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British Journal of Cancer (2012) 106, 1697–1701
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www.bjcancer.com

Germline *BRCA1* mutations increase prostate cancer risk

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BACKGROUND: Prostate cancer (PrCa) is one of the most common cancers affecting men but its aetiology is poorly understood. Family history of PrCa, particularly at a young age, is a strong risk factor. There have been previous reports of increased PrCa risk in male *BRCA1* mutation carriers in female breast cancer families, but there is a controversy as to whether this risk is substantiated. We sought to evaluate the role of germline *BRCA1* mutations in PrCa predisposition by performing a candidate gene study in a large UK population sample set.

METHODS: We screened 913 cases aged 36–86 years for germline *BRCA1* mutation, with the study enriched for cases with an early age of onset. We analysed the entire coding region of the *BRCA1* gene using Sanger sequencing. Multiplex ligation-dependent probe amplification was also used to assess the frequency of large rearrangements in 460 cases.

RESULTS: We identified 4 deleterious mutations and 45 unclassified variants (UV). The frequency of deleterious *BRCA1* mutation in this study is 0.45%; three of the mutation carriers were affected at age $\leqslant$65 years and one developed PrCa at 69 years. Using previously estimated population carrier frequencies, deleterious *BRCA1* mutations confer a relative risk of PrCa of $\sim$3.75-fold, (95% confidence interval 1.02–9.6) translating to a 8.6% cumulative risk by age 65.

CONCLUSION This study shows evidence for an increased risk of PrCa in men who harbour germline mutations in *BRCA1*. This could have a significant impact on possible screening strategies and targeted treatments.

British Journal of Cancer (2012) **106,** 1697–1701. doi:10.1038/bjc.2012.146 www.bjcancer.com
Published online 19 April 2012
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Keywords: prostate cancer; *BRCA1* gene; mutation screening; cancer risk

Prostate cancer (PrCa) is one of the most common cancers affecting men worldwide; in the United Kingdom, PrCa incidence has overtaken lung cancer with 37 000 cases and 10 000 deaths reported in the latest statistics since 2008. Worldwide there were $>$900 000 cases diagnosed and $\sim$250 000 deaths reported in 2008 (CRUK CancerStats August 2011). However, the underlying aetiology is complex and poorly understood compared with other common complex diseases. The only well-established risk factors are age (60% of men diagnosed with PrCa are $>$70 years old), family history (first-degree relatives have an $\sim$2-fold risk compared with the general population) and ethnic background (men of African descent have an increased risk of PrCa). Recently, common genetic variants (minor allele frequency $>$5%) that individually account for a moderate increase in PrCa risk (relative risk (RR) $<$2) were identified by the initial PrCa genome-wide association studies (GWAS; Eeles *et al*, 2008; Gudmundsson *et al*, 2008; Thomas *et al*, 2008), these and subsequent follow-up studies have now found $>$40 PrCa risk alleles (reviewed in Goh *et al*, 2012). Predisposition genes with a higher RR have proven more difficult to identify.

Studies on twins were instrumental in providing evidence for the existence of a substantial genetic component in PrCa risk, with identical twins being $\sim$5 times more likely to be concordant with PrCa compared with non-identical twins (Grönberg *et al*, 1994; Page *et al*, 1997). A large Scandinavian twin study into the causes of cancer reported that genetic factors accounted for 42% of the PrCa risk in their sample set and concluded that both rare highly penetrant variants and common polymorphisms with low penetrance contribute to PrCa risk (Lichtenstein *et al*, 2000).

Deleterious germline mutations in *BRCA1* and *BRCA2* increase the risk of breast and ovarian cancer in women, and co-aggregation of PrCa and breast cancer was initially established in epidemiological studies of breast cancer families (Thompson and Easton, 2001, 2002). For *BRCA1* mutation carriers the Breast Cancer Linkage Consortium reported an increase in PrCa risk in men aged $<$65 years with a RR of 1.82 (95% confidence interval (CI) 1.01–3.29, $P=0.05$), but no risk increase was seen for men $\geqslant$65 years. For *BRCA2* mutation carriers the study reported an increase in PrCa RR of 4.65 (95% CI 3.48–6.22) rising to 7.33 (95% CI 4.66–11.52) for men $<$65 years (The Breast Cancer Linkage Consortium, 1999). The frequency and risk estimates for *BRCA2* mutation carriers was addressed by several studies, most comprehensively it was recently evaluated in a large cohort by our group (Kote-Jarai *et al*, 2011), which reported an $\sim$8.6-fold increased risk of PrCa for *BRCA2* mutation carriers.

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Received 12 December 2011; revised 24 February 2012; accepted 25 March 2012; published online 19 April 2012

However, most *BRCA1* mutations reported in PrCa cases to date were identified through familial breast/ovarian cancer studies and PrCa-specific studies restricted to specific populations and assessment of founder mutations (Kirchhoff *et al*, 2004; Cybulski *et al*, 2008). Therefore, these studies are likely to be underpowered to assess accurately a *BRCA1* mutation carrier's risk of PrCa. Here, we report the first large-scale (913 cases) prostate centric study, using direct Sanger sequencing and multiplex ligation-dependent probe amplification (MLPA) to provide a comprehensive assessment of germline *BRCA1* genetic variation across the entire coding sequence and proximal promoter region of this gene in PrCa cases.

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). The majority (546, 83.4%) of patients with a known diagnosis method (655/913) had clinically presenting (not prostate-specific antigen (PSA) screened) disease. Case selection for this study was based primarily on age at diagnosis of $\leqslant$65 years (821 cases; age range 36–65 years); with a further cohort aged $>$65 years (92 cases; age range 66–88 years) with a family history of one or more first-degree relatives with PrCa (Table 1).

Mutation detection

Germline DNA was obtained from peripheral blood samples and extracted as reported previously (Edwards *et al*, 1997). A total of 29 primer pairs were designed using primer3 and exonprimer (Rozen and Skaletsky, 2000; http://ihg.gsf.de/ihg/ExonPrimer.html) to cover all exons, intron/exon junctions, proximal promoter and exon 1a/b region of the full-length NM_007294.3 mRNA transcript; primer sequences available on request. Using Qiagen multiplex PCR kit 206145 (Qiagen, Hilden, Germany), the 29 primer pairs were grouped into four multiplexs (3×7 multiplexes and 1×6 multiplex) and two singleton reactions. The amplicons were sequenced on an ABI3730 Genetic Analyzer using a 1/16th BigDye v3 protocol (Applied Biosystems, Foster City, CA, USA). Deleterious mutations were confirmed by Sanger sequencing using a different aliquot from stock DNA of each sample. Multiplex ligation-dependent probe amplification was performed on a subset of 460 samples using a modified protocol of the SALSA MLPA kit P002-C2 (MRC-Holland, Amsterdam, The Netherlands).

Sequence analysis

Mutation surveyor 3.97 (Softgenetics, State College, PA, USA) was used to analyse the sequencing data, using a region of interest covering 500 bp of the near promoter exons 1–3, 5–24 and also including $\pm$20 bp per exon to screen the intron/exon boundaries.

Table 1 Age range and family history of PrCa samples screened for *BRCA1* germline mutation

Age range (years)	Number of samples	% PrCa family history[a]	% Br/Ov family history[a]	% Samples deceased	Number of carriers	% Carriers
36–55	327	37.3	26.0	13.1	1	0.3
56–65	494	53.0	28.7	12.8	2	0.4
66–88	92	100.0	31.5	22.8	1	1
Total	913				4	

Abbreviations: BrCa = breast cancer; OvCa = ovarian cancer; PrCa = prostate cancer. Family history of (BrCa) and (OvCa) is also shown. [a]In first- and second-degree relatives.

Results were exported to Human Genome Variation Society nomenclature and converted to hg19 genomic coordinates using Mutalyzer 2.0 β-8, (http://www.mutalyzer.nl/) to allow variant annotation using ANNOVAR (Wildeman *et al*, 2008; Wang *et al*, 2010). Variants were designated as 'novel' if they were not described in dbSNP132, Breast cancer Information Core (BIC) or the Leiden Open Variation Database as of July 2011 (Szabo *et al*, 2000; Sherry *et al*, 2001; Fokkema *et al*, 2011).

Statistical methods

Estimates for *BRCA1* prevalence in the general population were derived from a study where a large population series and a set of high-risk breast cancer families from the United Kingdom were analysed incorporating the effect of BRCA1, BRAC2 and other genes to account for residual family history (Antoniou *et al*, 2002). Confidence intervals for the prevalence of *BRCA1* mutations among PrCa cases and hence the CIs on RR were computed using an exact binomial procedure. Age-specific cumulative risks of PrCa to age 65 in *BRCA1* mutation carriers were computed based on the RR estimated from this study and age-specific pooled incidence rates from five cancer registries in England for the year 2002 as reported in Ferlay *et al* (2007).

RESULTS

We screened 913 PrCa cases for germline mutations in the *BRCA1* gene using direct Sanger sequencing. After quality control (QC) exclusions, data from 886 PrCa cases were analysed. We identified four deleterious mutations (0.45%), of which three were frameshifts and one was a splice site mutation (Table 2). A subset of the PrCa cases (460) was also screened for large-scale rearrangements (LGRs) using MLPA, none were found in our sample set.

All four deleterious mutations had been previously recorded in BIC (http://research.nhgri.nih.gov/bic/). We also identified 45 missense variants (10 novel), 15 synonymous substitutions (5 novel) and 6 intronic variants (1 novel; Supplementary Table 1). The majority of deleterious mutations occurred in PrCa cases $<$65 years (3 out of 4) and all mutations are within two distinct functional domains of BRCA1; mutations c.68_69delAG (A) and c.212+1G>T (B) in the N-terminus RING finger domain and c.1954dupA (C) and c.2475delC (D) in the large central DNA-binding domain (Figure 1).

In addition we have also identified several missense unclassified variants (UV) and a deletion in the proximal promoter region, c.1-1462delC immediately 3′ of a CREB-binding site regulated by methylation (Mancini *et al*, 1998; Atlas *et al*, 2001; DiNardo *et al*, 2001).

The missense UVs identified were annotated by ANNOVAR against dbNSFP normalised (0–1) scores for SIFT, Polyphen2, LRT, MutationTaster and Phylop, (Chun and Fay, 2009; Kumar *et al*, 2009; Adzhubei *et al*, 2010; Pollard *et al*, 2010; Schwarz *et al*, 2010; Liu *et al*, 2011). In addition, all non-synonymous SNPs were annotated with Align-GVGD (Mathe *et al*, 2006; Tavtigian *et al*, 2008). The intersection of UVs predicted to be 'probably damaging' by both SIFT and Polyphen2 selects 13 of the 45 UVs found in the study, of which 11 are in BIC and 2 are novel (Supplementary Table 1). Of the BIC annotated mutations three are classed as 'of no clinical significance', the rest as 'of unknown significance', whereas 10 of these 13 variants have been classified as 'of no clinical significance' using multifactorial likelihood approaches (Vallée *et al*, 2011). This leaves three variants, H279Q, M1400R and M1783T (Supplementary Table 2) with some potential to be deleterious, although only M1783T is situated in a known functional domain (BRCT repeat 2).

Based on the previously estimated carrier frequency of *BRCA1* mutations in the United Kingdom (which estimated the allele

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Table 2 Patient information for protein-truncating mutation carriers

Sample ID	Nucleotide change	Age at diagnosis (years)	Tumour stage	Node stage	Metastases	Final gleason	Years to death	FH of prostate cancer	FH of Br or Ov	FH of other cancers in family
PR-1	c.68_69delAG	58	T1a	N0	M0	3+3	12 (Alive)	No	Mother Ov	No
PR-2	c.212+1G>T	57	T4	Nx	Mx	4+4	3	No	Sister Ov, aunt Br, grandmother Br	Mother bladder, grandfather colon
PR-3	c.1954dupA	51	Tx	Nx	M0	—	11	No	No	Brother Bladder
PR-4	c.2475delC	69	Tx	Nx	Mx	—	8	Father	No	No

Abbreviations: Br = breast cancer; Ov = ovarian cancer. Clinical and family history (FH) data are detailed here if available. Tx, Nx and Mx if status unknown.

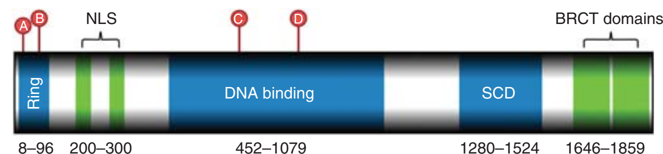

Figure 1 Schematic diagram of the positions of the deleterious mutations (A–D) found in this study within the *BRCA1* transcript. The locations of the functional domains are marked in blue or green.

frequency of *BRCA1* mutation to be 0.006 corresponding to a carrier frequency of 0.0012 (~1/1000), Antoniou *et al*, 2002) and the observed prevalence of *BRCA1* mutations in our series of PrCa cases of 4/886 (0.45%), we estimate that germline mutations in the *BRCA1* gene confer a RR of PrCa of ~3.75-fold, (95% CI 1.02–9.6).

DISCUSSION

This study utilised direct Sanger sequencing to screen the complete *BRCA1* coding transcript (NM_007294.3) in 913 PrCa patients, the majority of whom were clinically detected (83.4% of cases for which the diagnosis method was reported). This is the largest direct sequencing study of *BRCA1* reported so far in PrCa and therefore presents the most comprehensive assessment of the contribution of *BRCA1* mutations to PrCa risk.

After QC, 886 PrCa cases were included in our mutation analysis of the sequencing data and four pathogenic mutations were identified (0.45%). In the 460 samples, which were also submitted for MLPA, no LGRs were identified.

Of the four identified pathogenic mutations, c.68_69delAG (BIC 185delAG) is a well-known Ashkenazi-Jewish founder mutation (reported 1979 times in BIC), which has been extensively studied and has a frequency of ~1% in the general Ashkenazi-Jewish population. From the UK 2001 census, 266 740 people (0.5% UK population) are recorded Jewish as their religion; however, this is believed to be an underestimate due to the non-counting of secular Ashkenazi-Jews and the self-reporting nature of this statistic. A recent meta-analysis on six Ashekenazi-Jewish studies of PrCa (3005 cases and 6834 controls) by Fachal *et al* showed a non-statistically significant odds ratio 1.8 (95% CI 0.91–3.57; $P = 0.09$) for this particular mutation (Fachal *et al*, 2011). This correlates with the observation by Al-Mulla *et al*, 2009, where breast cancer patients with *BRCA1*-truncating mutations in exon 2 (28/30 mutations assessed were c.68_69delAG) had a significantly higher age of diagnosis compared with carriers of mutations in exons 11, 13 and 20, therefore suggesting protein-truncating mutations in exon 2 have a lower penetrance than would be expected in this gene. The c.68_69delAG mutation has also been found in the non-Ashkenazi-Jewish, Spanish and UK (Yorkshire) populations, with haplotype evidence for this mutation having arisen by independent mutational events in different populations (Neuhausen *et al*, 1996; Fackenthal and Olopade, 2007). Therefore, without detailed haplotype analysis it cannot be determined which ancestry is relevant in this study. The carrier of this mutation in our study was diagnosed with PrCa at 58 years and following treatment had 12 years disease-free survival.

The c.212+1G>T (BIC IVS5+1G>T) mutation disrupts the splice donor site, allowing read-through into the intron and termination of transcription at codon 64. This mutation has also been observed in a Spanish breast cancer study in a family with breast and bladder cancers (Diez *et al*, 2010). The patient with this mutation was diagnosed at 57 years and survived for only 3 years after diagnosis; he also has a close relative who was diagnosed with bladder cancer.

The c.1954dupA (BIC 2080insA) mutation is a frame-shift causing insertion, resulting in termination of transcription at codon 672 and has been reported as a Pakistani founder mutation (Liede *et al*, 2002).

The c.2475delC mutation (BIC 2594delC) is a frame-shift deletion, resulting in termination at codon 845. This mutation appears to be a Scandinavian/Northern European founder mutation from the ethnicity of the study samples recorded in BIC and in two Scandinavian studies (Johannsson *et al*, 1996; Thomassen *et al*, 2008).

The incidence of truncating *BRCA1* mutations in this study for PrCa cases diagnosed at ⩽65 years (3/802) is 0.37%, and 0.45% in all cases (4/886). Of the four pathogenic mutations identified, only one patient had a family history of PrCa (father, age unavailable), one patient had a family history of bladder cancer (brother, age unavailable) and two patients were from families with first-degree relatives with breast or ovarian cancer (details in Table 2). The small number of mutation carriers does not allow the statistical evaluation of the tumour characteristics in this study; however, we have conducted a clinical assessment of a much larger set of *BRCA1* and *BRCA2* mutation carriers and non-carrier PrCa cases, which indicates that both *BRCA1* and *BRCA2* mutations confer a more aggressive PrCa phenotype (Castro *et al*, submitted).

Based on the previously estimated carrier frequency of *BRCA1* mutations in the United Kingdom, we estimate that germline mutations in the *BRCA1* gene confer a RR of PrCa of ~3.7-fold and this translates to an 8.6% cumulative risk by the age of 65 years. Other studies have estimated higher frequencies of *BRCA1* mutation prevalence but these did not allow for the presence of other genetic effects (Whittemore *et al*, 2004). It is important to emphasise that in addition to the population frequency of *BRCA1* mutations various other factors could influence the estimates of RR. Also, if one or more of the missense changes (UVs) were pathogenic, the risk estimates would be higher. On balance our estimate of ~3.7-fold increased risk for *BRCA1* mutation carriers is accurate based on incidence rates in the United Kingdom. Several studies addressed the estimation of PrCa risk by modelling and incorporating various factors such as PSA level (Thompson *et al*, 2006) or the recently discovered common genetic variants found by GWAS (Macinnis *et al*, 2011). This latest model also has

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the capacity to incorporate clinical information and effect of rare genes once this becomes available.

These results suggest that routine testing of early onset PrCa cases for germline *BRCA1* mutations would further help to refine the prevalence and risk associated with *BRCA1* mutations and may be useful for guiding management options.

ACKNOWLEDGEMENTS

We acknowledge the NCRN nurses and Consultants for their work in the UKGPCS study. This work was supported by The Prostate Cancer Research Foundation (now Prostate Action) and by Cancer Research UK (grant numbers C5047/A7357, C1287/A10118, C1287/A5260, C5047/A3354, C5047/A10692, C16913/A6135 and C16913/A6835). We also thank the following for funding support: the Institute of Cancer Research and the Everyman Campaign, Prostate Research Campaign UK, the Orchid Cancer Appeal, the National Cancer Research Network, UK and the National Cancer Research Institute, UK. We acknowledge NHS funding to the NIHR Biomedical Research Centre at the Institute of Cancer Research and the Royal Marsden NHS Foundation Trust.

Conflict of interest

The authors declare no conflict of interest.

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

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