

# BMJ Open 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.<sup>1</sup> Localised prostate cancer (PC) has an excellent survival rate after 10 years.<sup>2</sup> However, 10–20% of men<sup>3 4</sup> 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

## Strengths and limitations of this study

- We describe the results of germline testing from a large cohort in the metastatic castrate-resistant prostate cancer setting, a population often overlooked for inherited mutation screening.
- These results have implications for the patient and their family members who may also be carrying the same genetic mutations.
- In lieu of a traditional replication set, we relied on previous estimates of mutation frequencies in this population which support our initial findings.
- Even though the entire exome was sequenced, we only analysed data from 157 genes with known association with inherited cancer risk, so there may be other functionally relevant mutations not discussed here.

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.<sup>4</sup> Recently, progress has been made and this survival has doubled since 2004.<sup>5</sup> 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.<sup>6</sup> Moreover, men with predisposing variants in *BRCA2* present clinically with more aggressive stage and grade,<sup>7</sup> leading to decreased survival compared with non-carriers.<sup>8</sup> Beyond *BRCA2*, panel-based screening of 191 men with familial PC identified pathogenic truncating mutations in *ATM*, *BRIP1*, *CHEK2*, *BRCA1*, *MUTYH*, *PALB2* and *PMS2* in 7–27% of cases.<sup>9</sup> 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 unselected populations.<sup>10</sup> Founder mutations, however, drive allele frequencies higher in Ashkenazi Jewish (2.9%)<sup>11</sup> and Icelandic populations (5.6%).<sup>7</sup> 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 ( $\geq 7$ ) and there is a close relative with breast ( $\leq 50$  years), ovarian, pancreas or prostate cancer (also Gleason score  $\geq 7$ ) (<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.<sup>12</sup> 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.

**Table 1** Descriptive statistics of the cohort

	Deleterious mutation		p Value
	Yes	No	
Age			
At primary diagnosis	64	66	0.047
At ADT	69.4	69.5	0.958
At the development of mCRPC	73.9	73.7	0.923
Family history			
Yes	10	45	0.785*
No	3	11	
Histology			
Adenocarcinoma	5	28	0.877†
Poorly differentiated carcinoma NOS	1	8	
Small-cell carcinoma	0	1	
Unknown	7	20	
Gleason score			
7	0	2	0.054*
8	0	15	
9	2	6	
10	4	13	
NS	7	21	

\*Linear model ANOVA.

†Pearson's  $\chi^2$  test.

ADT, androgen deprivation therapy; mCRPC, metastatic castrate-resistant prostate cancer; NS, not scored.

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 V.3.

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  $\times 20$  and more than 70% of the capture kit covered at  $\times 50$ . 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`

-c 4 -i PE 425,80 -x 5 -r 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,<sup>13</sup> 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) database.<sup>14</sup> 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),<sup>15</sup> reported as pathogenic in ClinVar<sup>16</sup> 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.<sup>17</sup>

### 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 discussion of 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*, *RBI*, *TSHR* 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*, *RBI* and *WRN* variants. None of these variants have been shown to be directly linked to mCRPC but are markedly 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. There 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

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

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.<sup>12</sup> 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.<sup>10</sup> 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%.<sup>18–19</sup> 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.<sup>20</sup>

## 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*<sup>9</sup> 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.<sup>21</sup> Even less is known about the role of *ATR*, *FANCL*, *MSR1*, *MUTYH*, *RBI*, *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.

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