* and prostate cancer was examined in strata defined by age at diagnosis (<65 versus ≥ 65 y), and by first-degree family history of prostate cancer, breast or ovarian cancer. To test effect modification, interaction terms between *BRCA* mutations and age (<65 versus ≥ 65 y) or family history of prostate cancer, or of breast or ovarian cancer were included in models containing the main effects in separate logistic regression models. The log likelihood of reduced models with main effects only were compared with the log likelihood of fully saturated models that also contained the interaction terms, using a likelihood ratio test to determine statistical significance (34).

We also examined the association between the *BRCA1* and *BRCA2* founder mutations and prostate cancer according to strata defined by Gleason score, and a composite measure of disease severity. Gleason score information was obtained from either biopsy or from surgical pathology reports. For these analyses, prostate cancer cases were grouped into two strata: those with Gleason scores of 2 to 6 and those with Gleason scores of 7 to 10. Advanced prostate cancer was characterized as either having a Gleason score of ≥ 7 or at least two of the following characteristics documented on the pathology report: positive tumor invasiveness, tumor present at resection margins, prostate capsule invasion, seminal vesicle involvement, and lymph node involvement. The frequency of founder mutations in *BRCA1* and *BRCA2* genes in each group of cases [i.e., those with high (7-10) and low (2-6) Gleason score cancers] was compared with the frequency of founder mutations among controls using polytomous logistic regression (35) models. SAS version 9.1 (SAS Institute) and STATA version 9 (STATA Corporation) were used for statistical analyses.

Results

The characteristics of 979 prostate cancer cases and 1,251 controls are presented in Table 1. Cases were slightly older at study participation compared with controls (mean age of 69.4 versus 68.3, respectively; $P = 0.01$). Cases were more likely than controls to report a first-degree family history of prostate cancer (28% versus 14%; $P < 0.0001$) but not of breast and/or ovarian cancers. The majority of cases (95%) and controls (98%) had undergone PSA testing or DRE for prostate cancer screening. The average age at diagnosis for prostate cancer cases was 64.5

years (range 43-87 years) and the majority of cases were diagnosed with prostate cancer because of an abnormal PSA or DRE test (85.2%). Most cases had a Gleason score of 2 to 6, 25% had a Gleason score of 7, and 12% had Gleason score 8 to 10; approximately half of the cases were classified as having an advanced form of prostate cancer (Table 1).

The prevalence of the three founder mutations in *BRCA1* or *BRCA2* was 3.0% and 1.8% among cases and controls, respectively (OR, 1.62; 95% CI, 0.91-2.85). There was an increased risk, although not statistically significant, of prostate cancer among *BRCA2* (6174delT) mutation carriers (OR, 1.92; 95% CI, 0.91-4.07) but not for carriers of either *BRCA1* mutation. In a combined analysis of *BRCA1* (185delAG) or *BRCA2* (6174delT) mutations there was an OR of 1.91 (95% CI, 1.05-3.48) for prostate cancer for carriers versus noncarriers. Because several studies have suggested that the risk of prostate cancer associated with mutations in *BRCA1* or *BRCA2* genes vary by age at diagnosis (15, 20, 36), we stratified our data into 2 groups: cases with age at diagnosis <65 and ≥ 65 years (Table 2). Interestingly, there was a statistically significant interaction ($P = 0.03$) between age ≥ 65 years at prostate cancer diagnosis and *BRCA1* 185delAG mutation-carrier status. Carriers of *BRCA1*-185delAG mutations who were diagnosed with prostate cancer at ages ≥ 65 years had an OR of 6.91 (95% CI, 1.37-34.9) compared with noncarriers in the same age category (Table 2). Among men diagnosed with prostate cancer at younger ages (<65 years), there were no associations for any of the three founder mutations.

Next, we examined association between the *BRCA1* and *BRCA2* founder mutations and prostate cancer according to clinical features of the disease using polytomous logistic regression (Table 3). Carriers of any of the three founder mutations in *BRCA1* or *BRCA2* had a statistically significant increased risk of having a high Gleason score (≥ 7) prostate cancer (OR, 2.61; 95% CI, 1.36-4.98), whereas no association was observed for those with a Gleason score <7. In mutation specific analyses, risk of high Gleason score cancer was increased for *BRCA2* (6174delT) mutation carriers (OR, 3.18; 95% CI, 1.37-7.34). By contrast, for the *BRCA1* gene, there was not a statistically significant increase in risk of high Gleason score disease (OR, 2.23; 95% CI, 0.84-5.86) for carriers of *BRCA1* 185delAG or 5382insC mutations. However, for mutation-carriers of *BRCA1* (185delAG) alone, there was a statistically significant increased risk of high Gleason score cancer, and the magnitude of association was similar to that observed for the *BRCA2* mutation (Table 3). Because some clinicians consider a high-grade cancer as having a Gleason score 8 to 10, we repeated the above analyses using this cutoff criterion. For Gleason score 8 to 10 tumors, the only statistically significant association was observed for *BRCA2* (6174delT) mutation carriers (OR, 3.60; 95% CI, 1.14-11.41) but not for any of the *BRCA1* mutations. Similar findings were also observed when cases were stratified according to severity of prostate cancer (data not shown).

Lastly, associations between founder mutations in *BRCA1* or *BRCA2* genes and prostate cancer were examined within strata defined by first-degree family history of prostate cancer, as well as breast and/or ovarian cancers (Table 4), which have been linked to mutations in *BRCA1* and *BRCA2* genes (15, 26). There were associations between *BRCA1* and *BRCA2* founder

Table 1. Selected characteristics of Ashkenazi Jewish prostate cancer cases and controls

Characteristics	Cases (n = 979)	Controls (n = 1,251)	P*
Age at participation (y)	n (%)	N (%)	0.01
<50	11 (1.1)	12 (1.0)	
50-59	146 (14.9)	284 (22.7)	
60-69	345 (35.2)	382 (30.5)	
70-79	365 (37.3)	427 (34.1)	
≥80	112 (11.4)	146 (11.7)	
First-degree family history of prostate cancer			<0.0001
Yes	276 (28.2)	179 (14.3)	
No	703 (71.8)	1072 (85.7)	
First-degree family history of breast cancer			0.58
Yes	161 (16.4)	195 (15.6)	
No	818 (83.6)	1056 (84.4)	
First-degree family history of ovarian cancer			0.50
Yes	33 (3.4)	36 (2.9)	
No	946 (96.6)	1215 (97.1)	
Smoking status			0.93
Nonsmoker	427 (43.6)	543 (43.4)	
Former smoker	523 (53.4)	668 (53.4)	
Current smoker	24 (2.5)	33 (2.6)	
Missing	5 (0.5)	7 (0.6)	
Education			0.90
High school or less	63 (6.4)	86 (6.9)	
Post secondary /Some college	155 (15.8)	193 (15.4)	
College degree	259 (26.5)	320 (25.6)	
Graduate/Professional degree	493 (50.4)	645 (51.5)	
Missing	9 (0.9)	7 (0.6)	
History of BPH			<0.0001
Yes	323 (33.0)	538 (43.1)	
No	616 (62.9)	682 (54.5)	
Unknown	40 (4.1)	31 (2.5)	
Age at prostate cancer diagnosis (y)			
<50	27 (2.8)		
50-59	242 (24.7)		
60-69	443 (45.3)		
70-79	234 (23.9)		
≥80	33 (3.4)		
Reason for prostate cancer diagnosis			
Abnormal PSA	741 (73.1)		
Abnormal DRE	123 (12.1)		
Symptoms	25 (2.5)		
TURP for BPH	15 (1.5)		
Other procedures	57 (5.6)		
Unknown	18 (1.8)		
Gleason score			
2-4	88 (9.0)		
5-6	456 (46.6)		
7	243 (24.8)		
8-10	115 (11.7)		
Missing	77 (7.9)		
Advanced prostate cancer †			
Yes	484 (49.4)		
No	435 (44.4)		
Missing	60 (6.1)		

Abbreviations: BPH, benign prostatic hyperplasia; TURP, transurethral resection of the prostate.

* χ^2 P value.

† Advanced prostate cancer was characterized as having either a Gleason score ≥ 7 , or at least 2 of the following characteristics documented on the pathology report: positive tumor invasiveness, tumor present at resection margins, prostate capsule invasion, seminal vesicle involvement, or lymph node involvement.

mutations, particularly 185delAG or 6174delT, and prostate cancer in the strata with no family history of prostate cancer (OR, 2.01; 95% CI, 1.05-3.85), and no family history of breast and/or ovarian cancer (OR, 3.30; 95% CI, 1.41-7.73) when comparing carriers versus noncarriers of *BRCA1*-185delAG or *BRCA2*-6174delT mutations. There was a statistically significant

interaction between mutation carrier status of either *BRCA1* (185delAG) or *BRCA2* (6174delT) and first-degree family history of breast and/or ovarian cancer ($P = 0.04$). Surprisingly, in men reporting a positive first-degree family history of breast and/or ovarian cancers, the association between the *BRCA1* (185delAG) or *BRCA2* (6174delT) founder mutations

and prostate cancer was not significant, although as expected the prevalence of *BRCA1* and *BRCA2* mutations was much higher in both cases and controls.

Discussion

In this study, we examined associations between founder mutations in *BRCA1* (185delAG, 5382incC) and *BRCA2* (6174delT) and prostate cancer in a large case-control study encompassing >2,000 men of Ashkenazi descent. There was an increased risk, although not statistically significant, of overall prostate cancer among *BRCA2* (6174delT) mutation carriers but not for carriers of either *BRCA1* mutation. In relation to clinical characteristics of prostate cancer, *BRCA2* mutation carriers had a 3.2-fold (95% CI, 1.4-7.4) increase in the risk of high Gleason score prostate cancer, but no associations were observed for lower grade cancer. For the *BRCA1* gene, there was not a statistically significant increase in risk of high Gleason score disease for the *BRCA1* founder mutations taken together, although carriers of *BRCA1* (185delAG) had an OR of 3.5 (95% CI, 1.2-10.3) for high-grade disease compared with controls. In

addition, we found that risk of prostate cancer associated with carrying these mutations was higher in men diagnosed at older ages (65 years or older) and in particular, for those with a *BRCA1* (185delAG) mutation and in men without a first-degree familial history of prostate, breast and/or ovarian cancers.

A number of previous studies have examined the associations between these *BRCA1/2* founder mutations and prostate cancer (24, 25, 27-30). Among Ashkenazi-Jewish men recruited in Washington D.C., Struwing et al. (24) estimated a lifetime risk of prostate cancer of 16% (95% CI, 4-30%) for *BRCA1/BRCA2* mutation carriers and 3.8% (95% CI, 3.3%-4.4%) for non-carriers. In that study, when data were stratified by mutation status in *BRCA1* or *BRCA2* gene, the risk of prostate cancer at age 70 years was higher for *BRCA1* mutation carriers (25%) compared with *BRCA2* mutation carriers (5%). A large case-only study conducted in Israel, ascertained a population-based collection of 940 paraffin specimens from Ashkenazim through the Israeli Population Registry (30). They detected a similar prevalence of the *BRCA1* (185delAG, 5382incC) and *BRCA2* (6174delT) mutations in their case set of 1.9%, 0.2%, and 1.5%, as found in the men with prostate cancer in the current

Table 2. Associations between Ashkenazi Jewish founder mutations in the *BRCA1* and *BRCA2* genes and prostate cancer

Prostate cancer (all ages)	Cases		Controls		OR* (95% CI)
	Carrier/noncarrier	n (%) †	Carrier/noncarrier	n (%) †	
Mutations					
<i>BRCA1</i>					
185delAG		11/965 (1.1)		7/1,234 (0.6)	2.03 (0.77-5.36)
5382incC		1/975 (0.1)		4/1,236 (0.3)	0.28 (0.03-2.68)
Any <i>BRCA1</i> mutation		12/966 (1.2)		11/1,236 (0.9)	1.39 (0.60-3.22)
<i>BRCA2</i> 6174delT		18/951 (1.9)		12/1,228 (1.0)	1.92 (0.91-4.07)
<i>BRCA1</i> (185delAG) or <i>BRCA2</i> (6174delT)		28/951 (2.9)		19/1,230 (1.5)	1.91 (1.05-3.48)
Any mutations in <i>BRCA1/BRCA2</i>		29/950 (3.0)		23/1,227 (1.8)	1.62 (0.91-2.85)
Age at diagnosis < 65 y					
Mutations					
<i>BRCA1</i> 185delAG		4/498 (0.8)		5/470 (1.1)	0.75 ‡ (0.20-2.81)
Any <i>BRCA1</i> mutation		5/498 (1.0)		6/470 (1.3)	0.79 (0.24-2.61)
<i>BRCA2</i> 6174delT		10/487 (2.0)		6/467 (1.3)	1.58 (0.57-4.39)
<i>BRCA1</i> (185delAG) or <i>BRCA2</i> (6174delT)		14/490 (2.8)		11/466 (2.3)	1.20 (0.54-2.67)
Any mutations in <i>BRCA1/BRCA2</i>		15/489 (3.0)		12/466 (2.5)	1.18 (0.55-2.56)
Age at diagnosis ≥ 65 y					
Mutations					
<i>BRCA1</i> 185delAG		7/467 (1.5)		2/764 (0.3)	6.91 ‡ (1.37-34.87)
Any <i>BRCA1</i> mutation		7/468 (1.5)		5/765 (0.7)	2.70 (0.82-8.87)
<i>BRCA2</i> 6174delT		8/464 (1.7)		6/761 (0.8)	2.63 (0.85-8.19)
<i>BRCA1</i> (185delAG) or <i>BRCA2</i> (6174delT)		14/461 (3.0)		8/764 (1.0)	3.69 (1.47-9.29)
Any mutations in <i>BRCA1/BRCA2</i>		14/461 (2.9)		11/761 (1.4)	2.61 (1.13-6.03)

NOTE: Missing values varied by mutation: *BRCA1* 185delAG (3 cases, 11 controls); *BRCA1* 5382incC (3 cases, 12 controls); *BRCA2* 6174delT (10 cases, 12 controls).

*ORs are adjusted for age at diagnosis (cases) and age at interview (controls); Reference category is nonmutation carriers.

† Percentages of mutation carriers in each category.

‡ *P* = 0.03 for interaction analysis between *BRCA1* (185delAG) or *BRCA2* (6174delT) mutation carrier status and age at diagnosis (<65 and ≥65 y). *P* values for interaction analyses between other mutation carrier categories and age at diagnosis were not statistically significant.

Table 3. Associations between founder mutations in the *BRCA1* and *BRCA2* genes and Gleason score

Gleason score	Controls		Cases: Gleason score 2-6		Cases: Gleason score 7-10	
	Carrier/noncarrier		Carrier/noncarrier		Carrier/noncarrier	
Mutations	n (%) [*]		n (%) [*]	OR [†] (95% CI)	n (%) [*]	OR [†] (95% CI)
<i>BRCA1</i> 185delAG	7/1,234 (0.6)		3/538 (0.6)	0.99 (0.25-3.92)	7/351 (1.9)	3.54 (1.22-10.31)
Any <i>BRCA1</i> mutation	11/1,236 (0.9)		4/539 (0.7)	0.82 (0.26-2.64)	7/351 (1.9)	2.23 (0.84-5.86)
<i>BRCA2</i> 6174delT	12/1,228 (1.0)		5/534 (0.9)	0.92 (0.31-2.67)	11/344 (3.0)	3.18 (1.37-7.34)
<i>BRCA1</i> (185delAG) or <i>BRCA2</i> (6174delT)	19/1,230 (1.5)		8/536 (1.5)	0.95 (0.41-2.20)	17/341 (4.8)	3.18 (1.62-6.24)
Any mutations in <i>BRCA1/BRCA2</i>	23/1,227 (1.8)		9/535 (1.7)	0.87 (0.39-1.92)	17/341 (4.8)	2.61 (1.36-4.98)

NOTE: Missing values varied by mutation: *BRCA1* 185delAG (3 cases, 11 controls); *BRCA1* 5382insC (3 cases, 12 controls); *BRCA2* 6174delT (10 cases, 12 controls).

^{*}Percentages of mutation carriers in each category.

[†]ORs and 95% CI were generated using polytomous logistic regression; models were adjusted for age at diagnosis (cases) and age at interview (controls); Reference category is nonmutation carriers.

report—1.1%, 0.1%, and 1.9%, respectively. However, Giusti and colleagues (30) used the *BRCA* mutation prevalence from the Washington D.C study (24) to estimate the association between *BRCA1/2* mutations and prostate cancer. Similar to our results, Giusti et al. reported that mutation carriers had twice the risk of prostate cancer, and as reported here, the *BRCA1* 5382insC mutation was not associated with prostate cancer (30). The lack of a detectable effect for this mutation could be related to its low prevalence in the population, or the effects of allelic heterogeneity. In another case study, 251 Ashkenazi Jewish men with prostate cancer recruited at Memorial Sloan-Kettering were also compared with 1,472 Ashkenazi male controls from the Washington D.C. study (24). They reported an OR of 4.8 (95% CI, 1.9-12.3) between *BRCA2* mutations and prostate cancer, whereas they did not detect an increased risk of prostate cancer in *BRCA1* mutation carriers (25). In contrast, three case-report studies of Ashkenazi prostate cancer patients (27–29) concluded that *BRCA1* or *BRCA2* mutations contribute little to prostate cancer susceptibility. However, these studies were relatively small compared with studies that have found a positive association, including the current one. Moreover, none of the previously reported case-control or case-only studies in Ashkenazi men was sufficiently powered to detect associations with the *BRCA1* and *BRCA2* founder mutations.

In support of a role for *BRCA2* mutations associated with prostate cancer, studies of families with breast and/or ovarian cancers who harbor disease-associated *BRCA2* mutations have reported that male family members who carry such mutations have an increased RR of prostate cancer (15–20, 26). In the Breast Cancer Linkage Consortium, *BRCA2* protein-truncating mutation carriers had a RR of 4.7 (95% CI, 3.5-6.2) for prostate cancer (15). In that study, the RR was higher in men diagnosed at ages <65 years (RR, 7.3; 95% CI, 4.7-11.5), compared with those diagnosed at older ages (>65 years). A Finnish study (17) of breast and/or ovarian cancer families also reported a 5-fold increase in the RR of prostate cancer in men carrying protein-truncating *BRCA2* mutations (RR, 4.9; 95% CI, 1.8-11.0). Similar findings were reported by Tulinius et al. (18), in an analysis of nearly a thousand breast cancer pedigrees from Iceland; first-degree male relatives of breast cancer patients carrying protein-truncating *BRCA2* mutations had a RR of 4.8

(95% CI, 3.3-6.3) for prostate cancer (18). Finally, in an analysis of a large number of *BRCA2* families from the Netherlands, there was a RR of 2.5 (95% CI, 1.6-3.8) for prostate cancer in *BRCA2* mutation carriers (20). When the analysis was stratified by age at diagnosis, the RR of prostate cancer was 8.0 (95% CI, 4.1-14.0) for *BRCA2* carriers diagnosed at ages <65 years. Also, studies of “early-onset” prostate cancer (age at diagnosis < 55 years) unselected for family history indicated that the prevalence of *BRCA2* mutations was higher in these cases (ranging from 0.8% to 2.3%) compared with the estimated prevalence of *BRCA2* mutations (~0.1%) in the general population (36, 37). Family-based studies examining risk of prostate cancer in *BRCA1* mutation carriers have reported small, but not statistically significant, or null results (16, 26). A recent study, which examined loss of heterozygosity in prostate tumors in a relatively small sample of 4 and 14 men with *BRCA1* and *BRCA2* mutations, respectively, found loss of heterozygosity at *BRCA2* locus in 71% of tumors, but none at the *BRCA1* locus (38). In the current study, the risk in both *BRCA1* (185delAG) and *BRCA2* (6174delT) carriers was higher in men ages ≥65 years. Similarly, Giusti et al. (30) and Streuwing et al. (24) reported an increased risk of prostate cancer in *BRCA1* (185delAG) carriers. The relatively lower OR of *BRCA2* (6174delT) for prostate cancer in the current study compared with other studies might be related to the location of this mutation inside the Ovarian Cancer Cluster Region (OCCR) of *BRCA2*. Mutations inside the OCCR of *BRCA2* have been associated with a lower risk of prostate cancer (20).

In the current study, Gleason score information was obtained from 92% of cases. Carriers of any of the three founder mutations in the *BRCA1* and *BRCA2* genes had a statistically significant increased risk of high Gleason score (≥7) prostate cancer (OR, 2.6; 95% CI, 1.4-5.0). In mutation-specific analyses, *BRCA2* mutation carriers had a 3.2-fold (95% CI, 1.4-7.4) increase in the risk of high Gleason score prostate cancer. For the *BRCA1* gene, there was not a statistically significant increase in risk of high Gleason score disease for either of the *BRCA1* founder mutations taken together, although carriers of *BRCA1* (185delAG) had an OR of 3.5 (95% CI, 1.2-10.3) for high-grade disease compared with controls. When analyses were repeated considering only

prostate tumors with a Gleason score of 8-10, association with the *BRCA2* mutation remained the same (OR, 3.6; 95% CI, 1.1-11.4) to those observed previously. However, none of the associations between any of the *BRCA1* founder mutations and Gleason score 8 to 10 tumors were statistically significant. This might be partly due to reduced power because only 12% of prostate cancer cases were diagnosed with a Gleason score 8 to 10 tumors. Our study did not have complete clinical information on tumor stage and PSA value at diagnosis and could not examine associations between founder mutations and these other clinical prognostic variables. Nevertheless, these data provide new insights into the risks associated with specific *BRCA1* and *BRCA2* founder mutations and prostate cancer, particularly for high-grade cancer, which might have important clinical implications.

In relation to family history of prostate cancer and other cancers, we found that the risk of prostate cancer associated with carrying these founder mutations was increased in Ashkenazi men who reported no family history (first-degree) of either prostate, breast, or ovarian cancer. The lack of an association in men with a first-degree family history of prostate cancer is similar to the lack of association between *BRCA1/BRCA2* mutations and prostate cancer reported in studies of hereditary prostate cancer (39–42). Because a family history of

breast and/or ovarian cancer is a strong predictor of carrying *BRCA1* and *BRCA2* mutations, it was not surprising that we observed an overall higher mutation prevalence in this subgroup of men. However, the lack of an association in men with a first-degree relative with breast and/or ovarian cancers, is likely related to an age difference between *BRCA1/BRCA2* mutation carrier controls with a family history of breast and/or ovarian cancer (average age of 62.8 years) and those with no family history (average age of 70.0 years). The age difference between the two control groups indicates that the former group is still at risk for prostate cancer as they transit to older ages. In fact, the prevalence of *BRCA1* founder mutations decreased with age in controls, which in part can explain the positive association observed between *BRCA1* mutations and prostate cancer in older men. In this study, the observed prevalence of *BRCA1* and *BRCA2* mutations in controls declined with age from 2.4%, 1.8%, and 1.6% in men <60, 60 to 70, and >70 years, respectively. Similarly, Hartge et al. (43) reported a decline in *BRCA1/2* founder mutations in the Washington, D.C. study in participants without cancer.

Our study has strengths and limitations. We originally estimated that a sample size of 1,800 Ashkenazi Jewish men would be sufficient to detect a 2-fold increase in prostate cancer risk with an accumulated mutation frequency of 2.0% based on

Table 4. Associations between founder mutations in *BRCA1* and *BRCA2* and prostate cancer, stratified by first-degree family history of prostate, breast or ovarian cancers

Mutations	Positive family history of prostate cancer			No family history of prostate cancer		
	Cases	Controls	OR* (95% CI)	Cases	Controls	OR* (95% CI)
	Carrier/ noncarrier	Carrier/ noncarrier		Carrier/ noncarrier	Carrier/ noncarrier	
	n (%) [†]	n (%) [†]	n (%) [†]	n (%) [†]		
<i>BRCA1</i> 185delAG	2/274 (0.7)	1/177 (0.6)	1.61 (0.14-18.10)	9/691 (1.3)	6/1,057 (0.6)	2.06 (0.71-5.92)
Any <i>BRCA1</i> mutation	2/274 (0.7)	1/178 (0.6)	1.60 (0.14-18.06)	10/692 (1.4)	10/1,058 (0.9)	1.38 (0.56-3.40)
<i>BRCA2</i> 6174delT	3/269 (1.1)	1/176 (0.6)	1.91 (0.20-18.60)	15/682 (2.2)	11/1,052 (1.0)	2.10 (0.93-4.70)
<i>BRCA1</i> (185delAG) or <i>BRCA2</i> (6174delT)	5/271 (1.8)	2/176 (1.1)	1.76 (0.34-9.24)	23/680 (3.3)	17/1,054 (1.6)	2.01 (1.05-3.85)
Any mutations in <i>BRCA1/BRCA2</i>	5/271 (1.8)	2/177 (1.1)	1.77 (0.34-9.27)	24/679 (3.4)	21/1,050 (2.0)	1.68 (0.91-3.09)

Mutations	Positive family history of breast or ovarian cancers			No family history of breast or ovarian cancers		
	Cases	Controls	OR* (95% CI)	Cases	Controls	OR* (95% CI)
	Carrier/ noncarrier	Carrier/ noncarrier		Carrier/ noncarrier	Carrier/ noncarrier	
	n (%) [†]	n (%) [†]	n (%) [†]	n (%) [†]		
<i>BRCA1</i> 185delAG	4/185 (2.1)	4/219 (1.8)	1.01 (0.24-4.28)	7/780 (0.9)	3/1,015 (0.3)	3.45 (0.86-13.63)
Any <i>BRCA1</i> mutation	4/186 (2.1)	5/219 (2.2)	0.86 (0.22-3.38)	8/780 (1.0)	6/1,017 (0.6)	1.81 (0.61-5.36)
<i>BRCA2</i> 6174delT	7/182 (3.7)	7/214 (3.2)	1.04 (0.35-3.10)	11/769 (1.4)	5/1,014 (0.5)	3.16 (1.07-9.29)
<i>BRCA1</i> (185delAG) or <i>BRCA2</i> (6174delT)	10/180 (5.3)	11/213 (4.9)	0.93 [‡] (0.37-2.29)	18/771 (2.3)	8/1,017 (0.8)	3.30 [‡] (1.41-7.73)
Any mutations in <i>BRCA1/BRCA2</i>	10/180 (5.3)	12/212 (5.4)	0.87 (0.36-2.11)	19/770 (2.4)	11/1,015 (1.1)	2.44 (1.14-5.22)

NOTE: Missing values varied by mutation: *BRCA1* 185delAG (3 cases, 11 controls); *BRCA1* 5382insC (3 cases, 12 controls); *BRCA2* 6174delT (10 cases, 12 controls).

*ORs are adjusted for age at diagnosis (cases) and age at interview (controls); Reference category is nonmutation carriers.

[†] Percentages of mutation carriers in each category.

[‡] P = 0.04 for interaction between mutation carrier status and family history of breast/ovarian cancer. Other P values for interaction analysis between mutation carrier status and family history of either prostate, or breast or ovarian cancers were not statistically significant.

the prevalence of *BRCA1/2* mutations in the Ashkenazi population (24, 44). Men were recruited using a novel strategy of recruitment by advertisement and were requested to provide all materials through the mail. As presented in Table 1, over 75% of the study population had at least a college degree that facilitated the completion of the self-administered questionnaire and self-obtained DNA sample. In fact, most cases provided their own pathology reports significantly reducing the labor involved in obtaining medical records. Nevertheless, such a recruitment strategy has the potential to introduce bias into the study sample. Therefore, these data should be interpreted in light of this recruitment strategy. Despite this fact, the data in Table 1 provide reassurance that not only were our cases comparable with controls with respect to demographic characteristics, but we also show that the prevalence of the three founder mutations in our study is similar to other large studies conducted in the Ashkenazi population (24). In addition, we collected detailed information on family history of prostate cancer and other cancers, as well as covariates that are important in understanding the association between *BRCA1* and *BRCA2* mutations and prostate cancer. This report is the first large study in men of Ashkenazi descent that recruited both cases and controls and did not depend on previous control cohorts to calculate the association between *BRCA1* and *BRCA2* mutations and prostate cancer. Because only 1% of cases and controls were <50 years old, we had limited power to examine associations with very early ages at diagnosis and thus our findings are relevant to men diagnosed with this disease at age ≥ 50 years.

Lastly, there was an average 5-year lag time between prostate cancer diagnosis and participation in the study among cases, which represents a challenge in assessing the role of *BRCA1* and *BRCA2* mutations in incident versus prevalent prostate cancer. To address this issue, we adjusted the analysis for age at prostate cancer diagnosis for cases and age at participation for controls. When we restricted our analyses to cases that were recruited in the study within a 5-year period after their cancer diagnosis ($n = 595$), the associations between *BRCA1/BRCA2* mutations and prostate cancer were similar to those observed using the entire case group ($n = 979$). Although the overall 5-year survival rate

of prostate cancer is 98% (45) and the majority of incident cases are still alive 10 years after their cancer diagnosis, we cannot rule out the possibility that some cases diagnosed with more advanced prostate cancer had subsequently died of the disease and were not part of this study population. Nevertheless, given the 3.2-fold increased risk of high Gleason score prostate cancer associated with the *BRCA2* founder mutation in this study, if men with high Gleason score (8–10) died before study participation, then our findings reflect an underestimate of the true association between *BRCA2* founder mutation and high Gleason score cancer.

In conclusion, the results of this study indicate that protein-truncating founder mutations in the *BRCA2* gene confer a 3-fold increased RR of high Gleason score prostate cancer. Although the two *BRCA1* mutations taken together were not associated with prostate cancer, the *BRCA1*-185delAG was associated with high Gleason score cancer. Associations between founder mutations and prostate cancer were stronger in men with no first-degree family history of breast and/or ovarian cancers but were unaffected by family history of prostate cancer. These findings highlight the importance of founder mutations, particularly those located in the *BRCA2* gene, in susceptibility to advanced disease. Further studies need to examine associations between *BRCA1* and *BRCA2* founder mutations and other clinical prognostic indicators of prostate cancer, including tumor stage and PSA at diagnosis, to better understand the clinical relevance of such mutations to prostate cancer susceptibility, prognosis, and its implications for genetic testing and counseling.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank Stacey Fishman, Mindy Ginsberg, Gloria Ho, Nicolas Schlecht, Victor Kamensky, Leonid Strizhevsky, Rochelle Demsky, Peggy Cottrel, Betsy Vibert, Kit Tam, and Lydia Chiu for their help with study design and data collection, and all men who participated in this study for their time, effort, and collaboration.

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