Rare germline mutations in the BRCA2 gene are associated with early-onset prostate cancer

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Studies of families who segregate BRCA2 mutations have found that men who carry disease-associated mutations have an increased risk of prostate cancer, particularly early-onset disease. A study of sporadic prostate cancer in the UK reported a prevalence of 2.3% for protein-truncating BRCA2 mutations among patients diagnosed at ages ≤55 years, highlighting the potential importance of this gene in prostate cancer susceptibility. To examine the role of protein-truncating BRCA2 mutations in relation to early-onset prostate cancer in a US population, 290 population-based patients from King County, Washington, diagnosed at ages <55 years were screened for germline BRCA2 mutations. The coding regions, intron–exon boundaries, and potential regulatory elements of the BRCA2 gene were sequenced. Two distinct protein-truncating BRCA2 mutations were identified in exon 11 in two patients. Both cases were Caucasian, yielding a mutation prevalence of 0.78% (95% confidence interval [95%CI] 0.09–2.81%) and a relative risk (RR) of 7.8 (95%CI 1.8–9.4) for early-onset prostate cancer in white men carrying a protein-truncating BRCA2 mutation. Results suggest that protein-truncating BRCA2 mutations confer an elevated RR of early-onset prostate cancer. However, we estimate that <1% of early-onset prostate cancers in the general US Caucasian population can be attributed to these rare disease-associated BRCA2 mutations.

British Journal of Cancer (2007) 97, 826–831. doi:10.1038/sj.bjc.6603929 www.bjcancer.com
Published online 14 August 2007
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Keywords: BRCA2 gene; protein-truncating BRCA2 mutations; prostate cancer; early-onset disease

Studies of families segregating BRCA2 mutations (BCLC, 1999; Johannsson et al, 1999; Errola et al, 2001; Tulinius et al, 2002; Bermejo and Hemminki, 2004; van Asperen et al, 2005), kin-cohort studies of cancer incidence among relatives of population-based breast or ovarian cancer cases (Loman et al, 2003; Risch et al, 2006), as well as studies of populations who harbour founder BRCA2 mutations (Sigurdsson et al, 1997; Struewing et al, 1997, Kirchhoff et al, 2004) suggest that men who carry a disease-associated BRCA2 allele have an increased relative risk (RR) of prostate cancer (two- to five-fold elevation). Two of the family studies in which data were stratified on age at diagnosis of prostate cancer reported that the RR was even higher (seven- to eight-fold increase) in men diagnosed before age 65 years (BCLC, 1999; van Asperen et al, 2005). These findings suggest that protein-truncating BRCA2 mutations may play a role in prostate cancer susceptibility. However, the majority of studies that have investigated the role of BRCA2 mutations in hereditary prostate cancer (HPC) families, which usually include men diagnosed with prostate cancer at younger ages, have reported no disease-associated mutations (Wilkens et al, 1999; Gayther et al, 2000; Sinclair et al, 2000; Agalliu et al, 2007).

To date, only one study has assessed the contribution of BRCA2 mutations in early-onset sporadic prostate cancer (Edwards et al, 2003). In a case series of 263 men in the UK diagnosed with prostate cancer at younger ages (≤55 years), and who were not selected on the basis of either breast or prostate cancer family history, the prevalence of protein-truncating BRCA2 mutations was 2.3% (95% confidence interval [95%CI] 0.8–5.0%) (Edwards et al, 2003). The estimated RR of prostate cancer was 23.0 (95%CI 9.0–57.0) among protein-truncating BRCA2 mutation carriers in that study. The main goal of the current study was to determine the frequency of germline protein-truncating BRCA2 mutations in early-onset prostate cancer patients in a US population.

MATERIALS AND METHODS

Study population

Case patients (n = 290) included in this analysis are Caucasian and African American men diagnosed with histologically confirmed adenocarcinoma of the prostate before age 55 years and who participated in one of two population-based case–control studies.
of prostate cancer conducted in King County, Washington. Case patients in the first study \((n = 168)\) were diagnosed from January 1, 1993 to December 31, 1996 (Stanford et al, 1999), and those in the second study \((n = 128)\) were diagnosed from January 1, 2002 to December 31, 2005. Incident cases in both studies were identified through the population-based Seattle-Puget Sound SEER cancer registry, which also provided information on Gleason score, tumour stage, and serum prostate-specific antigen (PSA) level at diagnosis. Case patients completed a structured in-person interview that collected information about demographic and lifestyle characteristics, medical history, prostate cancer screening history, and family history of prostate cancer and other cancers. After the interview, patients were asked to provide a blood sample.

A total of 429 eligible prostate cancer case patients aged <55 years were identified during the ascertainment periods. Of eligible patients, 381 (89%) were interviewed and 301 (79%) provided a blood sample. A total of 290 case patients with adequate amounts of DNA were sequenced for \(BRCA2\) mutations. The study was approved by the Institutional Review Board of the Fred Hutchinson Cancer Research Center and the National Human Genome Research Institute, and written informed consent was obtained from all study participants.

**BRCA2 gene sequencing**

Genomic DNA was purified from peripheral blood lymphocytes using a standard proteinase K digestion and alkaline lysis followed by phenol-chloroform extraction (Sambrook et al, 1989). All coding and non-coding regions of the \(BRCA2\) gene were screened for mutations using 47 primer pairs described previously (Malone et al, 2000, 2006; Agalliu et al, 2007). Exons 5 and 6 were amplified in a single fragment and longer exons were amplified using multiple PCR reactions. There were five amplicons for exon 10 (10A – 10E), 16 for exon 11 (11A – 11P), and two for each of the following exons: 14 (14A and 14B), 18 (18A and 18B), and 27 (27A and 27B). PCR reactions were performed in a 10 \(\mu\)l volume containing 5 ng of genomic DNA, 1 mM dNTPs, 1.5 mM, or 2.5 mM MgCl\(_2\), 1 \(\mu\)M of both the forward and reverse primers, and 0.25 U AmpliTaq DNA polymerase (Applied Biosystems, Foster City, CA, USA).

PCR products were treated using the ExoSAP-IT method (USB Corporation, Cleveland, OH, USA). DNA-sequencing reactions were carried out using BigDye\textsuperscript{TM} Terminator v3.1 Cycle Sequencing Kits (Applied Biosystems, Foster City, CA, USA) and sequence data were obtained on both forward and reverse PCR primers using the ABI 3730xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Contigs were assembled using PhredPhrap/Consed (Gordon et al, 1998). Variants were detected using Polyphred (Nickerson et al, 1997) and MutationSurveyor (http://www.softgenetics.com/mutationSurveyor.html) by two independent researchers. All variants were unanimously confirmed.

**Statistical analyses**

The 95% CI for the prevalence of protein-truncating \(BRCA2\) mutations among prostate cancer patients was computed using a Poisson-distributed events-table under the assumption that these mutations are rare and follow a Poisson distribution. The prevalence of germline protein-truncating \(BRCA2\) mutations in the general population was taken from a recently published study (Malone et al, 2006) and was used to estimate the RR of prostate cancer in men aged <55 years associated with carrying a \(BRCA2\) mutation.

A \(\chi^2\) test was used to evaluate whether the distribution of genotypes for single nucleotide polymorphisms (SNPs) in the \(BRCA2\) gene differed by race (Caucasians vs African Americans), family history of prostate cancer (yes vs no) or clinical characteristics such as Gleason score (2 – 6 or 7 = 3 + 4 vs 7 = 4 + 3 or 8 – 10), stage of cancer (localised vs regional or distant) or a composite measure of disease aggressiveness. The definition of more aggressive prostate cancer included a Gleason score of 7 = 4 + 3 or 8 – 10 or regional or distant stage or serum PSA \(> 20\) ng ml\(^{-1}\) at prostate cancer diagnosis; men with a Gleason score of 2 – 6 or 7 = 3 + 4 and local stage tumours and a serum diagnostic PSA level <20 ng ml\(^{-1}\) at diagnosis were classified as having less aggressive cancer. SAS version 9.1 (SAS Institute, Cary, NC, USA) was used for statistical analyses.

**RESULTS**

Table 1 shows characteristics of the 290 population-based prostate cancer patients screened for \(BRCA2\) mutations; 11% were African-American and 2% were Jewish. The proportion of patients that reported either a first- or second-degree family history of prostate, breast, or ovarian cancer was 37, 16, and 3%, respectively. Five percent reported a family history of prostate, breast, and/or ovarian cancers; 3% reported first-degree relatives with either prostate or breast cancers. Most of the patients (79%) reported that they had received at least one PSA test and/or digital rectal examination (DRE) in the 5-year period before diagnosis. The majority of the patients had localised tumours (73%), Gleason scores of 2–6 (59%), and 36% of them were classified as having tumours with more aggressive clinical features at diagnosis.

Two distinct protein-truncating \(BRCA2\) mutations were identified, yielding an overall prevalence of 0.69% (95%CI 0.08 – 2.49%). Table 2 shows characteristics of the two prostate cancer patients with protein-truncating \(BRCA2\) mutations. One of these mutations was a 5-bp deletion (4625 4629delACATT) and the other was a 2-bp deletion (4074_4075delIGT), both located in exon 11. Both cases with protein-truncating \(BRCA2\) mutations were Caucasian, one reported a first-degree relative with prostate cancer, but neither reported a family history of breast or ovarian cancer, and both were diagnosed with Gleason score 7 tumours. As the protein-truncating \(BRCA2\) mutations were detected in Caucasians only, the prevalence of such mutations in whites is 0.78% (95%CI 0.09 – 2.81%). Recently, Malone et al (2006) reported a weighted prevalence of protein-truncating \(BRCA2\) mutations of 0.1% (95%CI 0.0 – 0.3%) among US population-based Caucasian control women aged 35 – 64 years, assuming that the prevalence of disease-associated \(BRCA2\) mutations among Caucasian men in the general population is similar to the prevalence of such mutations reported for US Caucasian women, the estimated RR of prostate cancer in white men <55 years of age associated with carrying a protein-truncating \(BRCA2\) mutation is 7.78 (95%CI 1.80 – 9.37). In addition to the two protein-truncating mutations, 84 sequence variants were detected in the \(BRCA2\)-coding exons, intron – exon boundaries, and putative regulatory regions that were sequenced. One SNP was in the 5' UTR region, 19 (22.6%) were intronic SNPs, and 40 (47.6%) non-synonymous and 24 (28.6%) synonymous SNPs were in coding exons. Most of these 84 SNPs were rare; only 11 (13.1%) had a minor allele frequency of 3% or greater, and of these, only four resulted in an amino-acid change (Table 3). There were two SNPs that differed \((P < 0.05)\) between Caucasian and African-American patients; both the C allele of SNP 1341A>C located in exon 10 and the T allele of the intronic SNP (IVS17-14) were more common in white than African American patients (26.7 vs 7.6% and 48.8 vs 34.8%, respectively). The distribution of genotypes for the SNPs having a minor allele frequency of \(> 3\%\) did not vary substantially according to family history of prostate cancer or clinical features of the disease (data not shown). One exception was that the frequency of the T allele for the SNP 5971C>T located in exon 11 was higher in patients with more aggressive disease in comparison to cases with less aggressive clinical features, 5.2 vs 1.9%, respectively \((P = 0.02)\).
DISCUSSION

In this population-based study of 290 patients diagnosed with prostate cancer before age 55 years, there were two patients with germline protein-truncating BRCA2 mutations, yielding an overall prevalence of 0.69% (95%CI 0.08 – 2.49%). One of the cases with a BRCA2 mutation reported a first-degree relative with prostate cancer, but neither reported a family history of breast or ovarian cancer. As both disease-associated BRCA2 mutation carriers were Caucasian, the prevalence of such mutations in whites is 0.78% (95%CI 0.09 – 2.81%); the estimated RR of prostate cancer is 7.8 (95%CI 1.8 – 9.4) in Caucasian BRCA2 mutation carriers. Although this represents a substantially elevated RR of earlier-onset prostate cancer associated with carrying a germline protein-truncating BRCA2 mutation, such mutations are very rare (~0.1%) in the general US population (Malone et al, 2006). The estimated cumulative risk (i.e. penetrance) of prostate cancer to age 55 years in Caucasian men due to carrying a disease-associated BRCA2 mutation is about 1.1%, and the population attributable risk of prostate cancer in this same group is 0.7%.

In a hospital-based series (n = 263) of prostate cancer patients with early ages at diagnosis (<55 years) in the UK, Edwards et al (2003) reported a prevalence of germline protein-truncating BRCA2 mutations of 2.3% (95%CI 0.8 – 5.0%). The estimated RR

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Table 1 Characteristics of 290 population-based prostate cancer patients aged <55 years at diagnosis screened for BRCA2 mutations

| Ascertainment period | 1993–1996 (n = 162) | 2002–2005 (n = 128) | Total case patients (n = 290) |
|----------------------|----------------------|----------------------|-----------------------------|
| **Age at diagnosis (years)** | | | |
| 35–49 | 39 (24.1) | 58 (45.3) | 97 (33.4) |
| 50–54 | 123 (75.9) | 70 (54.7) | 193 (66.6) |
| **Race** | | | |
| Caucasian | 154 (95.1) | 103 (80.5) | 257 (88.6) |
| African-American | 8 (4.9) | 25 (19.5) | 33 (11.4) |
| **Jewish** | | | |
| No | 159 (98.1) | 126 (98.4) | 285 (98.3) |
| Yes | 3 (1.9) | 2 (1.6) | 5 (1.7) |
| **Family history of prostate cancer** | | | |
| None | 111 (68.5) | 71 (55.5) | 182 (62.8) |
| First-degree | 33 (20.4) | 40 (31.3) | 73 (25.2) |
| Second-degree only | 18 (11.1) | 17 (13.3) | 35 (12.1) |
| **Family history of breast cancer** | | | |
| None | 134 (82.7) | 109 (85.2) | 243 (83.8) |
| First-degree | 22 (13.6) | 15 (11.7) | 37 (12.8) |
| Second-degree only | 6 (3.7) | 4 (3.1) | 10 (3.4) |
| **Family history of ovarian cancer** | | | |
| None | 157 (96.9) | 123 (96.1) | 280 (96.6) |
| First-degree | 2 (1.2) | 3 (2.3) | 5 (1.7) |
| Second-degree only | 3 (1.9) | 2 (1.6) | 5 (1.7) |
| **Prostate cancer screening** | | | |
| None | 32 (19.8) | 28 (21.9) | 60 (20.7) |
| DRE only | 46 (28.4) | 25 (19.5) | 71 (24.5) |
| PSA and DRE | 84 (51.9) | 75 (58.6) | 159 (54.8) |
| **Gleason score** | | | |
| 2–4 | 19 (11.7) | — | 19 (6.6) |
| 5–6 | 82 (50.6) | 69 (53.9) | 151 (51.2) |
| 7 (3+4) | 39 (24.1) | 40 (31.3) | 79 (27.2) |
| 7 (4+3) | 9 (5.6) | 11 (8.6) | 20 (6.9) |
| 8–10 | 10 (6.2) | 6 (4.7) | 16 (5.5) |
| Missing | 3 (1.9) | 2 (1.6) | 5 (1.7) |
| **Stage of cancer** | | | |
| Localised | 114 (70.4) | 97 (75.8) | 211 (72.8) |
| Regional | 40 (24.7) | 29 (22.7) | 69 (23.8) |
| Distant | 8 (4.9) | 2 (1.6) | 10 (3.4) |
| **PSA at diagnosis (ng/ml)** | | | |
| 0.39 | 26 (16.1) | 29 (22.7) | 55 (19.0) |
| 4.0–9.9 | 77 (47.6) | 63 (49.2) | 140 (48.3) |
| 10.0–19.9 | 21 (12.9) | 15 (11.7) | 36 (12.4) |
| ≥20 | 19 (11.7) | 11 (8.6) | 30 (10.3) |
| Missing | 19 (11.7) | 10 (7.8) | 29 (10.0) |

*One or more prostate-specific antigen (PSA) test or digital rectal exam (DRE) in the 5-year period before diagnosis date. *Serum prostate-specific antigen (PSA) level at diagnosis.
of developing prostate cancer by age 56 years from a deleterious germline BRCA2 mutation was 23.0 (95%CI 9.0–57.0). To compute this RR estimate, Edwards et al (2003) used the average of the estimated prevalence (i.e., 0.12 and 0.07%) of germline disease-associated BRCA2 mutations in the general UK population, which was indirectly estimated from two prior studies of breast cancer in the UK (Peto et al, 1999; Antoniou et al, 2002). Interestingly, of the six prostate cancer patients carrying disease-associated BRCA2 mutations, 50% were diagnosed before age 50 and most had no family history of prostate cancer (five out of six) or breast cancer (four out of six) (Edwards et al, 2003).

Although the overall finding is consistent with our results, a lower prevalence of disease-associated BRCA2 mutations was observed in our population-based patient series. This difference may be due to different study designs and populations. Edwards et al (2003) utilised prostate cancer patients recruited through three different sources: Cancer Research UK/British Prostate Group, UK Familial Prostate Cancer Study, and the British Association of Urological Section of Oncology, and 90% of them had clinically detected prostate cancer. By contrast, our patients were ascertained from a population-based SEER registry. Most of these men had received a PSA and/or DRE test within the 5-year period before diagnosis, and 27% of the cases reported one or more clinical signs or symptoms at the time of diagnosis.

Studies of families with breast and/or ovarian cancer who harbour disease-associated BRCA2 mutations have reported that male family members who carry such mutations have an increased RR of prostate cancer (BCLC, 1999; Johannsson et al, 1999; Eerola et al, 2001; Tulinius et al, 2002; Bermejo and Hemminki, 2004; van Asperen et al, 2005). In the Breast Cancer Linkage Consortium cohort of 173 breast and/or ovarian cancer families, a RR of 4.7 (95%CI 3.5–6.2) was reported for prostate cancer among BRCA2

### Table 2 Characteristics of prostate cancer patients with germline protein-truncating BRCA2 mutations

| BRCA2 mutation | Exon location | Exon | Nucleotide change | Protein effect | Characteristics | Age at diagnosis (years) | Race | Family history of | Prostate cancer | Breast cancer | Ovarian cancer | Clinical characteristics |
|----------------|---------------|------|-------------------|----------------|------------------|------------------------|------|-------------------|---------------|--------------|--------------|-----------------------------|
|                |               |      |                   |                |                  |                        |      |                    |               |              |              |                             |
|                |               | 11   | 4625_4629delACATT  | STOP 1467      |                  | 51                     | Caucasian             | Yes (first-degree) | No            | No            |                             |
|                |               | 11   | 4074_4075delGT     | STOP 1284      |                  | 47                     | Caucasian             | No              | No            | No            |                             |

### Table 3 SNPs with a >3% minor allele frequency in the BRCA2 gene among 290 prostate cancer patients, by race

| Exon | Nucleotide position | Nucleotide change | Amino-acid change | Genotype | Total N = 290 | Caucasian N = 257 | African American N = 33 | P-value* |
|------|---------------------|-------------------|-------------------|----------|---------------|-------------------|-------------------------|----------|
| 202  | G > A               | 5’ UTR            |                   | GG       | 160           | 55.2              | 21                      | 63.6     | 0.51        |
|      |                     |                   |                   | AG       | 100           | 34.5              | 10                      | 30.3     |             |
|      |                     |                   |                   | AA       | 30            | 10.3              | 2                        | 6.1      |             |

Non-synonymous SNPs

10 1092 | A > C | N289H | AA | 269 | 92.8 | 240 | 93.4 | 29 | 87.9 | 0.25 |
|      | AC    |       | 21 | 7.2  | 17  | 6.6  | 4  | 12.1 |
| 10 1341 | A > C | N372H | AA | 170 | 58.6 | 142 | 55.3 | 28 | 84.8 | 0.004 |
|      | AC    |       | 98 | 33.8 | 93  | 36.2 | 5  | 15.2 |
| 11 3198 | A > G | N991D | AA | 268 | 92.4 | 240 | 93.4 | 28 | 84.8 | 0.08 |
|      | AG    |       | 22 | 7.6  | 17  | 6.6  | 5  | 15.2 |
| 11 5971 | C > T | T1915M | CC | 272 | 93.8 | 243 | 94.6 | 29 | 87.9 | 0.13 |
|      | CT    |       | 18 | 6.2  | 14  | 5.4  | 2  | 12.1 |

Synonymous or intronic SNPs

10 1592 | A > G | S455S | AA | 269 | 92.8 | 240 | 93.4 | 29 | 87.9 | 0.25 |
|      | AG    |       | 21 | 7.2  | 17  | 6.6  | 4  | 12.1 |
| 11 2456 | T > C | H743H | TT | 269 | 92.8 | 240 | 93.4 | 29 | 87.9 | 0.25 |
|      | CT    |       | 21 | 7.2  | 17  | 6.6  | 4  | 12.1 |
| 11 3623 | A > G | K1132K | AA | 134 | 46.2 | 121 | 47.1 | 13 | 39.4 | 0.68 |
|      | AG    |       | 121 | 41.7 | 105 | 40.9 | 16 | 48.5 |
| 11 4034 | T > C | V1269V | TT | 195 | 67.2 | 171 | 66.5 | 24 | 72.7 | 0.72 |
|      | CT    |       | 77  | 26.6 | 69  | 26.8 | 8  | 24.2 |
| 14 7469 | A > G | S2414S | AA | 170 | 58.6 | 155 | 60.3 | 15 | 45.5 | 0.23 |
|      | AG    |       | 104 | 35.9 | 89  | 34.6 | 15 | 45.5 |
| 17 IVS17-14 | T > C | in | TT | 70  | 24.1 | 64  | 24.9 | 6  | 18.2 |

*χ² P-value comparing the distribution of genotypes between Caucasian and African-American prostate cancer cases. a No homozygous variants were detected. b Two Caucasian men had missing data for this SNP and thus percentages do not add up to 100%.
protein-truncating mutation carriers (BCLC, 1999). In that study, the RR was higher in men diagnosed at ages <65 years (RR = 7.3, 95%CI 4.7 – 11.5). A Finnish study (Eerola et al, 2001) of 107 breast or ovarian cancer families reported a five-fold increase in the RR of prostate cancer among men carrying protein-truncating BRCA2 mutations (RR = 4.9, 95%CI 1.8 – 11.0). Similar findings were reported by Tulinius et al (2002) in an analysis of 995 breast cancer pedigrees from Iceland. In that study, 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 (Tulinius et al, 2002). Finally, van Asperen et al (2005) in a analysis of 139 BRCA2 families from the Netherlands reported an RR of 2.5 (95%CI 1.6 – 3.8) for prostate cancer among BRCA2 protein-truncating mutation carriers. When the analysis was stratified by the age at diagnosis, the RR of prostate cancer was 8.0 (95%CI 4.1 – 14.0) among protein-truncating BRCA2 mutation carriers diagnosed at ages <65 years.

In contrast to the above studies and the current analysis, most studies of HPC families investigating the contribution of protein-truncating BRCA2 mutations to prostate cancer susceptibility have reported null results (Wilkins et al, 1999; Gayther et al, 2000; Sinclair et al, 2000; Agalliu et al, 2007). Only one study conducted in the UK of 38 affected individuals from HPC families with two to five affected men per family reported a prevalence of 5.3% for protein-truncating BRCA2 mutations (Gayther et al, 2000). In that study, the two men with protein-truncating BRCA2 mutations were diagnosed with prostate cancer at ages ≤56 years, and both had a brother with prostate cancer. There is still uncertainty as to why BRCA2 families, ascertainment based on the presence of breast and/or ovarian cancer, have a higher RR of prostate cancer in men carrying protein-truncating BRCA2 mutation (BCLC, 1999; Johannsson et al, 1999; Eerola et al, 2001; Tulinius et al, 2002; Bermejo and Hemminki, 2004; van Asperen et al, 2005). By comparison, when HPC families that include members with a history of breast or ovarian cancer are screened for BRCA2 mutations, no excess of protein-truncating BRCA2 mutations is found (Agalliu et al, 2007).

A more complete understanding of the role of the BRCA2 gene in relationship with prostate cancer susceptibility may provide insight on prostate carcinogenesis. For example, the encoded BRCA2 protein is involved in DNA repair, transcription, and cell cycle control (Davies et al, 2001; Venkitaraman and Powell, 2003; Yoshida and Miki, 2004). BRCA2 interacts directly with RAD51, which is an essential protein for error-free double-strand DNA break repair by homologous recombination (Sharan et al, 1997; Wong et al, 1997; Xia et al, 2001; Pellegrini et al, 2002; Venkitaraman, 2002; Jackson, 2002; Powell et al, 2003). BRCA2 regulates both the intracellular localisation and DNA-binding ability of RAD51, and therefore loss of this control following BRCA2 inactivation may be a key event leading to genomic instability and carcinogenesis (Davies et al, 2001; Jackson, 2002; Yoshida and Miki, 2004). Whether prostate tissue is particularly susceptible to BRCA2 loss is not clear. The fact that protein-truncating mutations in the BRCA2 gene predispose to breast and ovarian cancers suggests that the hormonal nature of these cancers may be important. In relation to the prostate, a study found that BRCA2 functions as a co-activator for the androgen receptor (AR) in conjunction with histone acetyltransferase (Shin and Verma, 2003). This finding suggests a novel mechanism by which BRCA2 protein may exert its tumour suppressor function, by altering AR signalling.

Our study has strengths and limitations that should be considered when interpreting the results. The current study is population-based; patients were not selected on the basis of family history of prostate, breast, or ovarian cancer, which offers a less-biased assessment of the role of BRCA2 protein-truncating mutations in men from the general population. In addition, this is one of the largest such studies completed to date. A limitation of this study is that no screening for large genomic deletions in the BRCA2 gene was performed, as this method requires a relatively large amount of DNA per patient. It has been estimated that large genomic deletions account for an additional 10 – 15% of reported disease-associated BRCA2 mutations (Szabo and King, 1997). Thus, the prevalence of protein-truncating BRCA2 mutations might have been underestimated in this study. In addition, owing to the high cost of genetic sequencing and the rarity of germline BRCA2 protein-truncating mutations in the general population, it was not feasible to screen the large number of control men that would be required for direct case-control comparisons. Therefore, we used the previously reported prevalence of BRCA2 mutations in Caucasian control women from the general US population (Malone et al, 2006) to estimate the RR. There is no reason to believe that the prevalence of BRCA2 mutations in the general population would be substantially different between men and women, as the gene is located on chromosome 13. Malone et al (2006) reported a prevalence of 0.1% for germline BRCA2 protein-truncating mutations among US Caucasian control women. This prevalence is similar to the estimates of 0.12 and 0.07% for BRCA2 protein-truncating mutations in the general UK population (Peto et al, 1999; Antoniou et al, 2002). By contrast, a recent population-based study of ovarian cancer in Ontario, Canada, estimated a higher carrier frequency of protein-truncating BRCA2 mutations of 0.69% (95%CI 0.43% – 1.10%) in the general population (Risch et al, 2006). However, the Canadian estimate was based on data from ovarian cancer patients. In addition, the ovarian cancer population was enriched with subjects, such as Ashkenazi Jews, known to have a higher prevalence of BRCA2 disease-associated founder mutations (Risch et al, 2006).

In conclusion, the results of this study indicate that germline protein-truncating mutations in the BRCA2 gene confer an elevated RR (almost eight-fold) of developing early-onset prostate cancer in Caucasian men. However, the population attributable risk of prostate cancer in US Caucasians under age 55 due to carrying one of these rare germline protein-truncating BRCA2 mutation is estimated to be <1%. Given the relatively high cost of BRCA2 mutation screening and the rarity of these high-risk BRCA2 protein-truncating mutations in the general population, screening for these mutations in the general population would not be cost effective and is not currently recommended.

ACKNOWLEDGEMENTS

We thank Dr KE Malone for providing data from the CARE study. We thank all the men who participated in this study for their time, effort, and cooperation. This work was supported by Grants R01-CA56678 and R01-CA092579, and Contract N01-CN-05230 from the National Cancer Institute. Additional support was provided by the Fred Hutchinson Cancer Research Center, and the Intramural Program of the National Human Genome Research Institute.

REFERENCES

Agalliu I, Kwon EM, Zadory D, McIntosh L, Thompson J, Stanford JL, Ostrander EA (2007) Germline mutations in the BRCA2 gene and susceptibility to hereditary prostate cancer. Clin Cancer Res 13: 839 – 843

Antoniou AC, Pharoah PD, McMullan G, Day NE, Stratton MR, Peto J, Ponder BJ, Easton DF (2002) A comprehensive model for familial breast cancer incorporating BRCA1, BRCA2 and other genes. Br J Cancer 86: 76 – 83

British Journal of Cancer (2007) 97(6), 826 – 831 © 2007 Cancer Research UK
Bermejo JL, Hemminki K (2004) Risk of cancer at sites other than the breast in Swedish families eligible for BRCA1 or BRCA2 mutation testing. *Ann Oncol* 15: 1834 – 1841

Breast Cancer Linkage Consortium (BCLC) (1999) Cancer risks in BRCA2 mutation carriers. *J Natl Cancer Inst* 91: 1310 – 1316

Davies AA, Masson JY, Mcllwraith MJ, Stasiak AZ, Stasiak A, Venkitaraman AR, West SC (2001) Role of BRCA2 in control of the RAD51 recombination and DNA repair protein. *Mol Cell* 7: 273 – 282

Edwards SM, Kote-Jarai Z, Meitz J, Hamoudi R, Fan I, Tang J, Li S, Zhang S, Shaw PA, Narod SA (2006) Population BRCA1 and BRCA2 mutation frequencies and cancer penetrances: a kin-cohort study in Ontario, Canada. *J Natl Cancer Inst* 98: 1694 – 1706

Peto J, Collins N, Barfoot R, Seal S, Warren W, Rahman N, Easton DF, Evans C, Deacon J, Stratton MR (1999) Prevalence of BRCA1 and BRCA2 gene mutations in patients with early-onset breast cancer. *J Natl Cancer Inst* 91: 943 – 949

Powell SN, Kachnic LA (2003) Roles of BRCA1 and BRCA2 in homologous recombination, DNA replication fidelity and the cellular response to ionizing radiation. *Oncogene* 22: 5784 – 5791

Risch HA, McLaughlin JR, Cole DE, Rosen B, Bradley L, Fan I, Tang J, Li S, Zhang S, Shaw PA, Narod SA (2006) Population BRCA1 and BRCA2 mutation frequencies and cancer penetrances: a kin-cohort study in Ontario, Canada. *J Natl Cancer Inst* 98: 1694 – 1706

Sharan SK, Morimitsu M, Albrecht U, Lim DS, Regel E, Dinh C, Sands A, Eichele G, Hasty P, Bradley A (1997) Embryonic lethality and radiation hypersensitivity mediated by Rad51 in mice lacking BRCA2. *Nature* 386: 804 – 810

Shin S, Verma IM (2003) BRCA2 co-operates with histone acetyltransferases in androgen receptor-mediated transcription. *Proc Natl Acad Sci USA* 100: 7201 – 7206