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Published text:

BRCA2 is a moderate penetrance gene contributing to young-onset prostate cancer: implications for genetic testing in prostate cancer patients

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BACKGROUND: A family history of prostate cancer (PrCa) is a strong risk factor for the disease, indicating that inherited factors are important in this disease. We previously estimated that about 2% of PrCa cases diagnosed \( \leq 55 \) years harbour a BRCA2 mutation and PrCa among BRCA2 carriers has been shown to be more aggressive, with poorer survival.

METHODS: To further evaluate the role of BRCA2 in PrCa predisposition, we screened 1864 men with PrCa aged between 36 and 88 years. We analysed the BRCA2 gene using a novel high-throughput multiplex fluorescence heteroduplex detection system developed for the ABI3130xl genetic analyzer.

RESULTS: We identified 19 protein-truncating mutations, 3 in-frame deletions and 69 missense variants of uncertain significance (UV) in our sample set. All the carriers of truncating mutations developed PrCa at \( \leq 65 \) years, with a prevalence of BRCA2 mutation of 1.20% for cases in this age group.

CONCLUSION: Based on the estimated frequency of BRCA2 mutations in the United Kingdom we estimate that germline mutations in the BRCA2 gene confer an \( \sim 8.6\) fold increased risk of PrCa by age 65, corresponding to an absolute risk of \( \sim 15% \) by age 65. These results suggest that routine testing of early onset PrCa cases for germline BRCA2 mutations will further help to refine the prevalence and risk associated with BRCA2 mutations and may be useful for guiding management options.


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Keywords: prostate cancer; BRCA2 gene; mutation screening; cancer risk

Prostate cancer (PrCa) is the most common cancer in men living in the Western world, with a lifetime risk of \( \sim 1 \) in 8 for men in Europe and the United States (http://info.cancerresearchuk.org/cancerstats/types/prostate/). Its aetiology remains poorly understood and although many men will not develop a clinically relevant disease, it is recognised that some PrCa cases have a particularly poor prognosis. Although there are some histological and stage predictors of prognosis (Ross et al, 2002), multiple aetiologies, both hereditary and environmental, have been proposed to contribute to the development of PrCa. There is strong evidence that inherited genetic factors are important due to the significant familial aggregation of the disease in some men, particularly those affected at a young age (Edwards and Eeles, 2004). Some family studies have found an increased risk of PrCa among the relatives of breast cancer patients, suggesting a common genetic basis (Thiessen, 1974; Anderson and Badzioch, 1992; Tulinius et al, 1992). Clearer evidence has emerged of an increased risk of PrCa in carriers of BRCA1 and BRCA2 mutations ascertained via family history of breast cancer (Sigurdsson et al, 1997; Ford et al, 1994). Analyses of male mutation carriers in breast cancer families from the Breast Cancer Linkage Consortium (BCLC) found a relative risk (RR) of 4.65 (95% CI 3.48–6.22) of PrCa in male BRCA2 mutation carriers rising to 7.33 below the age of 65 years and RR 1.07 (0.75–1.34) in BRCA1 mutation carriers with a RR rising to 1.82 (1.01–3.29) for men under 65 years old (Thompson and Easton, 2001; Thompson and Easton, 2002). Furthermore, through an analysis of early onset PrCa cases, we previously estimated that the RR of PrCa in BRCA2 mutation carriers was \( \sim 23\) fold below age 56 (Edwards et al, 2003). Studies have been conducted in the Ashkenazi population investigating the association of the BRCA2 6174delT founder mutation with PrCa (Hamel et al, 2003). These have reported conflicting data and the majority of these studies have been limited by relatively small sample sizes and did not look for mutations other than the 6174delT founder mutation.

A study from Iceland reported that PrCa occurring in BRCA2 mutation carriers were more aggressive than those in non-carriers,
and had poorer survival (Sigurdsson et al., 1997). These individuals all carried a common founder mutation (999del5). Narod et al. (2008) reported that survival from PrCa in BRCA2 mutation carriers is shorter (median survival from diagnosis: 4 years) when compared with PrCa survival in BRCA1 carriers (median survival from diagnosis: 8 years). In PrCa where BRCA2 germline mutation status was unknown, allelic loss at the BRCA2 locus has been shown to be a prognostic factor for survival on univariate analysis (Edwards et al., 1998) implying a tumour suppressor mechanism for BRCA2 and thus predisposition to this disease in BRCA2 mutation carriers, but at that time it was not known if this is a surrogate for high tumour grade or due to the mutation per se (Willems et al., 2008). Recent data have shown that it is the mutation per se that is an independent prognostic factor for PrCa survival (Edwards et al., 2010b). This was also supported by an Australian study reporting a very similar finding (Thorne et al., 2011). Here we report a large study of nearly 2000 PrCa cases with varying ages of diagnosis to validate the potential association between germline BRCA2 mutation and increased risk of disease and to refine RR for PrCa among BRCA2 mutation carriers.

**MATERIALS AND METHODS**

**Samples**

A series of men with PrCa were recruited from the UK Genetic Prostate Cancer Study (UKGPCS) as reported previously (Eeles et al., 1997) and about 90% of these patients had clinically presenting (non-screen-detected) disease at diagnosis. Case selection for this study was based predominantly on age of disease onset ≤65 (1621 cases; range 36–65 years), with a further cohort aged >65 but with a family history of one or more first-degree relatives with PrCa (243 cases; range 66–88 years) selected to delineate the contribution of germline BRCA2 mutations to PrCa (Table 1).

**Mutation detection**

Germline DNA was obtained from peripheral blood samples and extracted as reported in previous articles (Edwards et al., 1997). The full coding region and exon–intron boundaries of BRCA2 were analysed using a high-throughput multiplex fluorescent heteroduplex analysis method. The PCR reactions were performed using the three-primer system described by NGRI Wessex (http://www.ngrl.co.uk/Wessex/downloads) and labelled with one of four dyes (FAM, VIC, NED, ROX). The PCR fragments were diluted 1:50 in H2O and pooled robotically into multiplex groups of up to 8 before mutation screening. These multiplexes were designed using a range of fragment sizes to prevent overlap of tags with interfering absorbance spectra. The BRCA2 PCR primer set used was a modification of that described by NGRI Wessex with alterations to enhance high throughput. This panel consisted of 46 PCR fragments, with larger exons covered by multiple overlapping fragments to ensure mutation detection within the primer regions. The primer sequences, dyes assigned to each fragment, PCR conditions and multiplexing information are available on request.

Multiplexed, dye-tagged PCR fragments were run on an ABI3130xl Genetic Analyzer in 1 × TTE running buffer, using a polymer comprising of 4.5% POP Conformational Analysis Polymer (CAP–Applied Biosystems, Carlsbad, CA, USA, P/N 4340379), 4 M urea and 1% TTE buffer. Polymer was filtered through a 0.5-μm filter before use and stored at 4°C for up to 1 month. Fragments were analysed for peak shifts corresponding to putative mutations in Genemapper v4.0 (Applied Biosystems) by visual inspection and using Bionumerics (Applied Maths, Sint-Martens-Latem, Belgium).

After confirmation of a peak shift, the samples were re-amplified by PCR from stock DNA and the genetic alteration was characterised by sequencing on an ABI3730 Genetic Analyzer with both forward and reverse PCR primers.

**RESULTS**

We screened 1864 PrCa cases for germline mutations in the BRCA2 gene. After quality control we excluded 32 samples (all aged ≤65) from the analysis. In the 1832 samples analysed we have identified 19 protein-truncating mutations, of which 16 were frameshift and 3 were nonsense mutations (Table 1). Of these deleterious mutations, 16 have been reported previously in breast and ovarian cases and these are listed on the Breast Cancer Information Core (BIC) database (http://research.nhgri.nih.gov/bic/), whereas 3 mutations were novel. In addition, we identified 3 in-frame deletions (1 novel) and 69 missense variants (13 novel) of uncertain significance (UV) (Supplementary Table 2), one common nonsense mutation at the 5’ end of the protein (rs11571833), which is considered to be benign, 31 synonymous substitutions (5 novel) and 35 in intronic variants. All the deleterious mutations were present in younger onset cases with age of disease diagnosis at

<table>
<thead>
<tr>
<th>Age range, years</th>
<th>Number of samples</th>
<th>With family history of PrCa (%)</th>
<th>With family history of Br/OvCa (%)</th>
<th>Percentage of samples deceased</th>
<th>Number of mutation carriers</th>
<th>Percentage of mutation carriers</th>
<th>Percentage of mutation carriers deceased</th>
</tr>
</thead>
<tbody>
<tr>
<td>36–55</td>
<td>632 (34.5%)</td>
<td>34.5</td>
<td>25.3</td>
<td>15.3</td>
<td>8</td>
<td>1.27</td>
<td>37.5</td>
</tr>
<tr>
<td>56–65</td>
<td>957 (52.2%)</td>
<td>50.6</td>
<td>24.3</td>
<td>12.4</td>
<td>11</td>
<td>1.15</td>
<td>81.8</td>
</tr>
<tr>
<td>66–88</td>
<td>243 (13.3%)</td>
<td>100.0</td>
<td>29.6</td>
<td>29.8</td>
<td>0</td>
<td>0.56</td>
<td>28.9</td>
</tr>
<tr>
<td>Total</td>
<td>1832</td>
<td>51.6</td>
<td>25.4</td>
<td>15.7</td>
<td>19</td>
<td>1.03</td>
<td>63.2</td>
</tr>
</tbody>
</table>

Abbreviations: BrCa = breast cancer; OvCa = ovarian cancer; PrCa = prostate cancer. A total of 1832 samples (age range 36–88) passed quality control, of which 1589 were aged ≤65 years at diagnosis. Protein-truncating mutations were enriched at younger age of diagnosis. Family history of BrCa and OvCa is also shown.
mutation carriers were affected at determined to be pathogenic in the future. All 19 deleterious samples). It is also possible that a proportion of the UVs will be deletions/rearrangements (no MLPA was conducted for this set of through-put methods) would have not been able to detect large mutation detection method we have used (as with other high one affected first degree relative with PrCa). In the 1832 samples diagnosed with prostate cancer. After quality control we included 1832 DNA samples in our analysis. Of these men, 1589 were clinically detected; 88.6%). The remaining 243 men (PrCa were diagnosed between nucleotides 1231 and 9253, predominantly within exons 10 and 11 (Table 2) indicating a more aggressive clinical course for the majority of the BRCA2 mutation carriers.

DISCUSSION

We have analysed the entire coding region and exon–intron boundaries of the BRCA2 gene from blood DNA of 1864 men diagnosed with prostate cancer. After quality control we included 1832 DNA samples in our analysis. Of these men, 1589 were diagnosed at ≤65 years (Table 1) and the majority presented with clinical symptoms (data available for 1374 patients, of whom 1218 were clinically detected; 88.6%). The remaining 243 men (PrCa diagnosed >65 years) had a family history of the disease (at least one affected first degree relative with PrCa). In the 1832 samples analysed we identified 19 protein-truncating mutations, which is likely to be an underestimate of the mutation frequency, as the mutation detection method we have used (with as other high through-put methods) would have not been able to detect large deletions/rearrangements (no MLPA was conducted for this set of samples). It is also possible that a proportion of the UVs will be determined to be pathogenic in the future. All 19 deleterious mutation carriers were affected at ≤65 years and no mutations were found in the older onset group with family history, which is concordant with a previous study (Agalliu et al 2007) where no association was found between BRCA2 mutation status and high familial risk of PrCa. Twelve mutation carriers had family history of PrCa among first- or second-degree relatives (63.2%). This represents a slight but not statistically significant excess (P = 0.24) as in the whole study 48.6% of cases ≤65 had some family history of PrCa. Nine patients had family history of breast or ovarian cancer among first- or second-degree relatives (47.4%) and this is significantly higher than for non-carriers in the study (21.3%, P = 0.024). Based on these data, the strongest predictors for the presence of a germline BRCA2 mutation are a young age of onset of PrCa and a family history of breast and/or ovarian cancer. The proportion of high-grade PrCa (Gleason score ≥8) was 63%, significantly higher than in non-carriers, 15%, (P < 0.0001) and similar results were reported previously by our group and by others (Edwards et al, 2010a; Gallagher et al, 2010). The clinical and statistical evaluation of a much larger set of BRCA2 mutation carrier and non-carrier PrCa cases is underway (Castro et al, manuscript in preparation). The prevalence of BRCA2 mutations in this study is 1.27% (8/632) for cases diagnosed ≤55 years, 1.20% (19/1589) for cases diagnosed ≤65 years and 0% (0/243) diagnosed >65 years; P = 0.14. Based on the previously estimated frequency of BRCA2 mutations in the United Kingdom of 0.16%, we estimated that germline mutations in the BRCA2 gene confer an increased RR of PrCa of ~8.6-fold (95% CI 5.1–12.6) by age 65 corresponding to an absolute risk of ~15% by age 65 years based on incidence rates in England from 2002. The estimate of RR from this study is similar to the 7.3-fold RR from the BLC study for patients diagnosed before age 65 years (Thompson and Easton, 2001). Our previous study reported a higher (~23-fold) RR (Edwards et al, 2003); although this was calculated from a much smaller cohort of cases all diagnosed at ≤55 years old and had a very wide confidence interval (95% CI 9–57), which overlaps that of this study. This current study provides more accurate BRCA2 mutation frequencies for PrCa cases and relative and absolute PrCa risk estimates for BRCA2 mutation carriers.

The protein-truncating mutations identified are situated between nucleotides 1231 and 9253, predominantly within exons 10 and 11 but not restricted to any specific domain (Figure 1). The majority, however, are within either the BRC repeat region (nucleotides 3006–6255), which is responsible for RAD51 binding or the OB DNA-binding domain (nucleotides 8007–9570), both of which have an important role in DNA damage repair (Kote-Jarai and Eeles, 1999; Venkitaraman, 2002).

In addition to the protein-truncating mutations, we identified 3 in-frame deletions and 69 missense UVs (Supplementary Table 1). We evaluated the missense variants using various predictive tools (SIFT, polyphen, Align GVGD, pMUT and PANTHER) and the majority of the mutation carriers (12/19) have died with relatively short survival (age of onset: 66). Twelve of the deleterious mutation carriers (12/19) have died with relatively short survival (age of onset: 66). Twelve of the deleterious mutation carriers (12/19) have died with relatively short survival (age of onset: 66). Twelve of the deleterious mutation carriers (12/19) have died with relatively short survival (age of onset: 66). Twelve of the deleterious mutation carriers (12/19) have died with relatively short survival (age of onset: 66). Twelve of the deleterious mutation carriers (12/19) have died with relatively short survival (age of onset: 66). Twelve of the deleterious mutation carriers (12/19) have died with relatively short survival (age of onset: 66). Twelve of the deleterious mutation carriers (12/19) have died with relatively short survival (age of onset: 66). Twelve of the deleterious mutation carriers (12/19) have died with relatively short survival (age of onset: 66). Twelve of the deleterious mutation carriers (12/19) have died with relatively short survival (age of onset: 66). Twelve of the deleterious mutation carriers (12/19) have died with relatively short survival (age of onset: 66). Twelve of the deleterious mutation carriers (12/19) have died with relatively short survival (age of onset: 66). Twelve of the deleterious mutation carriers (12/19) have died with relatively short survival (age of onset: 66). Twelve of the deleterious mutation carriers (12/19) have died with relatively short survival.
## Table 2

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Nucleotide change</th>
<th>Age at diagnosis</th>
<th>Years to death</th>
<th>Tumour stage</th>
<th>Node stage</th>
<th>Final Gleason</th>
<th>Metastases</th>
<th>Other cancers in the family</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR1</td>
<td>1231delA</td>
<td>65</td>
<td>4.5</td>
<td>T3b</td>
<td>Nx</td>
<td>M0</td>
<td>Alive (8)</td>
<td>No</td>
</tr>
<tr>
<td>PR2</td>
<td>1265delA</td>
<td>58</td>
<td>3</td>
<td>Tx</td>
<td>Nx</td>
<td>–</td>
<td>Father, uncle</td>
<td>Sister (Ov), grandmother (Br)</td>
</tr>
<tr>
<td>PR3</td>
<td>1787delATGAAACATCTTA</td>
<td>55</td>
<td>3.3</td>
<td>T2</td>
<td>N0</td>
<td>M0</td>
<td>Alive (8)</td>
<td>No</td>
</tr>
<tr>
<td>PR4</td>
<td>1813insA</td>
<td>60</td>
<td>4.4</td>
<td>T2b</td>
<td>N0</td>
<td>M0</td>
<td>Alive (8)</td>
<td>No</td>
</tr>
<tr>
<td>PR5</td>
<td>2807delAACA</td>
<td>59</td>
<td>3.3</td>
<td>T2b</td>
<td>N0</td>
<td>M0</td>
<td>Alive (8)</td>
<td>No</td>
</tr>
<tr>
<td>PR6</td>
<td>2836delGA</td>
<td>51</td>
<td>3.3</td>
<td>T1c</td>
<td>N0</td>
<td>M0</td>
<td>Alive (6)</td>
<td>No</td>
</tr>
<tr>
<td>PR7</td>
<td>3158T4G</td>
<td>46</td>
<td>5.5</td>
<td>T1c</td>
<td>N1</td>
<td>M0</td>
<td>Alive (10)</td>
<td>Father</td>
</tr>
<tr>
<td>PR8</td>
<td>4478delAAAG</td>
<td>54</td>
<td>4.4</td>
<td>T2c</td>
<td>N0</td>
<td>M0</td>
<td>Alive (18)</td>
<td>Brother, father, Sister (Br), aunt (Ov), daughter</td>
</tr>
<tr>
<td>PR9</td>
<td>3847delGT</td>
<td>56</td>
<td>4.3</td>
<td>T4</td>
<td>Nx</td>
<td>M0</td>
<td>Alive (2)</td>
<td>No</td>
</tr>
<tr>
<td>PR10</td>
<td>4478delAAAG</td>
<td>54</td>
<td>4.4</td>
<td>T2c</td>
<td>N0</td>
<td>M0</td>
<td>Alive (18)</td>
<td>Brother, father, Sister (Br), aunt (Ov), daughter</td>
</tr>
<tr>
<td>PR11</td>
<td>4877delAA</td>
<td>54</td>
<td>4.4</td>
<td>T3b</td>
<td>N1</td>
<td>M1</td>
<td>Alive (7)</td>
<td>Father, uncle</td>
</tr>
<tr>
<td>PR12</td>
<td>4877delAA</td>
<td>55</td>
<td>5.5</td>
<td>T2a</td>
<td>N0</td>
<td>M0</td>
<td>Alive (8)</td>
<td>No</td>
</tr>
<tr>
<td>PR13</td>
<td>4981delT</td>
<td>62</td>
<td>3</td>
<td>Tx</td>
<td>Nx</td>
<td>–</td>
<td>Two brothers</td>
<td>Sister (Br), aunt (Br), grandmother (Br)</td>
</tr>
<tr>
<td>PR14</td>
<td>5645C</td>
<td>57</td>
<td>4.4</td>
<td>T2a</td>
<td>N0</td>
<td>M0</td>
<td>Alive (11)</td>
<td>No</td>
</tr>
<tr>
<td>PR15</td>
<td>6405delCTTAA</td>
<td>53</td>
<td>3</td>
<td>T2a</td>
<td>N1</td>
<td>M0</td>
<td>Alive (3)</td>
<td>No</td>
</tr>
<tr>
<td>PR16</td>
<td>6405delCTTAA</td>
<td>48</td>
<td>5.5</td>
<td>T3</td>
<td>N0</td>
<td>M0</td>
<td>Alive (5)</td>
<td>No</td>
</tr>
<tr>
<td>PR17</td>
<td>6405delCTTAA</td>
<td>48</td>
<td>5.5</td>
<td>T3</td>
<td>N0</td>
<td>M0</td>
<td>Alive (5)</td>
<td>No</td>
</tr>
<tr>
<td>PR18</td>
<td>8904delC</td>
<td>56</td>
<td>3.3</td>
<td>T3a</td>
<td>N0</td>
<td>M0</td>
<td>Alive (4)</td>
<td>No</td>
</tr>
<tr>
<td>PR19</td>
<td>9253insA</td>
<td>57</td>
<td>5.5</td>
<td>T4</td>
<td>Nx</td>
<td>M1</td>
<td>Alive (2)</td>
<td>No</td>
</tr>
</tbody>
</table>

In conclusion, we have shown that the frequency of germline mutation in BRCA2 in PrCa patients is ~1.20% at <65 years. No mutations were found in cases diagnosed >65 in our series, suggesting that germline BRCA2 mutation is far more closely linked to a younger age of PrCa onset than to a family history of PrCa. With the advent of PARPi drugs, which preferentially target tumours with a BRCA null phenotype (Fong et al, 2009), germline testing of patients diagnosed at <65 years would be warranted as part of their cancer care pathway once testing becomes faster and cheaper.

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Supplementary Information accompanies the paper on British Journal of Cancer website (http://www.nature.com/bjc)

## REFERENCES


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