Loss of Heterozygosity at the BRCA2 Locus Detected by Multiplex Ligation-Dependent Probe Amplification is Common in Prostate Cancers from Men with a Germline BRCA2 Mutation

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Abstract Purpose: Prostate cancer risk is increased for men carrying a pathogenic germline mutation in BRCA2, and perhaps BRCA1. Our primary aim was to test for loss of heterozygosity (LOH) at the locus of the mutation in prostate cancers from men who carry a pathogenic germline mutation in BRCA1 or BRCA2, and to assess clinical and pathologic features of these tumors. Experimental Design: From 1,243 kConFab families: (a) 215 families carried a pathogenic BRCA1 mutation, whereas 188 families carried a pathogenic BRCA2 mutation: (b) of the 158 men diagnosed with prostate cancer (from 137 families), 8 were confirmed to carry the family-specific BRCA1 mutation, whereas 20 were confirmed to carry the family-specific BRCA2 mutation; and (c) 10 cases were eliminated from analysis because no archival material was available. The final cohort comprised 4 and 14 men with a BRCA1 and BRCA2 mutation, respectively. We examined LOH at the BRCA1 and BRCA2 genes using multiplex ligation-dependent probe amplification of DNA from microdissected tumor. Results: LOH at BRCA2 was observed in 10 of 14 tumors from BRCA2 mutation carriers (71%), whereas no LOH at BRCA1 was observed in four tumors from BRCA1 mutation carriers (P = 0.02). Under the assumption that LOH occurs only because the cancer was caused by the germline mutation, carriers of BRCA2 mutations are at 3.5-fold (95% confidence interval, 1.8-12) increased risk of prostate cancer. A high Gleason was the only distinct clinical feature. Conclusions: These observations are consistent with the idea that BRCA2, but not BRCA1, is a tumor suppressor of prostate cancer.

Prostate cancer, a common cancer diagnosed in Western men, is a major cause of morbidity and mortality. Risk factors include family history, especially if relatives are diagnosed at a young age (1). To date, the search for genes associated with a high individual risk has been unsuccessful, perhaps due to genetic heterogeneity. Studies are under way to find common genetic variants associated with a small increased risk (2).

Rare germline mutations in the breast cancer predisposition genes, BRCA1 and, in particular, BRCA2, have been implicated in susceptibility to prostate cancer (3). The issue is complicated by the fact that, except in populations with founder mutations, only indirect estimates of the prevalence of germline mutations in unaffected men are available. These estimates have wide confidence intervals (that have not necessarily been correctly reported). The prevalence of BRCA2 mutations in selected series of men with prostate cancer is low, around 1% to 2%, but these estimates are imprecise (4, 5). In addition, estimates of penetrance from mutation testing case series have not been collected systematically and have used information from relatives (6), leading to potential biases. The best data have come from modified segregation analysis of mutation-carrying families identified from studying multiple-case breast and ovarian cancer families. These data suggest that the risk of prostate cancer is 4.7-fold higher (95% confidence interval (95% CI), 3.5-6.2) in men carrying germline BRCA2 mutations than in the general population. This relative risk (RR) is estimated to be higher in carriers who are younger than 65 years (RR, 7.3; 95% CI, 4.7-11.5; ref. 3).

In the context of breast and ovarian cancers, BRCA1 and BRCA2 are classic tumor suppressor genes as first described by Knudson (7) for retinoblastoma. Breast tumors arising in
mutation carriers usually display inactivation of both copies of the corresponding gene (8–13); one allele is inactivated as a result of the inherited germline mutation, whereas the second allele is somatically inactivated, typically as a consequence of deletion (8–11) but less frequently by epigenetic changes (12–15). Whatever the mechanism, the absence of a functional wild-type allele from tumors fits the Knudson 2-hit model and suggests a causal link between the presence of a germline BRCA1 or BRCA2 mutation and the development of breast or ovarian malignancy.

BRCA1 and BRCA2 mutation carriers are on average at 10 or more times population risk of breast cancer, and more so for ovarian cancer (16). Therefore, although the proportion of breast and ovarian cancers in mutation carriers that arise independent of them being a mutation carrier is <10%, it is still >0%. That is, women who carry germline mutations may still develop breast or ovarian cancers via other pathways. Although rare, the proportion that does is a reflection of the extent of an increase in cancer risk experienced by carriers. In other words, if the increased risk of cancer in mutation carriers is RR-fold, then 100 × RR / (RR + 1) % of carriers who develop cancer will have done so through being a carrier. On average, the tumors of 100 / (RR + 1) % of carriers would not necessarily have the same biology or pathology as those that have been caused by the germline mutation. We shall use the reverse of this argument to estimate the increased risk of prostate cancers for germline mutation carriers under some assumptions.

To date, only two small studies have reported on loss of heterozygosity (LOH) in prostate cancers from BRCA2 mutation carriers (9, 17). In the first study, LOH analysis was done on prostate cancers from four brothers, at least three of whom carried a family-specific BRCA2 mutation. Two tumors showed loss of the wild-type allele for at least one informative microsatellite marker, whereas heterozygosity was retained in the remaining two tumors (17). The second study identified LOH in six of seven (86%) prostate cancers from men with germline BRCA2 mutations (9). This prevalence of LOH is higher than the two in 27 (7%) observed in so-called sporadic prostate cancers (P = 0.0001; ref. 18).

The primary aim of this study was to test for LOH at the locus of the mutation in prostate cancers from men who carry a germline pathogenic mutation in either BRCA1 or BRCA2. The secondary aim was to assess the clinical, epidemiologic, and pathologic features of the tumors from these men to see if they displayed any characteristic phenotype, as is the case for breast tumors from BRCA1, and to a lesser extent BRCA2, mutation carriers (19, 20).

**Materials and Methods**

**Subjects**

Verified cases of prostate cancer were identified by examination of the cancer family histories of multiple-case breast cancer families identified through Family Cancer Clinics and associated public hospitals in Australia and New Zealand by the Kathleen Cunningham Consortium for Research into Familial Breast Cancer (kConFab; see Mann et al. 21). In brief, a recruited family had to have either a strong family history of breast and/or ovarian cancer, or to be known to be segregating a mutation in BRCA1, BRCA2, ATM, or TP53. As of June 2007, kConFab had recruited 1,243 families. Personal and family cancer histories from all participants were collected by a kConFab Research Nurse using an epidemiology questionnaire developed by the Breast Cancer Family Registry (22).

Eligibility for inclusion in this prostate cancer study required each participant to be a carrier of a germline pathogenic mutation in either BRCA1 or BRCA2, have a confirmed diagnosis of prostate cancer, and for there to be access to archival tumor material. Mutation status was confirmed by either a predictive clinical mutation test or by extended genotyping within a family by the kConFab laboratory.

All prostate cancers were verified through the State or Centralised Cancer Registries or by a diagnostic pathology report. All participants gave informed consent and approval for the study was obtained from the human research ethics committee at the Peter MacCallum Cancer Centre.

**Nucleic acid isolation**

**LOH analysis.** Archival blocks for all tissues used were obtained from pathology laboratories in Australia and New Zealand. A 3- to 5-μm section stained with H&E was marked by a pathologist for tumor rich (>95%) regions. Tumor cells were microdissected from an overlaid unstained section. DNA was extracted from unstained sections of the formalin-fixed paraffin-embedded tissue using the DNeasy blood & tissue kit (Qiagen) and a modification of the protocol by Wu et al. (23), which involves a 3-d incubation at 56°C with the addition of 240 μg proteinase K at 24-h intervals.

**Methylation analysis.** The DNA from the paraffin-embedded tissues was extracted using the Puregene DNA Purification kit (Genta Systems) according to the manufacturer’s instructions for paraffin-embedded tissue.

**LOH testing**

Analysis of LOH was done using the multiplex ligation-dependent probe amplification (MLPA) technique, which allows for relative quantification of multiple DNA fragments in a single reaction (24). The BRCA1 and BRCA2 MLPA kits (MRC-Holland) were used as described by the manufacturer, using 55- to 100-ng tumor DNA. MLPA reactions were analyzed on the ABI 3100 Prism Genetic Analyser (Applied Biosystems), and dosage profiles were assembled using the GeneMarker software (v.1.2; SoftGenetics) by comparison to a reference sample without large genomic rearrangements in BRCA1 or BRCA2. As an additional quality assurance measure, samples known to carry large genomic rearrangements (BRCA1 del exon 5 and BRCA2 del exons 14_16) were also included in each set of MLPA reactions. In the BRCA2 MLPA probe set used (P045), exons 5 and 23 are not represented. LOH analysis using MLPA was done on all tumor samples from eligible participants. Samples yielding dosage quotients of 0.8 to 1.2 were scored as negative for LOH, whereas dosage quotients <0.6 were scored as positive for LOH.

To confirm LOH, PCR amplification using the HotStar Taq protocol was carried out as described by the manufacturer (Qiagen) on both normal (either blood DNA or DNA extracted from normal prostate tissue) and tumor tissue. The amplified DNA was then analyzed by sequencing using Big Dye v.3.1 (Applied Biosystems).

**BRCA1 promoter hypermethylation analysis**

The BRCA1 methylation status of each tumor was assessed using methylation-specific PCR (25) after treatment of tumor DNA with sodium bisulfite. Primers were derived from Esteller et al. (26) and the corresponding PCR protocol was replicated.

**Clinical and histopathologic features**

Clinical data that included date of birth, age at diagnosis of prostate cancer, mutation classification, ethnicity, family history of cancer, prostate-specific antigen level at diagnosis, stage of disease at diagnosis (27), and the age and cause of death where available were obtained from the epidemiology questionnaires completed at the time of participation and from the diagnostic pathology reports. Information on the histopathologic features of all prostate cancer cases was also obtained from the diagnostic histopathology reports for men diagnosed between 1991 and 2007. All tissues had been fixed in 10% neutral
buffered formalin. The clinical diagnostic pathology slides for each participant were blinded and reviewed by an independent prostate cancer pathologist who assessed tumor volume; Gleason score; extent of tumor; resection margins; extraprostatic extension; perineural, neurovascular, and lymphovascular invasion; androgenetic features; and the presence of high-grade prostatic intraepithelial neoplasia. For a subset of participants, nodal and distant metastatic involvement was also assessed.

Statistical methods

Under the assumption that having LOH at the same gene as the germline mutation meant that the prostate cancer had been caused by the mutation, the RR of prostate cancer associated with carrying a germline mutation is estimated by \( RR = \frac{N}{\left(\frac{N - n}{n}\right)} \), where \( N \) is the total number of affected carriers and \( n \) is the number of these for which their tumor exhibited LOH at the relevant gene. The 95% CI was estimated by assuming that \( n \) has a Binomial \( \left(\frac{N}{n}\right) \) distribution with \( P = \frac{n}{N} \). Proportions were compared using Fisher's exact test.

Results

A total of 137 families were identified as segregating a known pathogenic \( BRCA1 \) or \( BRCA2 \) mutation and having at least one man with a verified diagnosis of prostate cancer. Of the 158 men with prostate cancer in these families, DNA testing confirmed that 8 carried a family-specific \( BRCA1 \) mutation and 20 carried a family-specific \( BRCA2 \) mutation. Ten of these men were excluded from the study due to our inability to access an archival specimen of their prostate tumor, as in most cases, the archival material was >20 years old.

We therefore studied 18 subjects, 4 of whom were carriers of \( BRCA1 \) mutations, and the remaining 14 of \( BRCA2 \) mutations (Table 1). The mutations were as follows: frameshift (three \( BRCA1 \) and seven \( BRCA2 \)), nonsense (three \( BRCA2 \)), splice site (one \( BRCA1 \) and one \( BRCA2 \)), and there was a single large genomic rearrangement in \( BRCA2 \). Figure 1 shows the location of each mutation mapped to the corresponding exon, for both \( BRCA1 \) and \( BRCA2 \), demonstrating that the mutations were not clustered into a single region of either of the two genes.

LOH

\( BRCA2 \) mutation carriers. LOH at the \( BRCA2 \) locus was detected by MLPA in 10 (71%) tumors from men with a \( BRCA2 \) mutation (Table 1). Allele dosage across the entire \( BRCA2 \) gene was reduced by \( \sim 50\% \) (dosage quotient, <0.6; Fig. 2A and B). Figure 2A shows the MLPA profile for subject 12. This man carried the \( BRCA2 \) del exons 19_20 large genomic rearrangement. In addition to illustrating LOH across all exons of \( BRCA2 \), the MLPA profile indicated complete loss of exons 19 and 20, confirming his germline mutation status. Figure 2B shows the MLPA profile of subject 20. A 50% reduction in allele dosage was observed for all 25 represented exons, consistent with LOH across the coding region of the \( BRCA2 \) gene. As the specific location of this mutation (\( BRCA2 \) 7895_7986 ins A) does not overlap the binding site of the MLPA probe for this exon, an allele dosage of \( \sim 0.5 \) was observed for exon 18 due to the loss of the wild-type allele. LOH extended beyond the \( BRCA2 \) gene, as shown by the reduction in allele dosage of probes that flank the 5’ and 3’ ends of \( BRCA2 \). The exact breakpoints of the recombination event remain unknown.

All four remaining men with \( BRCA2 \) mutations were determined not to have LOH at \( BRCA2 \) because no reduction in allele dosage was observed by the MLPA analysis (subjects 9, 10, 16, and 22). Figure 2C is the MLPA profile for subject 9. The profile illustrates a 50% reduction in the allele dosage of exon 7 as the \( BRCA2 \) mutation overlaps the binding site for the MLPA probe. The allele dosages of all remaining exons across the \( BRCA2 \) gene are between 0.8 and 1.2, consistent with the presence of both alleles.

To determine if the heterogeneity of the tumor may mask the demonstration of LOH due to a greater amount of lower Gleason score DNA being present in the overall microdissected material, the H&E-stained slides were reviewed and areas of specific Gleason score regions were identified within each tumor. For three of the four tumors that did not show LOH of \( BRCA2 \), tumor cells were microdissected separately from both lower (subject 10, Gleason score 6) and higher (subjects 9 and 16, Gleason score 7) Gleason score regions. DNA was extracted and analyzed for LOH as previously described. LOH at \( BRCA2 \) was still not observed when comparing lower or only higher Gleason score regions (data not shown).

The quality of DNA extracted from the formalin-fixed paraffin-embedded tumor samples was sufficient to amplify and sequence five subjects. For three subjects showing LOH, sequencing of both normal and tumor tissue confirmed loss of the wild-type allele. Sequencing of two tumors, for which LOH was not observed, confirmed the retention of the wild-type allele. Figure 3A illustrates loss of the wild-type allele for subject 21, whereas Fig. 3B illustrates the retention of the wild-type allele at the mutation locus for subject 9. These results are consistent with those obtained by MLPA.

\( BRCA1 \) carriers. None of the tumor samples from the four \( BRCA1 \) carriers were found to have LOH at the \( BRCA1 \) mutation locus (Table 1) or to have any reduction in allele dosages across \( BRCA1 \).

LOH in other family cancers diagnosed in \( BRCA2 \) mutation carriers. In cases where no LOH was observed at \( BRCA2 \), archival tumor blocks were retrieved, where possible, for the breast and ovarian cancers diagnosed in other mutation-carrying relatives. Table 2 shows that LOH was observed in DNA extracted from the breast tumor of the mutation-carrying sister of subject 9. LOH was not observed in the ovarian tumor of the second-degree \( BRCA2 \) mutation-carrying relative of subject 16. We also obtained a primary breast tumor specimen for subject 10 and found that LOH was observed, a finding consistent with previous studies of LOH in breast cancers from \( BRCA2 \) mutation carriers (9).

Clinical and histopathologic features

The mean age at diagnosis of prostate cancer was significantly younger for \( BRCA2 \) carriers compared with \( BRCA1 \) carriers [58.2 years (range, 43-77) versus 71.8 years (range, 58-83); \( P = 0.03 \); Table 1]. Prostate-specific antigen readings up to 1 month before surgery were available for 12 subjects and ranged from 4.4 to 74 ng/mL. A radical prostatectomy was done on seven subjects ages 43 to 71 years, a transurethral resection of the prostate (TURP) procedure was done on 5 subjects ages 65 and 77 years, and a prostate biopsy was taken from two subjects. One subject was diagnosed as a result of a pelvic mass and another at postmortem review. Two subjects had their diagnosis of prostate cancer via metastatic lymph node sampling at ages 58 and 63 years, respectively. All subjects had high Gleason scores of 7 to 9 (median score, 9) and all were diagnosed with at least stage II disease. A family history of
prostate cancer was confirmed for seven subjects (one BRCA1 mutation carrier and six BRCA2 mutation carriers) but none had more than two first- or second-degree relatives affected. All six of these BRCA2 mutation carriers were shown to have LOH at BRCA2 in their tumors. The only subject with a family history not to show LOH was a BRCA1 mutation carrier (subject 1). In addition to the diagnosis of prostate cancer, 3 subjects had been diagnosed with another primary cancer: subject 10 had breast and renal cancer at age 74 years followed by melanoma at age 75 years; subject 8 had breast cancer at age 61 years; and subject 4 had colon cancer at age 58 years.

**Risk estimation**

Under the assumption that LOH at the locus of a germline mutation occurs only because the cancer was caused by the germline mutation, the increased risk of prostate cancer associated with a germline mutation in BRCA2 was estimated to be 3.5-fold (95% CI, 1.8-12). Under this assumption, there was

### Table 1. Characteristics of the identified mutation carriers

<table>
<thead>
<tr>
<th>Subject no</th>
<th>Mutation*</th>
<th>FHx PrCa</th>
<th>FHx of cancer</th>
<th>LOH</th>
<th>DX PSA (ng/mL)</th>
<th>Stage</th>
<th>Other verified cancer</th>
<th>Age at death</th>
<th>Cause of death</th>
<th>Surgical description</th>
<th>Gleason score (sum)</th>
</tr>
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<tbody>
<tr>
<td>3</td>
<td>BRCA1 IVS 3-1 G&gt;T</td>
<td>None</td>
<td>7 FDR, 5 SDR, 5 TDR BrCa, 1 FDR &amp; 3 SDR OvCa</td>
<td>No</td>
<td>83 N/A</td>
<td>IV</td>
<td>84 Cancer</td>
<td>Pelvic mass</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>BRCA1 2080 del A</td>
<td>1 SDR</td>
<td>4 FDR, 2 SDR BrCa</td>
<td>No</td>
<td>58 N/A</td>
<td>IV</td>
<td>74 Cancer</td>
<td>Lymph node</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>BRCA1 5385_5386 ins C</td>
<td>None</td>
<td>4 FDR, 2 SDR BrCa</td>
<td>No</td>
<td>78 7</td>
<td>II</td>
<td>82 Not cancer related</td>
<td>TURP</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>BRCA1 5622_5687 del 62</td>
<td>None</td>
<td>5 FDR BrCa</td>
<td>No</td>
<td>68 6.5</td>
<td>III</td>
<td>Radial prostatectomy</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>BRCA2 478 G&gt;T</td>
<td>None</td>
<td>1 FDR, 1 SDR, 1 TDR BrCa, 1 SDR ReCa, 1 TDR Mel</td>
<td>No</td>
<td>43 4.8</td>
<td>II</td>
<td>Radial prostatectomy</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>BRCA2 542 T&gt;G</td>
<td>2 TDR</td>
<td>4 FDR, 4 SDR BrCa</td>
<td>Yes</td>
<td>64 N/A</td>
<td>IV</td>
<td>65 Prostate Cancer</td>
<td>Postmortem</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>BRCA2 767_768 ins AT</td>
<td>None</td>
<td>1 FDR, 1 SDR, 1 TDR BrCa, 1 FDR OvCa</td>
<td>No</td>
<td>71 4.4</td>
<td>II</td>
<td>Radial prostatectomy</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>BRCA2 2041_2042 ins A</td>
<td>1 SDR</td>
<td>2 FDR, 2 SDR BrCa</td>
<td>Yes</td>
<td>77 N/A</td>
<td>II</td>
<td>86 TURP</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>BRCA2 2041_2042 ins A</td>
<td>1 SDR</td>
<td>2 FDR, 1 SDR, 1 TDR BrCa, 1 FDR OvCa</td>
<td>Yes</td>
<td>62 N/A</td>
<td>II</td>
<td>Radial prostatectomy</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>BRCA2 4075_4076 del GT</td>
<td>None</td>
<td>2 SDR, 1 TDR BrCa</td>
<td>No</td>
<td>52 9.7</td>
<td>III</td>
<td>Radial prostatectomy</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>BRCA2 5514 T&gt;A</td>
<td>None</td>
<td>1 FDR, 2 SDR BrCa</td>
<td>Yes</td>
<td>65 4.4</td>
<td>II</td>
<td>Colon 68 Cancer</td>
<td>TURP</td>
<td>9</td>
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</tr>
<tr>
<td>23</td>
<td>BRCA2 6174 del T</td>
<td>1 FDR</td>
<td>1 FDR BrCa, 1 FDR UtCa</td>
<td>Yes</td>
<td>63 8.8-9.5</td>
<td>II</td>
<td>Radical prostatectomy</td>
<td>Prostate biopsy</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>BRCA2 7895_7896 ins A</td>
<td>1 SDR</td>
<td>3 FDR, 2 TDR BrCa</td>
<td>Yes</td>
<td>41 67</td>
<td>II</td>
<td>Radial prostatectomy</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>BRCA2 IVS 17-1 G&gt;C</td>
<td>1 FDR</td>
<td>2 FDR, 3 SDR, 1 TDR BrCa, 2 FDR OvCa</td>
<td>Yes</td>
<td>67 15</td>
<td>II</td>
<td>Prostate biopsy</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>BRCA2 IVS 17-1 G&gt;C</td>
<td>None</td>
<td>5 FDR, 1 SDR BrCa</td>
<td>Yes</td>
<td>63 65.3</td>
<td>IV</td>
<td>Lymph node 9</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>10</td>
<td>BRCA2 8525 del C</td>
<td>None</td>
<td>1 SDR, 1 TDR BrCa</td>
<td>No</td>
<td>77 11.5</td>
<td>II</td>
<td>Breast, renal 83</td>
<td>TURP</td>
<td>9</td>
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<td></td>
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<tr>
<td>12</td>
<td>BRCA2del exon 19_20</td>
<td>None</td>
<td>1 FDR, 1 SDR, 1 TDR BrCa, 2 FDR OvCa</td>
<td>Yes</td>
<td>70 74</td>
<td>IV</td>
<td>Prostate cancer</td>
<td>TURP</td>
<td>9</td>
<td></td>
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<tr>
<td>8</td>
<td>BRCA2 9132 del C</td>
<td>None</td>
<td>1 FDR, 1 SDR, 1 TDR BrCa, 1 SDR OvCa</td>
<td>Yes</td>
<td>64 10.3</td>
<td>III</td>
<td>Breast</td>
<td>Radial prostatectomy</td>
<td>9</td>
<td></td>
<td></td>
</tr>
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</table>

**Abbreviations:** FHx PrCa, family history of prostate cancer; FDR, first-degree relative; SDR, second-degree relative; TDR, third-degree relative; FHx of cancer, family history of other cancers; BrCa, breast cancer; OvCa, ovarian cancer; ReCa, renal cancer; Mel, melanoma; UtCa, uterine cancer; Dx, age at diagnosis; PSA, prostate-specific antigen.

*All mutations classified as pathogenic by kConFab mutation classification subcommittee (guidelines at http://www.kconfab.org).
no evidence from this study that germline mutations in BRCA1 are associated with prostate cancer risk.

**Discussion**

This is the first study to show LOH in prostate tumors of known BRCA2 pathogenic mutation carriers at the specific mutation locus. Although other studies have shown that LOH is common in prostate tumors from known BRCA2 mutation carriers, microsatellite markers mapped to the BRCA2 region on chromosome 13q were used (9, 28–30). We found that LOH occurs frequently at the BRCA2 locus with loss of the wild-type allele. Although the sample size is small, these results indicate that BRCA2 has the hallmarks of a tumor suppressor and is the likely cause of the prostate cancer in a substantial proportion of carriers who are diagnosed with the disease. However, it is unclear why some prostate tumors from carriers did not show LOH. It is possible that epigenetic gene silencing and haplonsufficiency may be involved, as has been described for BRCA1-related breast cancers (31, 32) and other tumor suppressor genes (26, 33). It is important to note, however, that currently no data exists to support the association of such mechanisms with BRCA2-related prostate cancer. Alternatively, there may be somatic mutations in the BRCA2 gene that could not be detected by MLPA.

Nevertheless, prostate cancer is a common disease and the RR conferred by being a BRCA2 mutation carrier does not seem to be as extreme as it is for breast and ovarian cancers (see Discussion below).

It is therefore possible that some or all of the tumors in which no LOH was detected were so-called “sporadic” prostate cancers arising through mechanism(s) unrelated to BRCA2. If this hypothesis is correct, the risk of prostate cancer in BRCA2 mutation carriers was estimated from our study to be 3.5 times that of men in the general population. This risk estimate has considerable statistical imprecision (95% CI, 1.8-12), which could be reduced by testing a larger number of prostate tumors from carriers of BRCA2 mutations for LOH. For example, if we pool our data with those from the seven BRCA2 carriers previously reported (9), the RR estimate becomes 4.2 with a 95% CI of 2.5 to 13.

Previous studies have tried to estimate the increased risk of prostate cancer associated with being a BRCA2 mutation carrier. The Breast Cancer Linkage Consortium estimated the RR to be 4.7 (95% CI, 3.5-6.2) overall and higher for carriers younger than 65 years (RR, 7.3; 95% CI, 4.7-11.5; ref. 3). Another study of multiple-case breast cancer families (34) estimated the cancer risks of first-degree relatives of known BRCA2 mutation carriers who had not been tested for mutation status. The authors reported a 2.5-fold (95% CI, 1.6-3.8) increase in risk of prostate cancer. Although this might imply about a 5-fold increased risk for carriers, their estimate is difficult to interpret without knowing precisely how family members in that study were selected for family-specific mutation testing. In another study, Tulinius and colleagues (35) examined the relatives of breast cancer probands with a germline Icelandic founder mutation in BRCA2 and found that first- and second-degree relatives were at increased risk of prostate cancer by 4.8-fold (95% CI, 3.3-6.3) and 2.2-fold (95% CI, 1.6-3.0), respectively. These estimates are consistent with male carriers of the 999del5 BRCA2 mutation being at ~10-fold increased risk of prostate cancer (36). The authors of a recent Icelandic study also reported that the BRCA2 999del5 mutation carried by all 29 men in this analysis had

**Fig. 1.** Position of family-specific pathogenic mutations within BRCA1 and BRCA2. Schematic represents coding region. Arrows, position of mutations for each prostate cancer case within the gene. Broken arrow, tumor cases where heterozygosity was retained. Solid arrows, LOH in tumor cases.
advanced disease at diagnosis and a poor survival outcome (37). It may not solely be the mutation status that contributes to aggressive disease as suggested, it may also be important to take into account epidemiologic features to the overall contribution to disease development.

There have been several attempts to estimate penetrance from studies of unselected prostate cancer cases. A UK study of 263 men diagnosed with prostate cancer at or before the age of 55 years found six BRCA2 mutation carriers (2.3%; 95% CI, 0.8-5%; ref. 5). Based on a population carrier prevalence of 0.1% derived from modeling population-based UK breast cancer families (38), the authors reported a 23-fold increased risk. However, the 95% CI was large (9- to 27-fold) and did not take into account the imprecision of the population carrier estimate. Taking this into account, the confidence interval becomes even larger (6- to 92-fold). More recently, Antoniou and colleagues (39) have updated their estimate of population carrier frequency to 0.2% (95% CI, 0.12-0.30), in which

Fig. 2. BRCA2 MLPA profile illustrating the presence/absence of LOH in prostate tumor specimens. Light blue trace, a patient with no genomic rearrangements; dark blue trace, the prostate cancer patient. Green line, 50% reduction in copy number of exons. Orange line, no reduction in copy number of exon. *, MLPA control probes located upstream and downstream of the BRCA2 gene. A, subject 12, LOH. Red arrows, complete loss of exons 19 and 20, confirming the presence of the BRCA2 large genomic deletion of exons 19 and 20. B, subject 20, LOH across BRCA2.
case, the estimated increased risk of prostate cancer would be reduced to 11-fold with 95% CI from 3- to 46-fold. Another population-based study in the USA found two BRCA2 mutation carriers in 290 unselected men with prostate cancer (0.7%; 95% CI, 0.09-2.81%; ref. 40). The authors assumed a population carrier prevalence of 0.1% and reported a 7.8-fold increased risk. Using the revised population prevalence from Antoniou et al. (39), the estimated risk in the United States study becomes 3.9-fold with 95% CI from 0.4- to 13-fold; i.e., the carrier prevalence in these men with prostate cancer was not significantly different from the prevalence in the population, whether it be 0.1% or 0.2%. The 95% CI of 1.8 to 9.4 reported in that paper (for an estimate of 7.8) requires adjustment. For prostate cases selected from multiple-case prostate cancer families, two BRCA2 mutation carriers were found in 38 tested men from the United Kingdom (41), but these were brothers. No BRCA2 mutations were found in the 266 tested men from a US study (4).

A recent paper reported on the accrual, similar to ours, of prostate cancers arising in men with a known BRCA1 or BRCA2 mutation (IMPACT study) detected from multiple-case breast cancer families (EMBRACE study). They found 20 mutation carriers, 16 with mutations in BRCA2 and 4 with mutations in BRCA1 (42). Our study found 28 carriers, 20 with mutations in BRCA2, and 8 with mutations in BRCA1. That is, BRCA2 carriers were thrice more prevalent when the data were combined (36 versus 12; P < 0.001). If selection of carriers had been independent of gene, and if BRCA1 mutation status is not, or at most weakly, associated with prostate cancer risk, these observations would be consistent with an increased risk for BRCA2 mutation carriers of 3.0-fold (95% CI, 1.7-7).

Therefore BRCA2 mutations are associated with an increased risk of prostate cancer but the magnitude of risk is unlikely to be >10-fold, as evidenced by the upper limits of the confidence intervals from other studies, including ours, and the failure to observe more than one multiple-case prostate cancer family segregating mutations in that gene of nearly 300 tested (4). The studies of unselected case series are relatively uninformative on this issue, given the rarity of carriers, lack of direct knowledge about carrier frequency in the population, and hence, wide confidence intervals. That is, in the setting of prostate cancers, these mutations are unlikely to be “high risk” in the sense of exhibiting a high-lifetime risk, as is the case for breast and ovarian cancers.

In our study, LOH was not observed in the prostate tumors of the four BRCA1 mutation carriers. To determine if the germline mutation in BRCA1 could have had a role in the development of these prostate cancers through gene silencing, promoter hypermethylation analyses were carried out. DNA from five tumors from BRCA1 mutation carriers (four prostate cancers and one breast cancer as a control) was tested for BRCA1 promoter methylation. The DNA from three prostate tumors failed to amplify with either methylated or unmethylated-specific primers. Neither the DNA of the remaining prostate tumor, nor DNA from the breast tumor, had abnormal BRCA1 promoter methylation (data not shown). The Breast Cancer Linkage Consortium has previously reported an increased risk of prostate cancer for BRCA1 mutation carriers but only for men younger than 65 years (RR, 1.8; 95% CI, 1.0-3.3; P = 0.05; ref. 43). Given the marginal statistical significance and subgroup analysis, this may reflect a type II error, especially in light of the absence of specific laboratory analysis of LOH at BRCA1 in prostate cancers arising in carriers of BRCA1 mutations or, as in this study, the failure to detect LOH at BRCA1 or to show promoter hypermethylation of the BRCA1 gene. Further larger studies examining LOH in prostate cancers from BRCA1 mutation carriers will be needed to determine if germline mutations in BRCA1 are associated with prostate cancer.
This study shows the potential importance of inquiring about the family cancer history of men with prostate cancer in families with multiple cases of breast and/or ovarian cancer, or even unselected cases of very early-onset breast cancer (44) because this may lead to the identification of families in which BRCA2 mutations are segregating with prostate disease. A larger data set and a clinical follow-up will also contribute to understanding the prognosis and survival outcomes for carriers.

**Table 2. Characteristics of subjects with germline BRCA2 mutations but no LOH in their prostate tumors at BRCA2**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Mutation carrier</th>
<th>Mutation description</th>
<th>LOH result</th>
<th>Other family members (cancer type, mutation-carrier status, LOH result)</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>Yes</td>
<td>BRCA2 478 C&gt;T</td>
<td>No LOH</td>
<td>N/A</td>
</tr>
<tr>
<td>9</td>
<td>Yes</td>
<td>BRCA2 767_768 ins AT</td>
<td>No LOH</td>
<td>BrCa, +ve, LOH</td>
</tr>
<tr>
<td>16</td>
<td>Yes</td>
<td>BRCA2 4075_4076 del GT</td>
<td>No LOH</td>
<td>OvCa, +ve, no LOH</td>
</tr>
<tr>
<td>10</td>
<td>Yes</td>
<td>BRCA2 8525 del C</td>
<td>No LOH</td>
<td>BrCa,* +ve, LOH</td>
</tr>
</tbody>
</table>

*Breast tumor from prostate cancer affected subject 10.
of a pathogenic BRCA2 mutation. Future research, such as the recently established IMPACT study (45), may provide evidence to support screening advice and optimal clinical management to help reduce the burden of prostate cancer.

Acknowledgments

We thank Professor Joe Sambrook for his continual help, advice, and guidance throughout the development of this project and preparation of the manuscript: Eveline Niedermay, all of the kConFab research nurses and staff, the heads and staff of the Family Cancer Clinics, and the Clinical Follow Up Study for their contributions to this resource, and the many families who contribute to kConFab; Dr David Huntsman from the British Columbia Cancer Agency, Vancouver for his technical advice and contribution to the manuscript; and Dr Daryl Thompson from the Peter MacCallum Cancer Centre, Melbourne for his assistance.

Disclosure of Potential Conflicts of Interest

There were no potential conflicts of interest.

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Loss of Heterozygosity at the BRCA2 Locus Detected by Multiplex Ligation-Dependent Probe Amplification is Common in Prostate Cancers from Men with a Germline BRCA2 Mutation

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