Frequent germline deleterious mutations in DNA repair genes in familial prostate cancer cases are associated with advanced disease

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Background: Prostate cancer (PrCa) is one of the most common diseases to affect men worldwide and among the leading causes of cancer-related death. The purpose of this study was to use second-generation sequencing technology to assess the frequency of deleterious mutations in 22 tumour suppressor genes in familial PrCa and estimate the relative risk of PrCa if these genes are mutated.

Methods: Germline DNA samples from 191 men with 3 or more cases of PrCa in their family were sequenced for 22 tumour suppressor genes using Agilent target enrichment and Illumina technology. Analysis for genetic variation was carried out by using a pipeline consisting of BWA, Genome Analysis Toolkit (GATK) and ANNOVAR. Clinical features were correlated with mutation status using standard statistical tests. Modified segregation analysis was used to determine the relative risk of PrCa conferred by the putative loss-of-function (LoF) mutations identified.

Results: We discovered 14 putative LoF mutations in 191 samples (7.3%) and these mutations were more frequently associated with nodal involvement, metastasis or T4 tumour stage (P = 0.00164). Segregation analysis of probands with European ancestry estimated that LoF mutations in any of the studied genes confer a relative risk of PrCa of 1.94 (95% CI: 1.56–2.42).

Conclusions: These findings show that LoF mutations in DNA repair pathway genes predispose to familial PrCa and advanced disease and therefore warrants further investigation. The clinical utility of these findings will become increasingly important as targeted screening and therapies become more widespread.

Prostate cancer (PrCa) is one of the most commonly diagnosed cancers in men worldwide (Jemal et al, 2011), representing the second most common cause of male cancer-related deaths in the United States, the third in the European Union and the sixth worldwide with over 250 000 deaths per year (Ferlay et al, 2013). Established risk factors for PrCa include age, family history of PrCa and ethnicity, although many common genetic variants that individually contribute to moderate increased risk have also been identified (Goh et al, 2012). Prostate cancer has also been observed to aggregate with other types of familial cancer, in particular with breast and ovarian cancers (Hemminki and Chen, 2005). Several genes that were initially implicated as high risk for breast or...
ovarian cancer predisposition, for example, *BRCA1*, *BRCA2*,
*CHEK2* and *BRIP1*, have subsequently been shown to increase
the risk of PrCa as well (Dong et al, 2003; Cybulski et al, 2004;
Agalliu et al, 2007; Kote-Jarai et al, 2009). This suggests that shared
genetic and/or environmental factors may be causal for multiple
cancer types. Further evidence for pleiotropy has come from the
recent Collaborative Oncological Gene-environment Study
(COGS), a multicancer mega-consortium and previous genome-
wide association studies (GWAS), which report sharing of
common loci between cancers and especially hormonal-related
malignancies (Bojesen et al, 2013; Eeles et al, 2013; Garcia-Closas
et al, 2013; Kote-Jarai et al, 2013; Michaillidou et al, 2013).
Genomic instability is the hallmark of most cancers and therefore
the investigation of DNA repair pathways in hereditary cancer risk
is widely established, for example, mutations in the DNA
mismatch repair (MMR) pathway cause hereditary non-polyposis
colon cancer (HNPCC/Lynch syndrome) and mutations in
Fanconi anaemia (FA) genes, which include *BRCA2*, *BRIP1* and
*PALB2*, predispose carriers to multiple cancers including PrCa
(Silva et al, 2009; Kottemann and Smorgorzewska, 2013).

We propose that additional moderate penetrance genes for PrCa
have yet to be discovered and these will be facilitated by the recent
development of massively parallel sequencing. This allows the
candidate gene study to be easily expanded both in terms of depth
and breadth, enabling the targeting of multiple genes across
multiple samples in a single experiment. In this study, we
investigated whether deleterious mutations in a set of DNA repair
genes have a role in familial PrCa predisposition. We selected for
this study the BROCA tumour suppressor gene set designed by
Walsh et al (2010), which comprises known high- and moderate-
risk breast/ovarian cancer genes from the *BRCA*–Fanconi anaemia
complex and also genes involved in rare multiorgan cancer
syndromes (Table 1); some of these overlap with previously known
genes implicated in PrCa predisposition.

### MATERIALS AND METHODS

**Study population.** We selected a series of men with PrCa from the
UK Genetic Prostate Cancer Study (UKGPCS; UKCRN ID 869)
(Eeles et al, 1997), based primarily on their PrCa family history.
Subjects were eligible if they had two or more relatives affected by
PrCa; a total of 191 men were included in this study. Germline
DNA was isolated from peripheral whole blood samples using the
Nucleon DNA purification system (GE Healthcare Life Sciences,
Pittsburgh, PA, USA) or methods described in Edwards et al
(1997). The study was approved by the Royal Marsden NHS Trust,
Local Research Ethics Committee.

**Target capture enrichment and sequencing.** A custom Agilent
SureSelect bait library (Agilent Technologies, Santa Clara, CA,
USA) was used to target capture 22 genes from germline DNA.
Capture regions were designed to cover coding, non-coding and
intronic sequences with an additional 10 kilobase (kb) genomic
sequence flanking each gene. After repetitive DNA elements were
masked, the total DNA targeted was 939 kb (Walsh et al, 2010).
Sequencing libraries were prepared in batches of 48 and each
sample was ‘barcoded’ with a 6 base pair (bp) index to allow
multiplexed sequencing. An initial batch of 48 libraries was
prepared using standard Agilent protocols, while the remaining 3
batches of 48 libraries used a modified Agilent protocol with
prehybridisation pooling to allow 3 libraries to be captured at once
with a single bait library (Cummings et al, 2010). All libraries were
clustered and sequenced on Illumina cBOT and HiSeq 2000
instruments (Illumina, San Diego, CA, USA), using v.2 flowcells
and Truseq reagents to produce 2 × 78 bp ‘paired-end’ reads and a
6 bp ‘index’ read.

**Sequencing data analysis and variant annotation.** Raw sequenc-
ing data were base-called and demultiplexed using Illumina
CASAVA software (v.1.8.1, Illumina) and purity filtered reads were
removed to produce paired FASTQ files. Each set of paired FASTQ
files was aligned using BWA 0.5.8 to ‘The 1000 Genomes Project’
Phase 1 reference, human_g1k_v37.fasta (Li and Durbin, 2009).
The ‘Best Practice Variant Detection with the Genome Analysis
Toolkit (GATK)’ v.3 for targeted resequencing was implemented
with Picard v.1.52 and the GATK (all tools v.1.0.5216M except
Unified genotyper v.1.6-9-g47df7bb) (DePristo et al, 2011) when
realigning, recalibrating and genotyping. Variants were annotated
using a February 2013 build of ANNOVAR using the summar-
ize_annovar.pl script (Wang et al, 2010). This maps variants to
RefSeq genes, known variation from dbSNP137 and annotates the
predicted functional consequence of missense variants using six
software tools (SIFT, PolyPhen-2, LRT, MutationTaster, phyloP
and GERP + +) from the dbNSFP v.1.3 (Liu et al, 2011). Additional
clinical variant annotation was obtained from NCBI ClinVar (last
accessed July 2013; http://www.ncbi.nlm.nih.gov/clinvar/).

**LoF mutation definition and validation.** Putative loss-of-func-
tion (LoF) mutations were defined as variants that are protein
truncating or result in significant alteration of the protein
sequence. This encompasses stop codon gain/loss, insertion/
deletion frameshifts or splice site loss variants.

Loss of function variants were validated by Sanger sequencing
in the probands and in other family members if available. Polymerease chain reaction amplicons were designed in Primer-Z
(Tsai et al, 2007), except for the *PMS2* variant, where

### Table 1. BROCA 22 tumour suppressor gene set

| Gene     | RefSeq accession | Involvement in rare multiorgan cancer syndromes |
|----------|------------------|-------------------------------------------------|
| ATM      | NM_000051.3      |                                                 |
| BARD1    | NM_000465.2      |                                                 |
| BRCA1    | NM_007294.3      |                                                 |
| BRCA2    | NM_000059.3      | Fanconi anaemia                                  |
| BRIP1    | NM_032043.2      | Fanconi anaemia                                  |
| CDH1     | NM_004360.3      | Hereditary diffuse gastric cancer                |
| CHEK2    | NM_007194.3      |                                                 |
| MLH1     | NM_000249.3      | HNPCC/Lynch                                     |
| MLH3*    | NM_001040108.1   | HNPCC/Lynch                                     |
| MRE11A   | NM_005591.3      |                                                 |
| MSH2     | NM_000251.2      | HNPCC/Lynch                                     |
| MSH6     | NM_000179.2      | HNPCC/Lynch                                     |
| MUTYH    | NM_001128425.1   | Familial adenomatous polyposis-2                |
| NBN      | NM_002485.4      | Nijmegen breakage syndrome                      |
| PALB2    | NM_024675.3      | Fanconi anaemia                                  |
| PMS1    | NM_000534.4      | HNPCC/Lynch                                     |
| PMS2     | NM_000535.5      | HNPCC/Lynch                                     |
| PTEN     | NM_000314.4      | Cowden                                          |
| RAD50    | NM_005732.3      |                                                 |
| RAD51C   | NM_058216.1      | Fanconi anaemia                                  |
| STK11    | NM_000455.4      | Peutz-Jeghers                                   |
| TP53     | NM_000546.5      | Li–Fraumeni                                     |

Abbreviations: HNPCC – hereditary non-polyposis colorectal cancer; RefSeq – NCBI Reference Sequence Database.

* Added since the study by Walsh et al (2010).
* Disputed as a Lynch syndrome gene.
Frequency of deleterious mutations in PrCa

Published primers were used from De Vos et al (2004) because of the pseudogene *PMS2CL* (primer sequences available in Supplementary Table S1), sequenced on a ABI3730 Genetic Analyzer using a 1/16th BigDye v.3 protocol (Applied Biosystems, Foster City, CA, USA) and analysed using Mutation surveyor v3.97 (Softgenetics, State College, PA, USA), against the appropriate RefSeq accession sequence (Table 1).

Statistical and segregation analysis. We investigated the correlations between LoF mutation status and clinical features using Fisher’s exact test, Mann–Whitney U-test or the Mantel–Haenszel test for linear trend; patients with missing data for a particular clinical feature were excluded from that analysis (Table 3). All statistical analyses were performed using R 2.15.1, ‘stats’ and ‘vcdExtra’ 0.5-7 packages (R Core Team, 2012; Friendly, 2013).

To obtain an estimate of the average PrCa risk conferred by the LoF mutations identified, we carried out a modified segregation analysis using information on 186 probands of European ancestry. This was implemented in the pedigree analysis software MENDEL (Lange et al, 2013). The analysis was based on PrCa occurrence in male family members. Unaffected male subjects were censored at the age of 85 years, the age at death or last observation, whichever occurred first. As no proband was found to carry LoF mutations in the age of 85 years, the age at death or last observation, whichever occurred first. As no proband was found to carry LoF mutations in more than one gene, we assumed a genetic model where all identified LoF mutations across all genes represent the alleles of a single genetic locus and assumed that all alleles conferred the same relative risk of PrCa. We assumed that the PrCa incidence depends on the underlying genotype through a model of the form: \( \hat{\lambda}(t) = \lambda_0(t) \exp(\beta g) \), where \( \lambda_0(t) \) is the baseline incidence at age \( t \) in non-mutation carriers, \( \beta \) is the log risk ratio associated with the LoF mutation and \( g \) takes value 0 for non-mutation carriers and 1 for LoF mutation carriers. The overall PrCa incidence and overall possible genotypes in the model were constrained to agree with the population incidences for England and Wales in the period 1993–1997 (Parkin et al, 2002). We assumed that the total mutation carrier frequency in the model was equal to the sum of mutation carrier frequencies in the genes, as estimated in previous studies (total mutation frequency = 1.4%; frequencies obtained from UK studies (European ancestry) where available or NHLBI GO Exome Sequencing Project (ESP); last accessed November 2013; http://evs.gs.washington.edu/EVS/) (Antoniou et al, 2002; The CHEK2 Breast Cancer Case–Control Consortium, 2004; Thompson et al, 2005; Seal et al, 2006; Rahman et al, 2007). The models were parameterised in terms of the log-relative risk ratios for PrCa. Parameters were estimated using maximum-likelihood estimation. To adjust for ascertainment, we modelled the conditional likelihood of all family phenotypes and mutation status of all tested family members (including the index/proband), given the disease phenotypes of all family members.

RESULTS

Patient characteristics. Of the 191 men with \( \geq 3 \) PrCa cases in their family, 128 men also had at least 1 relative affected by breast, ovarian or colon cancer. Ethnicity was known for 72% (137 of our series, with 96% (131) of white European descent, and the remainder of our set also included two men of black African descent, three men of black Caribbean descent and one Ashkenazi Jewish man. The method of diagnosis was available for 69% of patients, with an even split between clinically detected and PSA screened patients (64 and 68 patients, respectively).

Sequencing and variant quality control. All samples reached the required coverage threshold of 20 \( \times \) read depth across 80% of the target regions. The median value of average target region read depth was 135.85, and *STK11* had about half as much median read coverage compared with the rest of the target genes (76.8; Figure 1). After QC filtering, 7138 exonic and splice site variants were genotyped, corresponding to 300 unique variants. Of the initial 14 unique putative LoF mutations identified, a *BRCA2* stop-gain K3326X (rs11571833) was classed as ‘non-pathogenic’ in ClinVar (Wu et al, 2005) and consequently removed from further analysis. Non-carriers were defined as patients who did not carry an LoF mutation, a predicted deleterious SNV by all six in silico tools in dbNSFP v.1.3 or an SNV classed as ‘pathogenic’ in the NCBI ClinVar database for a disease other than PrCa (Table 3 and Supplementary Tables S2 and S3). Therefore 14 LoF mutation carriers and 140 non-carriers were selected for further analysis.

Frequency and type of LoF mutations. Thirteen LoF mutations in eight genes were identified in 14 familial PrCa cases (Figure 2 and Table 2). We found three frameshift mutations and one stop-gain mutation in *BRCA2*; two stop-gain mutations in *ATM*; a recurring stop-gain mutation in *BRIPI* affecting two families and two frameshift mutations in *CHEK2*. One mutation was found in each of *BRCA1*, *MUTYH*, *PALB2* and *PMS2*. Five of the 13 unique LoF variants found were not listed in dbSNP137, the 1000 Genomes Project (April 2012 data release) and the NHLBI GO ESP (ES6500SI data release). No subject carried more than one LoF mutation; therefore, 7.3% (14 of 191 men) of these familial cases were carriers of a deleterious mutation in 1 of the 22 tumour suppressor genes investigated here.

LoF mutations and clinical characteristics. Table 3 shows clinical characteristics of LoF carriers vs non-carriers. Median age at diagnosis in LoF carriers was very similar to non-carriers: 58.5 and 59.0 years, respectively (\( P = 0.334 \)), and median presenting PSA was higher in carriers compared with non-carriers but the difference was not significant (11.10 vs 8.25 ng ml\(^{-1} \); \( P = 0.156 \)). Gleason scores were categorised into three groups: \( \leq 6, 7 \) and \( \geq 8 \). There was no significant association between LoF carrier status and the Gleason grade groups (\( P = 0.312 \)), or when analysed against high grades only (Gleason \( \geq 8 \); \( P = 0.193 \)). There was also no significant association between LoF carrier status and tumour stage trend (\( P = 0.476 \)), or when analysed against high tumour stage (T3–T4; \( P = 0.704 \)). However, there was a significant association between LoF carrier status and the presence of nodal involvement (42.9% vs 13%; \( P = 0.0014 \)) and metastasis (30.0% vs 6.3%; \( P = 0.043 \)).
LoF mutations and advanced PrCa. To further investigate the associations seen with nodal involvement and metastasis, we applied the AJCC Stage IV prognostic grouping for advanced disease, as defined either by nodal involvement, metastasis or primary tumour grade of T4 (Edge et al, 2010). We performed a logistic regression on LoF carrier status vs Stage IV status, controlling for the effect of age at PrCa diagnosis. This showed that LoF mutation carriers have significantly higher odds of having advanced disease (OR 15.09, 95% CI: 2.95–95.81, \(P = 0.00164\)) (Table 3). Even after excluding the BRCA2 mutations (as these have been shown to be associated with poorer prognosis; Castro et al, 2013), the association remained significant (\(P = 0.00285\)), indicating that this correlation is independent of BRCA2 LoF status.

LoF mutations and family history of cancer. We also investigated the role of the proband’s family history on the odds of having an LoF mutation; however, no association was found with the total number of PrCa cases within a family (\(P = 0.808\)). We then used a modified risk prediction algorithm developed by Macinnis et al (2011) to assess the association between LoF mutations and family history of PrCa with and without 25 common risk SNPs, but no significant association was found with either (\(P = 0.456\) and \(P = 0.856\), respectively). However, this is not unexpected as all study cases were selected for having multiple relatives diagnosed with PrCa and only a subset of DNA repair genes were tested. On the other hand, LoF carriers were more likely to have a family history of breast cancer than non-carriers (OR 3.94, 95% CI: 1.07–18.10, \(P = 0.023\)) and there was also a significant trend with the increase in the total number of breast cancer cases within a family (\(P = 0.00354\)). Table 3 shows the 191 families grouped by family history of cancers other than PrCa and the percentage of LoF mutations found in each group.

LoF mutations and familial segregation. Of the 13 unique LoF mutations, 8 were frameshift and 5 were stop-gain. The eight affected genes represent four DNA damage repair or response pathways; homologous recombination (HR) and FA, ataxia–telangiectasia-mutated signalling (ATM), MMR and base excision repair (BER). As might be expected, mutations in the ATM and MMR pathways were observed in families where PrCa coaggregated with colon cancer cases (three of the four families), whereas mutations affecting the HR/FA pathway were found mainly in families with breast cancer, and to a lesser extent in families with ovarian cancer in addition to PrCa (six of the families) (Table 2). The only mutation unique to families with only PrCa reported was a previously known (rs137852986) stop-gain mutation c.2392C>T (p.(Arg798*)) in the BRIP1 gene that was present in two families.

The most frequently mutated gene in this study was BRCA2, with four protein truncating mutations. Three of these were in exon 11 (two frameshifts and one stop-gain), and a stop-gain in exon 25. Of the four men affected, we had additional DNA available from other family members in the two men with exon 11 frameshifts. We found partial segregation of the c.4981del (p.(Tyr1661Ilefs*9); Supplementary Figure S5) mutation, where the proband had two brothers with PrCa: one diagnosed (Dx) at 67 years who did not carry the mutation, whereas the other did and died of PrCa 4 years after diagnosis (69 years) at 73 years. The second exon 11 frameshift c.4876_4877del (p.(Asn1626Serfs*12); Supplementary Figure S6) was not present in the proband’s brother with PrCa.

Two mutations were found in the ATM gene. An exon 50 stop-gain c.7327C>T (p.(Arg2443*); Supplementary Figure S1) was found in a young proband (Dx 59 years) and his affected brother also carried the same mutation (Dx 61 years); furthermore, the proband had an additional MSH2 (NM_000251.2) c.1275A>G substitution at the –2 position in the 3’ end of exon 7, which has been characterised as causing partial exon skipping at the RNA level (Pagenstecher et al, 2006). The second ATM mutation, a stop-gain c.7777C>T (p.(Gln2593*)) in exon 52 was present in a family (Supplementary Figure S2) where the father had colon cancer; four out of eight brothers had PrCa and a sister was reported to have had an unspecified leukaemia. We confirmed the mutation in the proband (Dx 65 years) and in a brother (Dx 68 years, the only available sample). It is also worth noting that both brothers also had a secondary cancer diagnosis of the colon.

We identified two mutations in the CHEK2 gene. An exon 12 frameshift c.1263del (p.(Ser422Valfs*15)) was found in a family with multiple PrCa cases. DNA samples were available for five brothers, two of the three PrCa cases carried the mutation (Dx 65 years and 72 years and non-carrier Dx 74 years). Of the two unaffected brothers, one was also a carrier of this mutation (Supplementary Figure S10). The second CHEK2 mutation was an exon 8 frameshift c.869del (p.(Asn290Thrfs*14)), which was present in a patient diagnosed with PrCa at the age of 53 years, but did not segregate with his father, the only available sample (Dx 68 years); therefore, it is likely that this mutation was inherited from the maternal line, which contains two colon cancers (in the proband’s mother and grandfather; Supplementary Figure S11).

Only one variant was observed in more than one family: the BRIP1 stop-gain mutation c.2392C>T (p.(Arg798*)), first discovered in FA and later described in breast cancer and PrCa families (Levrant et al, 2005; Seal et al, 2006; Kote-Jarai et al, 2009). This mutation resides in exon 17 and has an MAF of 0.02% in 4300 European Americans from the ESP variant server. In the first unaffected brothers, one was also a carrier of this mutation (Supplementary Figure S2) where the father had colon cancer; four out of eight brothers had PrCa and a sister was reported to have young at 59 and 56 years (Supplementary Figure S9). In the second family there were three PrCa cases, but the only DNA available was from the proband that was diagnosed at 59 years (Supplementary Figure S8).

In four other genes, a single mutation was found: the BRCA1 mutation c.4065_4068del (p.(Asn1355Lysfs*10), Dx 58 years) was identified in a family with multiple (4) breast cancers and also had four PrCas (Supplementary Figure S3). The PALB2 frameshift mutation c.3507_3508del (p.(His1170Phefs*19), Dx 58 years) carrier had an affected brother (Dx 63 years) who also carried...
| ID  | Gene   | HGVS nomenclature                  | Mutation outcome  | Age of onset (years) | Tumour stagea | Nodal stageb | Metastasesb | Gleason score | PSA (ng mL⁻¹) | Years to death | Family history of PrCa³ | Mutation segregationb,c | Family history of other cancer² |
|-----|--------|------------------------------------|-------------------|---------------------|---------------|--------------|-------------|---------------|---------------|----------------|------------------------|--------------------------|-----------------------------|
| Prs1| ATM    | NM_000051.3:c.7227C>T: p.Arg2410P | Stop-gain         | 59                  | TX            | NX           | MX          | NA            | NA            | Alive (19)    | Brother (61), father (80), grandfather (m) | Brother = (61)           | Sister, CoCa, uncle (p) CoCa |
| Prs2| ATM    | NM_000051.3:c.7777C>T: p.Gln2593P | Stop-gain         | 65                  | T1b           | NX           | M0          | 3            | ±3           | NA            | Alive (20)    | 3 × brothers (68, 69, NA) | Brother = (68)           | Father CoCa               |
| Prs3| BRCA1  | NM_0007294:3:c.4065,4066del: p(Asn1355ysf*110) | Frameshift        | 58                  | T2a           | NX           | MX          | 3            | ±4           | 6.34         | Alive (6)     | Father and 3 uncles (p) | NA                        | 4 × Cousins (p) BrCa       |
| Prs4| BRCA2  | NM_000039.3:c.4876,4877del: p(Asn1626serf*1) | Frameshift        | 54                  | T3b           | N1           | M1          | 4            | ±4           | 70.1         | Alive (11)    | Father (67), uncle (m,63), 4 × cousins (m: 51, 55, 56, 57) | Cousin = (58)            | Cousin (m) BrCa            |
| Prs5| BRCA2  | NM_000039.3:c.4072del: p.Tyr1661del | Frameshift        | 62                  | TX            | NX           | M0          | 3            | ±4           | 13.3         | Alive (17)    | 2 × brothers (67, 69) | Brother = (62), brother = (67) | Sister, aunt (m), grandmother (m) BrCa; aunt (m) CoCa |
| Prs6| BRCA2  | NM_000039.3:c.5909C>A: p.Ser1970P | Stop-gain         | 71                  | T2b           | NX           | MX          | NA            | 11.1          | 12           | Father, 2 × brothers | NA                        | Sister (30) and aunt (p) BrCa |
| Prs7| BRCA2  | NM_000039.3:c.9382C>T: p.Arg3128P | Stop-gain         | 41                  | T2a           | N0           | M0          | 3            | ±4           | 3.09         | Alive (6)     | Uncle (p), uncle (m) | NA                        | Mother (41), aunt (m); 48, aunt (p) BrCa, aunt (m) OvCa (51) |
| Prs8| BRIP1  | NM_003243.2:c.2392C>T: p.Arg798P | Stop-gain         | 59                  | T1c           | N0           | M0          | 3            | ±3           | 10.5         | Alive (10)    | Father, grandfather (p) | NA                        | NA                          |
| Prs9| BRIP1  | NM_003243.2:c.2392C>T: p.Arg798P | Stop-gain         | 46                  | T1           | N0           | M1          | NA            | NA            | 6            | 3 × half-brothers (m) | Unaffected brother =; 2 × half-brothers =; (m: 59, NA) | Half-brother (m) StCa, uncle (p) BoCa |
| Prs10|CHEK2  | NM_0007194:3:c.1268del: p.Glu426del | Frameshift        | 65                  | T2            | N0           | M0          | 3            | ±3           | 11           | Alive (16)    | 3 × brothers (67, 72, 74), 2 × cousins (p: 57, 68) | Brother = (72), brother = (74), unaffected brother + and unaffected brother — | Father melanoma, sister BrCa |
| Prs11|CHEK2  | NM_0007194:3:c.1268del: p.Glu426del | Frameshift        | 53                  | TX            | N1           | M0          | 4            | ±5           | 14.2         | Alive (6)     | Father (68), grandfather (p) | Father = (68)            | Mother, grandfather (m), grandmother (p) CoCa; aunt (p) BrCa |
| Prs12|MUTYH  | NM_001128425.1:c.940C>T: p.Gln314P | Stop-gain         | 55                  | TX            | NX           | MX          | 3            | ±3           | 7            | Alive (6)     | 2 × brothers (69, NA), uncle | Brother = (69)           | Aunt (m) BrCa               |
| Prs13|PALB2  | NM_0024675:3:c.3507_3508del: p.His1170P<del| Frameshift        | 58                  | T3            | NX           | M1          | NA            | 91.1          | 2            | 2 × brothers (63, NA), uncle (m) | Brother = (63)           | Mother BoCa, sister, grandmother (m) BrCa |
| Prs14|PMS2   | NM_000535.5:c.2186,2187del: p.Leu729del | Frameshift        | 51                  | T4            | N1           | M0          | 4            | ±5           | 20.9         | 13           | Father, 2 × half uncles (p), grandfather (p) | NA                        | Aunt (m) BrCa               |

Abbreviations: BoCa = bone cancer; BrCa = breast cancer; CoCa = colon cancer; HGVS = Human Genome Variation Society; m = maternal; NA = not available; OvCa = ovarian cancer; p = paternal; PrCa = prostate cancer; PSA = prostate-specific antigen; StCa = stomach cancer.

*TX, NX or MX is used if tumour, nodal or metastases status is unknown.

²Age of onset is marked within parentheses where available.

³Positive and negative mutation status marked by + or — respectively.
Table 3. Clinical characteristics of LoF mutation carriers

| Clinical characteristic | No. | % | No. | % | LoF vs non-carriers (P-value) |
|-------------------------|-----|---|-----|---|-----------------------------|
| Age (years)             |     |   |     |   |                             |
| Median                  | 58.5|   | 41–71| | 59                           | 47–82| 0.334 |
| PSA at diagnosis (ng ml⁻¹) |     |   |     |   |                             |
| Median                  | 11.1|   | 3.09–91.12| | 8.25                         | 0.04–259| 0.156 |
| Gleason score           |     |   |     |   |                             |
| Gleason ≤6              | 5   | 35.71| 62 | 44.29| 0.312 |
| Gleason 7               | 2   | 14.29| 27 | 19.29|     |
| Gleason ≥8              | 3   | 21.43| 15 | 10.71|     |
| Unknown                 | 4   | 28.57| 36 | 25.71|     |
| Tumour stage            |     |   |     |   |                             |
| T1                      | 3   | 21.43| 38 | 27.14| 0.476 |
| T2                      | 4   | 28.57| 45 | 32.14|     |
| T3                      | 2   | 14.29| 25 | 17.14|     |
| T4                      | 1   | 7.14 | 2  | 1.43 |     |
| TX                      | 4   | 28.57| 31 | 22.14|     |
| Nodal stage             |     |   |     |   |                             |
| ND                      | 4   | 28.57| 76 | 54.29| 0.00141|
| NI                      | 3   | 21.43| 1  | 0.71 |     |
| NX                      | 7   | 50.00| 63 | 45.00|     |
| Metastasis              |     |   |     |   |                             |
| MO                      | 7   | 50.00| 74 | 52.86| 0.0431|
| MI                      | 3   | 21.43| 5  | 3.57 |     |
| MX                      | 4   | 28.57| 61 | 43.57|     |
| AJCC prognostic groups  |     |   |     |   |                             |
| I–III                   | 3   | 21.43| 68 | 48.57| 0.00121|
| IV                      | 5   | 35.71| 7  | 5.00 |     |
| Unknown                 | 6   | 42.86| 75 | 46.43|     |

Abbreviations: AJCC = American Joint Committee on Cancer; LoF = loss-of-function mutation; PSA = prostate-specific antigen.

Table 4. LoF mutations by family history of PrCa and other cancers

| No. of PrCa in family | 1 × BrCa/OvCa | 2 × BrCa/OvCa | 3 + BrCa/OvCa | 1 + CoCa | BrCa/OvCa and CoCa | PrCa only | Total |
|-----------------------|---------------|---------------|---------------|----------|--------------------|-----------|-------|
| 3 +                   |               |               |               |          |                    |           |       |
| No. of families       | 24            | 11            | 15            | 47       | 31                 | 63        | 191   |
| LoF genes             | CHEK2, BRCA2, PMS2 | BRCA2 | BRCA1, BRCA2, PALB2 | 2 × ATM | CHEK2, BRCA2, MUTYH | BRIP1 |
|                       | (3 (12.5%))   | 1 (9.1%)      | 3 (20%)       | 2 (4.2%) | 2 (6.5%)           | 2 (3.2%)  |       |

Abbreviations: BrCa = breast cancer; CoCa = colon cancer; LoF = loss of function; OvCa = ovarian cancer; PrCa = prostate cancer.

DISCUSSION

We have analysed the coding sequences of 22 tumour suppressor genes in 191 familial PrCa cases in the United Kingdom and found that 7.3% (14 of 191) of these cases were carriers of a putative LoF mutation. These mutations showed partial segregation with PrCa within the families, which is consistent with previous observations of moderately penetrant genes mutated within families. The eight affected genes represent four DNA damage repair or response pathways: HR and FA, ATM, MMR and BER. The most frequently mutated gene was BRCA2, which is in concordance with previous studies showing BRCA2 as the most strongly associated PrCa predisposition gene identified to date (Kote-Jarai et al, 2011).
There is currently an international targeted PrCa screening study in men with germline mutations in BRCA1 and BRCA2 (IMPACT; Mitra et al., 2011). Our study provides further evidence that ATM, CHEK2, BRCA1 and BRIP1 are also involved in familial PrCa predisposition (Dong et al., 2003; Angélec et al., 2004; Kote-Jarai et al., 2009; Leongamornlert et al., 2012).

Most importantly, we have shown that LoF mutation carriers were more likely to have advanced disease, defined by nodal involvement, metastasis or primary tumour grade of T4 (OR 15.09, 95% CI: 2.95–95.81, P = 0.00164). We have previously shown that BRCA2 LoF mutation carriers have significantly reduced survival and present with a more aggressive disease (Castro et al., 2013); however, even when excluding the BRCA2 LoF mutations, this significant association was preserved. This finding could have important clinical implications as men with deleterious germline mutations in these genes should be considered for more intensive screening and treatment. Furthermore, some of the genes studied here are in the HR repair pathway, in which targeted agents such as poly (ADP-ribose) polymerase inhibitors (PARPi) can be considered.

In addition to the clearly deleterious mutations, we identified several missense variants; 13 of these are predicted deleterious by a consensus of six in silico tools (Supplementary Table S2). Therefore, some of these may be classified in the future as deleterious, and accordingly, our estimated frequency of deleterious mutation presented here is likely to be an underestimate. Previous studies have shown that common SNPs identified by GWAS can be used to stratify cumulative risk of PrCa. We were able to calculate a 25 SNP risk score using the algorithm developed by Macinnis et al. (2011); the distribution of the median scores between of LoF carriers vs non-carriers were 0.223 vs 0.194, respectively, but this was not statistically significant (P = 0.456). This would seem to suggest that, in our sample set, there is no correlation between putative LoF carrier status and PrCa risk score using the common risk SNPs and family history data. This perhaps is not surprising as all our subjects had strong family history and the non-carriers may have additional rare LoF mutations in genes not tested here.

To further investigate the association of LoF carrier status with additional breast cancer family history, we applied the BOADICEA model, commonly used in clinical practice to predict the probability that a proband carries a BRCA1 or BRCA2 mutation. BOADICEA models the genetic susceptibility to disease using only the LoF mutation carriers only in terms of the effects of BRCA1 and BRCA2. This model predicted that 8.6% of our probands (15 of the 175 cases that were possible to be scored) had a combined BRCA1 and BRCA2 mutation carrier probability ≥ 10% and therefore would have been recommended to have genetic testing in the United Kingdom under the upcoming (Q3 2013) National Institute for Health and Care Excellence (NICE) guidelines for familial breast cancer. Of the 14 men with a LoF mutation, only three (PR54, 6 and 7; Table 2) of the five BRCA1/2 mutation carriers would have been eligible for genetic testing in the United Kingdom based on these new guidelines (Supplementary Figures S1–14). On the basis of our results, we would therefore recommend that in the future a panel of DNA repair genes should be tested in PrCa families with 3 or more PrCa cases.

The limitations of this study include the lack of events to allow overall survival analysis and the lack of functional evidence to enable the characterisation of the potentially pathogenic missense mutations. Nonetheless, our results suggest that mutations in a wider range of DNA repair genes, other than BRCA2, predispose to PrCa and that such cases are more likely to have advanced disease. Therefore, this warrants further investigation of an expanded set of genes within these pathways in a larger sample set. Also, we highlight that current genetic testing criteria would only have identified 3 of the 14 LoF carriers with breast cancer family history, and therefore current PrCa testing guidelines are likely to be inadequate in the era of personalised genomic medicine.

To our knowledge, this is the first study to apply second-generation sequencing to screen for germline mutations in multiple DNA repair genes in a familial PrCa cohort. We identified frequent deleterious mutations in these genes and the mutation carriers were more likely to present with advanced disease. These findings present strong evidence that genes in DNA repair pathways are good candidates for PrCa predisposition. The clinical utility of these and future findings within these pathways should become increasingly important as targeted screening (such as is undertaken in IMPACT; Mitra et al., 2011) and targeted therapies such as PARPi (Sandhu et al., 2013) become more widespread. If we can more effectively screen these men, clinicians can potentially offer more tailored screening, staging and treatment pathways.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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