Determining the frequency of pathogenic germline variants from exome sequencing in patients with castrate-resistant prostate cancer

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ABSTRACT

Objectives: To determine the frequency of pathogenic inherited mutations in 157 select genes from patients with metastatic castrate-resistant prostate cancer (mCRPC).

Design: Observational.

Setting: Multisite US-based cohort.

Participants: Seventy-one adult male patients with histological confirmation of prostate cancer, and had progressive disease while on androgen deprivation therapy.

Results: Twelve patients (17.4%) showed evidence of carrying pathogenic or likely pathogenic germline variants in the ATM, ATR, BRCA2, FANCL, MSR1, MUTYH, RB1, TSHR and WRN genes. All but one patient opted in to receive clinically actionable results at the time of study initiation. We also found that pathogenic germline BRCA2 variants appear to be enriched in mCRPC compared to familial prostate cancers.

Conclusions: Pathogenic variants in cancer-susceptibility genes are frequently observed in patients with mCRPC. A substantial proportion of patients with mCRPC or their family members would derive clinical utility from mutation screening.

Trial registration number: NCT01953640; Results.

INTRODUCTION

Prostate cancer is the most prevalent cancer in men and is the second leading cause of cancer deaths in men.1 Localised prostate cancer (PC) has an excellent survival rate after 10 years.2 However, 10–20% of men3 4 will develop metastatic hormone-sensitive PC followed by metastatic castrate-resistant PC (mCRPC). The initial treatment for hormone-sensitive disease is hormonal ablation, which is successful in controlling disease progression before castrate resistance emerges in the vast majority of patients. The median overall survival of mCRPC in 2004 was observed to be 18 months.4 Recently, progress has been made and this survival has doubled since 2004.5 While many factors contribute to the development of PC and progression to mCRPC, evidence suggests that pathogenic germline variants in known cancer predisposition genes such as BRCA2 can increase the risk of developing PC up to fivefold.6 Moreover, men with predisposing variants in BRCA2 present clinically with more aggressive stage and grade,7 leading to decreased survival compared with non-carriers.8 Beyond BRCA2, panel-based screening of 191 men with familial PC identified pathogenic truncating mutations in ATM, BRIPL, CHEK2, BRCA1, MUTYH, PALB2 and PMS2 in 7–27% of cases.9 After excluding...
**BRCA2**, loss-of-function mutations were highly enriched in advanced disease, supporting the notion that multiple genes are involved in the hereditary pathogenesis of advanced PC.

Currently, it is not routinely recommended that patients diagnosed with PC have genetic testing, without some other mitigating factor such as a personal or family history of cancer, since the frequency of pathogenic **BRCA2** variants is low (<1%) in unscreened populations. Founder mutations, however, drive allele frequencies higher in Ashkenazi Jewish (2.9%) and Icelandic populations (5.6%). Current National Comprehensive Cancer Network (NCCN) guidelines suggest screening for **BRCA1** and **BRCA2** mutations (also known as hereditary breast and ovarian cancer (HBOC) syndrome) if they present with a high Gleason score (>7) and there is a close relative with breast (<50 years), ovarian, pancreas or prostate cancer (also Gleason score >7) (http://www.nccn.org/).

In contrast to PC, very little is known about the prevalence of inherited pathogenic germline variants in patients who have progressed to mCRPC stage after initial treatments. To our knowledge, only one study has investigated the contribution of germline mutations in the mCRPC setting from comprehensive next-generation sequencing. In it, Robinson et al made the observation that 1% of mCRPC cases had germline mutations in ATM and 6% carried mutations in **BRCA2**. No mention was made of inherited mutations in other known cancer predisposition genes.

In this study, we evaluated germline variants in men participating in the Prostate Cancer Medically Optimized Genome-Enhanced Therapy (PROMOTE) study at Mayo Clinic. The primary aim of PROMOTE is to identify molecular signatures of resistance and response to a standard hormonal therapy in mCRPC. Another objective, which is presented here, is to investigate the frequency of pathogenic germline variants in men with mCRPC and develop a protocol for return of clinically actionable results.

**METHODS**

**Patient eligibility**

Seventy-one male patients were selected if they were at least aged 18 years with histological confirmation of PC and had progressive disease while on androgen deprivation therapy (see Table 1). Patients were recruited from three Mayo Clinic sites (Rochester MN, Jacksonville FL and Scottsdale AZ). All studies were conducted in accordance with the Institutional Review Board at Mayo Clinic and after obtaining patient consent.

**DNA sequencing analysis**

Genomic DNA was extracted from peripheral blood mononuclear cells using the QIAGEN FlexiGene chemistry on an AutoGen Flex Star. The red cells were lysed to allow the white cells to be pelleted by centrifugation. Pellets were lysed and treated with protease to remove proteins from the sample. DNA was precipitated out with isopropanol and washed with 70% ethanol. DNA was then resuspended in Tris-EDTA (TE) buffer and quantified using a spectrophotometer.

Paired-end libraries were prepared following the manufacturer’s protocol (Agilent) using the Bravo liquid handler (Agilent). The concentration and size distribution of the libraries was determined on an Agilent Bioanalyzer DNA 1000 chip. Whole exome capture was performed using a modified Agilent V4+UTR kit, which spans 71.45 Mb of coding exons, plus an intronic region tiling of the androgen receptor gene. Concentration and size distribution of the libraries was determined on Qubit (Invitrogen) and Agilent Bioanalyzer DNA 1000 chip for quality control purposes. Exome libraries were loaded one sample per lane onto Illumina TruSeq v3 paired-end flow cells at concentrations of 9 pM following Illumina’s standard protocol using the Illumina cBot and TruSeq rapid paired-end cluster kit V3.

On average, 200–300 million 100 bp paired-end reads were sequenced per sample. A sample passed initial quality control if more than 90% of the capture region was covered at a minimum of ×20 and more than 70% of the capture kit covered at ×50. Unless otherwise specified, all bioinformatics tools were run under default configuration. Reads were aligned to the hg19 reference genome using Novoalign (http://novocraft.com; VN: V2.07.13) with the following options: -hdrhd off -v 120.
-4 i PE 425,80 x 5 1 Random. Realignment and recalibration was performed using GATK (VN: 2.7-4-g6f46d11) Best Practices V.3. Germline variations were called with GATK’s UnifiedGenotyper.

**Variant annotation and prioritisation**

SAVANT (VN:1.1.0) was used to annotate the functional impact of variants. Using the BioR toolkit, several annotation sources were also added to aid in filtering/prioritising genetic variants. Common variants were excluded if they were seen at more than 1% allele frequency in the Exome Sequencing Project (ESP) data. Prioritising genetic variants. Common variants were excluded if they were seen at more than 1% allele frequency in the Exome Sequencing Project (ESP) database. Variants were then further restricted to a subset of 157 target genes (see online supplementary table S1) associated with hereditary cancer risk. Any variant that was reported as a ‘disease-causing mutation’ in the Human Gene Mutation Database (HGMD), reported as pathogenic in ClinVar or was predicted to be protein truncating (frameshift, nonsense or splice site variants) underwent comprehensive review by a certified genetic counsellor. This included review of allele frequency data, variant databases, literature and in silico predictions. Each variant was classified as benign, likely benign, likely pathogenic, pathogenic or unknown significance in accordance with American College of Medical Genetics (ACMG) guidelines.

**Return of results**

Return of results was offered to patients with a pathogenic or likely pathogenic variant in one of the genes that was determined to be medically actionable, which was defined as having existing medical management guidelines. Return of results was not considered for childhood-onset conditions, autosomal recessive conditions (as it was not possible to determine whether two variants identified within a gene were in cis or in trans) or carrier status for autosomal recessive conditions. Patients were eligible for return of results if they were not already aware of the result through clinical testing and if they had opted in to receive results on the consent form at the time of enrolment.

Patients eligible for return of results were contacted by a study coordinator and offered a telephone or in-person consultation with a certified genetic counsellor for discussing risks, benefits and limitations of receiving germline research results. A second consultation with a certified genetic counsellor was then offered for results disclosure. Given that the results were generated in a research laboratory, if patients had elected to receive results, confirmatory testing in a Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory would have been recommended prior to using the results to make medical management decisions.

**RESULTS**

Between June 2013 and August 2014, 71 patients with mCRPC were recruited in the study. Germline variant analysis was completed on 69 patients. Table 1 highlights the demographics of the study cohort. No significant associations were found between having a pathogenic or likely pathogenic variant and the age at primary disease, age at androgen insensitivity or metastasis. There was also no association between having a pathogenic or likely pathogenic variant and time to progression between primary diagnosis and castrate resistance (p=0.26, Student’s t test) or between castrate resistance and metastasis (p=0.83, Student’s t test). We did not observe any significant associations related to the histology and pathogenic variant status, or family history (defined as other first-degree relative with cancer). One sample was identified as adenocarcinoma but did not have enough tumour (<5%) for identifying Gleason score (table 2).

In all, 12 pathogenic or likely pathogenic variants were identified in nine genes: ATM, ATR, BRCA2, FANCL, MSRI, MUTYH, RBL, TSER, and WRN. ATM and BRCA2 were the only genes mutated in more than one patient. All of the pathogenic variants identified were previously labelled as pathogenic/disease causing by either ClinVar or HGMD, except for the ATR, MSRI, RBL and WRN variants. None of these variants have been shown to be directly linked to mCRPC but are marked enriched for other cancer types.

**Return of results**

At the initiation of the study, 68/69 participants opted in to receive clinically actionable germline results on the consent form at the time of enrolment. Of the 12 pathogenic or likely pathogenic variants, only three were considered medically actionable and met the criteria for return of results (all in BRCA2). These patients were recontacted by a study coordinator to offer a consultation with a genetic counsellor for results disclosure. One patient changed his mind and verbally opted out of knowing his results. The second patient proceeded with an initial consultation with a genetic counsellor and planned to discuss the information with his family prior to deciding whether or not to proceed with results disclosure. The third patient’s daughter called to ask questions about what the genetic counselling appointment entailed. After speaking with the genetic counsellor for more information, she indicated her interest in proceeding with a genetic consultation with her father. After 10 months, neither of these two patients returned for results disclosure.

A family history of cancer was documented in the medical records of all three patients. The first patient reported a brother who died of PC, six female relatives with postmenopausal breast cancer and a niece with uterine cancer. This was report of a ‘cancer gene’ in the family, but further details were not available. The second patient reported ‘a history of pancreas cancer’ in his family, but further details were not available. Finally, the third patient reported that his mother, brother and son had been diagnosed with colon cancer, a son had...
Table 2  Pathogenic and likely pathogenic germline variants in patients with mCRPC

<table>
<thead>
<tr>
<th>Gene</th>
<th>Transcript</th>
<th>Variant</th>
<th>Protein effect</th>
<th>PROMOTE classification</th>
<th>Pathogenic in ClinVar</th>
<th>Disease causing in HGMD</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATM</td>
<td>NM_000051.3</td>
<td>c.7271T&gt;G; p.Val2424Gly</td>
<td>Missense</td>
<td>Pathogenic</td>
<td>Yes</td>
<td>Yes</td>
<td>Ataxia telangiectasia</td>
</tr>
<tr>
<td>ATM</td>
<td>NM_000051.3</td>
<td>c.3245_3247delinsTGAT; p.His1082LeufsX14</td>
<td>Frameshift</td>
<td>Pathogenic</td>
<td>Yes</td>
<td>Yes</td>
<td>Ataxia telangiectasia</td>
</tr>
<tr>
<td>ATR</td>
<td>NM_001184.3</td>
<td>c.4957C&gt;T; p.Arg1653X</td>
<td>Stop gained</td>
<td>Likely pathogenic</td>
<td></td>
<td></td>
<td>Familial cutaneous telangiectasia and cancer syndrome</td>
</tr>
<tr>
<td>BRCA2</td>
<td>NM_000059.3</td>
<td>c.469_470delAA; p.Lys157ValfsX25</td>
<td>Frameshift</td>
<td>Likely pathogenic</td>
<td>Yes</td>
<td>Yes</td>
<td>Hereditary breast/ovarian cancer syndrome</td>
</tr>
<tr>
<td>BRCA2</td>
<td>NM_000059.3</td>
<td>c.6444dupT; p.Ile2149TyrfsX2</td>
<td>Frameshift</td>
<td>Pathogenic</td>
<td>Yes</td>
<td>Yes</td>
<td>Hereditary breast/ovarian cancer syndrome</td>
</tr>
<tr>
<td>BRCA2</td>
<td>NM_000059.3</td>
<td>c.9513_9516delACTT; p.Leu3172AlafsX44</td>
<td>Frameshift</td>
<td>Pathogenic</td>
<td>Yes</td>
<td></td>
<td>Hereditary breast/ovarian cancer syndrome</td>
</tr>
<tr>
<td>FANCL</td>
<td>NM_001114636.1</td>
<td>c.1111_1114dupATTA; p.Thr372AsnfsX13</td>
<td>Frameshift</td>
<td>Likely pathogenic</td>
<td></td>
<td></td>
<td>Fanconi anaemia</td>
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<tr>
<td>MSR1</td>
<td>NM_138715.2</td>
<td>c.183delT; p.Phe61LeufsX10</td>
<td>Frameshift</td>
<td>Likely pathogenic</td>
<td></td>
<td></td>
<td>Hereditary prostate cancer</td>
</tr>
<tr>
<td>MUTYH</td>
<td>NM_001128425.1</td>
<td>c.1187G&gt;A; p.Gly396Asp</td>
<td>Missense</td>
<td>Pathogenic</td>
<td>Yes</td>
<td>Yes</td>
<td>MUTYH-associated polyposis</td>
</tr>
<tr>
<td>RB1</td>
<td>NM_000321.2</td>
<td>c.1960+1G&gt;C</td>
<td>Splice donor</td>
<td>Likely pathogenic</td>
<td></td>
<td></td>
<td>retinoblastoma</td>
</tr>
<tr>
<td>TSHR</td>
<td>NM_000369.2</td>
<td>c.1349G&gt;A; p.Arg450His</td>
<td>Missense</td>
<td>Pathogenic</td>
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<td></td>
<td>Thyroid carcinoma</td>
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<tr>
<td>WRN</td>
<td>NM_000553.4</td>
<td>c.525delG; p.Trp175X</td>
<td>Frameshift</td>
<td>Likely pathogenic</td>
<td></td>
<td></td>
<td>Werner syndrome</td>
</tr>
</tbody>
</table>

Bolded genes are associated with increased prostate cancer risk.
HGMD, Human Gene Mutation Database; mCRPC, metastatic castrate-resistant prostate cancer; PROMOTE, Prostate Cancer Medically Optimized Genome-Enhanced Therapy.
bone cancer and a sister had pancreatic cancer. While all three patients would likely have fulfilled NCCN criteria for HBOC testing, there was no documentation of referrals to medical genetics, consultations with medical genetics or genetic testing in the patients’ medical records.

**DISCUSSION**

Here we report our findings of inherited pathogenic mutations in known cancer predisposition genes from the exome sequences of 69 mCRPC cases. We observed a high frequency of pathogenic germline variants in patients with mCRPC (17.4%; 12/69). Our finding is consistent with a recent report in which germline mutations in BRCA2 were found in 5.3% of 150 mCRPC cases. Mutation frequencies reported here are also similar to those reported for ATM (2/69, 2.9% vs 2/150, 1.3%) and BRCA1 (0/69, 0% vs 1/150, 0.6%) genes, although our small sample size prevents a robust estimate of true allele frequencies in this population.

Clear links have established BRCA2 as a predisposition gene for PC. However, the frequency of pathogenic variants in BRCA2 is low—on the order of 0.5–1% in unselected populations. When we compare the frequency of pathogenic BRCA2 variants with PC from non-mCRPC patients, the enrichment is striking. The pathogenic BRCA2 variant frequency (4.2%) in patients with mCRPC is much higher than has been reported in the context of familial PC. In a large study of 266 familial PC cases, pathogenic BRCA2 variants were not observed (p<0.01, Fisher’s exact test), although smaller studies (22 families) have observed mutation frequencies near 5%. We also observed a higher frequency of pathogenic BRCA2 variants in patients with mCRPC compared with early-onset PC cases (p<0.06, Fisher’s exact test). In a study of 1589 men diagnosed with PC under age 65, only 1.2% were found to have pathogenic BRCA2 mutations.

**Other genes**

Excluding the ATM and BRCA2 genes, MUTYH is the only other gene that has previously been identified in these cohorts. Leongamornlert et al. found one stop-gain mutation in 191 individuals with familial PC, with an enrichment in metastatic cases. MUTYH mutation carriers are not thought to be at increased risk for PC, at least in the case of individuals with a family history of colorectal cancer. Even less is known about the role of ATR, FANCL, MSRI, MUTYH, RBR, TSHR and WRN in PC or mCRPC.

Finally, we note that the three patients with clinically actionable BRCA2 mutations would likely have met NCCN criteria for HBOC screening but did not undergo medical genetic consultations and/or testing previously. It is unknown if genetic screening and counselling is being offered in clinical practice as often as recommended by national guidelines to families with a history of multiple cancers. While interesting, the small sample size precludes us from determining whether or not this is significant; thus, more studies in this area are warranted.

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**Contributors** MSE, TMK and KS classified and reviewed variants. SNH, PTV, HS, LW and KRK were responsible for the DNA sequencing and informatics. REC, JPS, PB, JEE-P and RQ coordinated the statistical analysis. WT, MK and AHB led the patient recruitment effort. The entire study was supervised and directed by RW, LW and MK. SNH, MSE and MK wrote the paper.


**Competing interests** None declared.

**Ethics approval** Institutional Review Board.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Data sharing statement** No additional data are available.

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