original reports

Pathogenic Germline DNA Repair Gene and *HOXB13* Mutations in Men With Metastatic Prostate Cancer

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abstract

PURPOSE Germline mutations in DNA repair (DR) genes and susceptibility genes *CDKN2A* and *HOXB13* have previously been associated with prostate cancer (PC) incidence and/or progression. However, the role and prevalence of this class of mutations in metastatic PC (mPC) are not fully understood.

PATIENTS AND METHODS To evaluate the frequency of pathogenic/likely pathogenic germline variants (PVs/LPVs) in men with mPC, this study sequenced 38 DR genes, *CDKN2A*, and *HOXB13* in a predominantly white cohort of 317 patients with mPC. A PC registry at the University of Utah was used for patient sample acquisition and retrospective clinical data collection. Deep target sequencing allowed for germline and copy number variant analyses. Validated PVs/LPVs were integrated with clinical and demographic data for statistical correlation analyses.

RESULTS All pathogenic variants were found in men self-reported as white, with a carrier frequency of 8.5% (DR genes, 7.3%; *CDKN2A*/*HOXB13*, 1.2%). Consistent with previous reports, mutations were most frequently identified in the breast cancer susceptibility gene *BRCA2*. It was also found that 50% of identified PVs/LPVs were categorized as founder mutations with European origins. Correlation analyses did not support a trend toward more advanced or earlier-onset disease in comparisons between carriers and noncarriers of deleterious DR or *HOXB13* G84E mutations.

CONCLUSION These findings demonstrate a lower prevalence of germline PVs/LPVs in an unselected, predominantly white mPC cohort than previously reported, which may have implications for the design of clinical trials testing targeted therapies. Larger studies in broad and diverse populations are needed to more accurately define the prevalence of germline mutations in men with mPC.

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INTRODUCTION

Despite improvements in the diagnosis and treatment of prostate cancer (PC), it remains the most common cancer diagnosed in US men and is the second leading cause of cancer-related death.[1] Major recognized risk factors for PC include increasing age, African heritage,[2,3] and family history of PC.[4] It was previously determined that PC risk is elevated in families with hereditary breast and ovarian cancer (HBOC) because of deleterious mutations in *BRCA1* and *BRCA2*.[5,6] Similarly, there is evidence of elevated PC risk in families with hereditary nonpolyposis colorectal cancer or Lynch syndrome.[7,8]

Although there is strong evidence for heritability in PC, few moderate to highly penetrant gene mutations that contribute to PC susceptibility have been identified. Linkage analysis and candidate gene studies led to the identification of a recurrent mutation (G84E) in the *HOXB13* gene that increases risk of PC[9,10] and contributes to approximately 5% of hereditary PCs worldwide.[11] In a meta-analysis of US PC cases (n = 9,461) and controls (n = 5,039), *HOXB13* G84E carriers had a statistically significantly increased risk of developing PC compared with noncarriers (odds ratio, 5.10; 95% CI, 3.21 to 8.10; $P < .00001$).[12] Recent studies of men with PC have also frequently found a correlation between mutations in DNA repair (DR) genes and an elevated risk of development of metastatic PC (mPC) and/or lethal PC.[13-15] In a study of 7 castration-resistant mPC (mCRPC) cohorts, presumed pathogenic germline variants were found highly enriched in DR genes, occurring at a frequency of 11.8%.[14]

In this study, we evaluated the prevalence and possible associated clinical phenotypes of pathogenic/likely pathogenic variants (PVs/LPVs) in DR genes, *CDKN2A*, and *HOXB13* in 317 men treated for mPC.

ASSOCIATED CONTENT

Appendix

Author affiliations and support information (if applicable) appear at the end of this article.

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Metastatic disease characteristics in this cohort included both de novo mPC and mPC progression after diagnosed biochemical recurrence.

PATIENTS AND METHODS

Patient Cohort

The study cohort comprised 317 men who were currently being treated for mPC at the University of Utah Huntsman Cancer Institute (n = 166) or who had been treated but had since died (n = 151). Cohort demographic and baseline clinical characteristics are summarized in Table 1. Patient selection was based on mPC disease state and was agnostic to age at diagnosis, ethnic background, and known familial predisposition to cancer. Clinical data for all patients in the cohort were extracted from the electronic medical record and included age at diagnosis, Gleason score, and baseline and follow-up laboratory results, including prostate-specific antigen (PSA) level. All aspects of the study were approved by the University of Utah Institutional Review Board. Because family history of cancer was not included in the patient clinical data, patients with mPC were identified in the Utah Population Database (UPDB) for available genealogy data.[16]

TABLE 1. Baseline Cohort Demographic and Clinical Characteristics (N = 317)

Characteristic	No. (%)
Age at mPC diagnosis, years	
Median	70
IQR	64-77
Age at death, years (n = 146)	
Median	75
IQR	68-82
Race	
Non-Hispanic white	306 (96.5)
Hispanic	4 (1.3)
Non-Hispanic black	1 (0.3)
Asian or Pacific Islander	5 (1.6)
Other	1 (0.3)
Gleason score (n = 293)	
≤ 6	36 (12.3)
7	74 (25.3)
8-10	183 (62.5)
Disease type	
mPC with no BR diagnosis	171 (54)
BR progression to mPC	146 (46)
PSA at diagnosis, ng/mL (n = 280)	
Median	16.1
IQR	6.8-87.0

NOTE. Clinical stratification data not available for all patients.
Abbreviations: BR, biochemical recurrence; IQR, interquartile range; mPC, metastatic prostate cancer; PSA, prostate-specific antigen.

Sequencing and Bioinformatic Processing

Genomic DNA extracted was from peripheral whole blood samples obtained from 317 patients with mPC. A custom QIAseq Targeted DNA Panel (QIAseq V3; Qiagen, Hilden, Germany) was designed for full exon and splice site coverage with or without 2 bp of 40 cancer-associated genes, including 38 DR genes, *CDKN2A,* and *HOXB13* (Appendix Table A1). Next-generation sequencing libraries were built using the Qiagen GeneRead DNA I Amp Kit in accordance with manufacturer instructions. Targeted sequencing was performed on an Illumina HiSEquation 2500 (San Diego, CA) in accordance with Illumina standard protocol.

Sequencing results were aligned to GRCh37 and post-processed following recommendations from GATK Best Practices.[17] Fastq reads and alignments were processed with custom scripts[18] for extracting and deduplicating alignments based on the QIAseq unique molecular indexes. Variants were called with GATK HaplotypeCaller and annotated using Ensembl Variant Effect Predictor.[19] Mean target read depth coverage across all targets was approximately 350×, with the exception of *ATR* exons 31 to 34, which failed to amplify across all samples and were excluded from analysis.

To identify putative large indels (> 40 bp), per-amplicon mean read depth data were generated for all passing samples via alignment against amplicon bed file targets. Cross-sample, cross-target normalizations were performed, and normalized read depth data were reviewed for targets with ≥ 200× coverage for significant differences from mean target depth of coverage (standard deviation, ± 4). The copy number variation detection process was developed and executed internally.

Variant Classification and Validation

Preliminary selection of candidate PVs/LPVs included the following classification resources: American College of Medical Genetics and Genomics (ACMG) PVS1 null variants (nonsense, frameshift, splice site ± 2bp of exon boundaries, initiation codon, and single- or multiexon deletion),[20] ClinVar classifications,[21] and REVEL missense scores ≥ 0.5 (sensitivity, 76%; specificity, 89%).[22] High- and moderate-penetrance variants with preliminary classification as PVs/LPVs were submitted to genetic counselors (GCs; W.K. and S.E.G.), who evaluated conflicting interpretations and identified the classification with the strongest clinical relevance. All verified PVs/LPVs occurred in genes included on the ACMG list and/or in the National Comprehensive Cancer Network guidelines on HBOC and colorectal cancer (version 1.2018; Table 2). Whether carrier status of these PVs/LPVs currently influences cancer preventative care and/or treatment is indicated in the first column of Table 2 (data provided by GCs). The Non-Finnish European (NFE) Genome Aggregation Database (gnomAD;

TABLE 2. PVs/LPVs (n = 28) in Patients With mPC (n = 27)[a]

Clinical	Gene	dbSNP or ClinVar ID	Allele Change	Amino Acid Change	Consequence	ClinVar	No.	ExAC MAF
PC, T	*ATM*	rs869312756	c.5762+1G>A	—	SV	LP	1	—
PC, T	*BRCA1*	rs80357662	c.1556delA	p.Lys519ArgfsTer13	FS	P	2	—
PC, T	*BRCA1*	rs397507247	c.5329dupC	p.Gln1777ProfsTer74	FS	P, RF	1	0.00016
PC, T	*BRCA2*	rs80359636	c.7069_7070delCT	p.Leu2357ValfsTer2	FS	P	2[b]	0.00003
PC, T	*BRCA2*	rs80359306	c.1813dupA	p.Ile605Asnfs	FS	P	2	—
PC, T	*BRCA2*	rs80359604	c.658_659delGT	p.Val220IlefsTer4	FS	—	1	0.00005
PC, T	*BRCA2*	rs80359405	c.3847_3848delGT	p.Val1283LysfsTer2	FS	P	1	0.00009
PC	*CDKN2A*	rs587780668	c.9_32dup	p.Ala4_Pro11dup	DUP	LP, P	1	—
PC	*CHEK2*	rs555607708	c.1100delC	p.Thr367Metfs	FS	P	5	0.00177
T	*FANCA*	rs756367276	c.2839dupT	p.Ser947PhefsTer4	FS	LP, P	1	0.00001
PC, T	*PMS2*	RCV000581876	c.1120C>T	p.Gln374Ter	NS	P	1	—
	ATR	rs756702410	c.3709delC	p.Leu1237SerfsTer2	FS	—	1	
	BARD1	2:215595194_-/ GAAGAAAAGTATGTGAACAG	c.1922_1941dup20	p.Arg641Argfs	FS	—	1	0.00008
	BLM	RCV000528231	c.1129delG	p.Glu377SerfsTer6	FS	P	1	—
	BLM	rs200389141	c.1642C>T	p.Gln548Ter	NS	P	1	0.00018
	HOXB13	rs138213197	c.251G>A	p.Gly84Glu	MS (0.45)	P, LP, RF	3	0.00215
	MUTYH	rs34612342	c.527A>G	p.Tyr176Cys	MS (0.96)	P	1	0.00157
	MUTYH	rs36053993	c.1178G>A	p.Gly393Asp	MS (0.95)	P	1	0.00278
	MUTYH	rs34126013	c.712C>T	p.Arg238Trp	MS (0.79)	LP, P	1	0.00009

NOTE. Pathogenicity rating supported by National Comprehensive Cancer Network, expert, or other consensus evidence for increased cancer risk. Indicated variants may influence preventative care (PC) and/or treatment (T; column 1).

Abbreviations: dbSNP, Single Nucleotide Polymorphism Database; DUP, duplication; ExAC, Exome Aggregation Consortium; FS, frameshift; LP, likely pathogenic; MAF, minor allele frequency; mPC, metastatic prostate cancer; MS, missense (revel score); NS, nonsense; P, pathogenic; PV/LPV, pathogenic/likely pathogenic germline variant; RF, risk factor; SV, splice variant.

[a]One patient carried 2 PV/LPVs: *BARD1* p.Arg641Argfs and *CHEK2* p.Thr367Metfs.

[b]*BRCA2* p.Leu2357ValfsTer2 carriers were first cousins.

groups contributing exome and genome variant data to this resource are provided online[23]) cohort[24] was used for variant minor allele frequency (MAF) comparisons. Additional variants of interest (VOIs) were identified based on the classification pipeline used for the mPC PVs/LPVs and GC feedback. To assist with classification, VOIs were additionally annotated and stratified with scores generated through wAnnovar[25] and BayesDel[26] (if available). VOI retention required a deleterious LRT and/or FATHM score, an average GERP++/SiPhy conservation rank score > 0.75, and a BayesDel predicted deleterious score. The reported maximum allele frequency reflected the highest MAF found in gnomAD germline populations.[24] Passing VOIs are listed in Appendix Table A2.

All reported PVs/LPVs were validated with Sanger sequencing using custom primers and Invitrogen Platinum Hot Start PCR Master Mix (Carlsbad, CA) for standard targets and Thermo Scientific Phusion Green HS High Fidelity PCR Master Mix (Waltham, MA) for high GC content (amplicon GC base content > 65%) and long-range amplicons > 5 kb.

Statistical Analysis

Prevalence and distribution of PVs/LPVs across the mPC cohort were analyzed for possible correlation between disease characteristics and treatment outcomes in general and in response to androgen-deprivation therapy (ADT). PV/LPV-carrier disease characteristics and clinical outcomes were compared against those of noncarrier patients to determine if underlying genotypes correlated with discrete phenotypes. Comparisons between the carrier and noncarrier groups included serum PSA levels at initial diagnosis using an unequal variance Welch *t* test, age at PC progression landmarks using an unpaired *t* test, and stratified Gleason score burden comparisons using a 2-sided Fisher's exact test. A Pearson's χ^2 test was used to calculate Gleason score percentage overall *P* value. MAF *P* values between PV/LPV carriers and noncarriers were calculated using a 2-sided Fisher's exact test. A *P* value < .05 (2 sided) was considered statistically significant. mPCa MAF significance evaluations were based in part based on The Cancer Genome Atlas Research Network control data.[27]

RESULTS

Identification of Deleterious Variants

To determine the frequency of PVs/LPVs in DR genes and susceptibility genes *CDKN2A* and *HOXB13*, we analyzed the complete coding and with or without 2 splice sites of these regions of interest in 317 patients with mPC. Overall, we identified 28 PVs/LPVs in 27 carriers (1 patient carried 2 PVs/LPVs), resulting in a carrier frequency of 8.5% (DR genes, 7.3%; *CDKN2A*/*HOXB13* G84E, 1.2%; Table 2). *HOXB13* G84E carrier frequency of 0.95% was not significantly enriched in the mPC cohort compared with the NFE genomAD carrier frequency of 0.49% (n = 64,053; $P = .202$).

All identified PVs/LPVs occurred in patients self-reported as white. The functional consequences of these mutations included 22 truncating alterations and 5 single-nucleotide polymorphisms. No copy number variants were validated in qualifying patients. Eighteen (64%) of the 28 total PVs/LPVs currently influenced PC preventative care and/or treatment. Of the genes that harbored deleterious mutations, *BRCA2* and *CHEK2* were the most represented, with overall patient carrier frequencies of 1.9% in *BRCA2* and 1.6% in *CHEK2* (Fig 1).

Additionally, there were 52 VOIs found across 23 sites that did not meet PV/LPV classification requirements but are provided in Appendix Table A2 for reference as possible risk factors. Appendix Table A2 includes a truncating nonsense *BRCA2* variant (p.Lys3326Ter) with a carrier frequency of 2.5%.

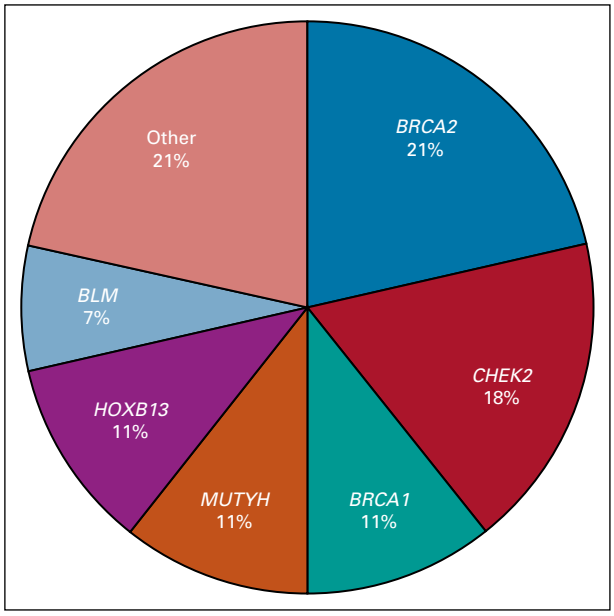

FIG 1. Frequency distribution of genes containing pathogenic/likely pathogenic germline variants (PVs/LPVs). The other category represents genes for which number of PVs/LPVs is n = 1.

Statistical Correlation Analyses

No statistically significant differences in baseline characteristics between PV/LPV carrier and noncarrier patients were observed (Appendix Table A3). Additionally, no significant correlation was found between DR PV/LPV status and response to ADT. In this retrospective mPC case study, in which men received ADT standard care that did not recommend quarterly scans, disease progression during ADT was determined based on PSA progression per Prostate Cancer Working Group 2 criteria, unless radiographic or clinical progression occurred before PSA progression (data not shown). Because of the inherent treatment heterogeneity within a retrospective disease state–based cohort, it is important to note that several patients in our mPC cohort were treated with PARP inhibitors during clinical trials and that 1 patient was treated with olaparib outside of a protocol and experienced clinically meaningful benefit.[28] A cross-study comparison of PV/LPV carrier frequencies in DR genes *ATM*, *BRCA1*, *BRCA2*, *MLH1*, *MSH2*, *MSH6*, *PALB2*, and *RAD51C* in mPC cohorts is reviewed in Appendix Table A4, showing an overall PV/LPV rate of 6.3% (107 of 1691) in men with mPC.

Characterization of Founder Mutations

PVs/LPVs were searched against published founder mutations to further characterize the at-risk carrier patient population through the origins of the mutations. No founder mutations originating outside of Europe were identified, a finding reflective of the self-reported white ethnic background of 97% of the mPC cohort. Of the 28 total PVs/LPVs listed in Table 2, 50% are previously identified and published European founder mutations and include the pathogenic Ashkenazi Jewish founder mutation *BRCA1* n.5382insC (Table 3).

DISCUSSION

In a cohort of men with mPC, predominantly of European ancestry, we observed that 23 (7.2%) of the 317 men harbored ≥ 1 pathogenic variants in DR genes. A direct comparison of the frequency of PVs/LPVs found in the 20 DR genes reported in the Pritchard et al[14] mPC trial against the same 20 genes in our mPC cohort showed a lower occurrence in the mPC cohort (11.8% *v* 5.7%). The DR frequency observed in our mPC cohort was somewhat lower than frequencies reported by 4 comparable PC studies for the 8 DR genes in common across all of the groups (Appendix Table A4). However, the enrichment of rare DR germline mutations within our cohort was consistent with the hypothesis that mutations in DR genes increase mPC risk.

Two factors likely contributing to the lower germline DR mutation frequency in our study are the absence of enrichment for both known family history of cancer and Ashkenazi Jewish founder mutations in *BRCA1* and *BRCA2*. Regarding family history of PC, prior studies have suggested that men with ≥ 1 first-degree relatives who have

TABLE 3. Founder Mutations

Gene	Published Founder ID	Hg19 HGVSp	No.	Origin	Reference
BLM	c.1642C>T	p.Gln548Ter	1	Central and Eastern Europe	58, 59
BRCA1	n.5382insC	p.Gln1777ProfsTer74	1	Central and Eastern Europe[a]	60
BRCA1	n.1675delA	p.Lys519ArgfsTer13	2	Northern Europe[b]	61
CHEK2	c.1100delC	p.Thr367Metfs	5	Northern and Eastern Europe	62
HOXB13	p.G84E	p.Gly84Glu	3	Northern Europe	63
MUTYH	p.G393D	p.Gly393Asp	1	European	64
MUTYH	p.Y176C	p.Tyr176Cys	1	European	64

NOTE. Origins of 50% of metastatic prostate cancer pathogenic/likely pathogenic germline variants are documented founder mutations.
Abbreviation: HGVSp, Human Genome Variation Society description of variant at protein level.
[a]Ashkenazi Jewish.
[b]Norwegian.

been diagnosed with a noncutaneous cancer are more likely to harbor deleterious DR mutations.[14,29] UPDB genealogy data were not available for a majority of men in our cohort (189 [60%] of 317 patients), and just 63% of the men with available UPDB family history were identified as having ≥ 1 first-degree relatives with cancer; therefore, we suspect that our cohort was not highly enriched for this important risk factor. Additionally, although *BRCA1* and *BRCA2* Ashkenazi Jewish founder mutations are important risk factors for oncogenesis, the patient demographics in our mPC cohort reflect the primarily European ancestry of the local aging population, which makes the single Ashkenazi Jewish *BRCA1* founder mutation occurrence in this cohort incidental to our findings. Examples of Ashkenazi Jewish elevated *BRCA1* and *BRCA2* founder mutation carrier frequencies across multiple types of cancer (eg, HBOC, PC, and pancreatic cancer) can be found in numerous publications, including elevated carrier frequencies identified in breast cancer cohorts both with (14.2%[30]) and without (10.3%[31] and 11.7%[32]) a family history of cancer. *BRCA1*/*BRCA2* founder mutation carrier frequencies in unselected Ashkenazi Jewish men with PC have been reported at 3.2% (n = 940) for *BRCA2* only[33] and a combined frequency of 5.2% (n = 251) for *BRCA1* and *BRCA2*,[34] a higher overall *BRCA1*/*BRCA2* carrier frequency than identified in our mPC cohort (2.8% *v* 5.2%).

Known deleterious founder mutations were highly represented in our mPC cohort, representing 50% of the identified PVs/LPVs in the predominantly white mPC cohort (97%). The identified founder mutations were all of European origin. Knowledge of the commonality of DR and *HOXB13* founder mutations in men with mPC has clinical utility, because coupling patient ethnic data with relevant founder mutations and locally enriched population risk factors may increase the success of preliminary germline testing.[35] Importantly, clinically relevant penetrance, risk, and treatment efficacy data are likely to be enriched for founder mutations, because they have been the focus of multiple previous studies.

A particular Northern European founder mutation, *HOXB13* G84E, has a strong correlation with increased lifetime risk of PC[36] and poor prognosis in some studies.[37] The *HOXB13* gene is expressed in the prostate from early developmental stages into adulthood and has been found to affect prostate cell proliferation and differentiation,[38,39] as well as androgen receptor regulation.[40] To quantify family history impact on expected carrier rates, a meta-analysis of *HOXB13* G84E frequencies in European ancestry PC cases was reviewed.[12] It was found that the mutation occurred at a rate of 5.11% (n = 2,272) in PC cohorts with a family history[10,11,41] compared with 0.95% (n = 8,917) in family history–nonselective PC cohorts (> 70% without family history[10,42-44]; $P < .00001$). The observed *HOXB13* G84E carrier rate in this family history–unselective study was found to be in alignment with comparable PC studies. The prevalence of *HOXB13* G84E in this mPC cohort is a reflection of the Northern European genetic background of many historical Utah settlers and may be an important PC risk factor in the local community. No trend toward early-onset disease in age at initial diagnosis or age at metastasis in G84E carriers was observed (data not shown).

It is also well known that deleterious germline mutations in DNA repair gene *BRCA2* increase the risk of prostate oncogenesis. A particular *BRCA2* C-terminus truncating mutation (p.Lys3326Ter) was validated in 8 patients with mPC (carrier frequency, 2.5%; MAF, 0.013), functionally resulting in the loss of the second of 2 nuclear localization signal (NLS) sequences within the C-terminal region. Although p.Lys3326Ter is a truncating mutation in *BRCA2*, it was not included as a PV/LPV in our report because of the current ClinVar classification for hereditary cancer as benign with 1 conflicting PV interpretation.[45] One of the 4 ClinVar benign interpretations concluded that p.Lys3326Ter is a significant but low-risk allele for HBOC (Int. Genetics / Lab. Corp. of America). However, this mutation has been reported in recent publications to be a low- to moderate-risk variant that alters *BRCA2* activity in multiple types of cancer.[45-52] The role of this mutation as

a risk modifier when coupled with a *BRCA1* LPV/PV has also been previously explored; Raffaele et al[53] classified this mutation as a powerful modifier in early-onset neoplasia when coupled with a *BRCA1* PV, reporting increased pathogenicity in heterogeneous carriers. Interestingly, 1 of the *BRCA2* p.Lys3326Ter mutations in the mPC cohort cooccurred with Ashkenazi Jewish founder mutation *BRCA1* p.Gln1777ProfsTer74. Overall, the 2.5% carrier frequency of *BRCA2* p.Lys3326Ter found in the mPC cohort is elevated compared with the genomAD NFE carrier frequency of 1.7% (n = 64,427). However, it is important to note that although *BRCA2* p.Lys3326Ter was enriched in the mPC cohort, the estimated maximum MAF of individual PVs/LPVs occurring in *BRCA2* is approximately 0.003,[54] and the approximately 0.009 NFE gnomAD MAF is above that threshold. A consideration of these data, including an elevated mPC MAF and possible loss of functional efficiency via a diminishment of the NLS, supports a mild-penetrance risk classification for *BRCA2* p.Lys3326Ter for hereditary cancer-predisposing syndrome.

This study has some limitations. First, because the cohort in this study was predominantly of European descent, complementary studies of non-European mPC cohorts would provide more comprehensive DR gene and *HOXB13* distribution and prevalence estimates. Second, statistical comparisons of the clinical data of mPC PV/LPV carriers versus mPC noncarriers were confounded in part by the low number of PV/LPV carriers present (n = 27) and the high degree of heterogeneity of patient disease characteristics and treatment courses. The underlying genotypic and phenotypic diversity in this disease highlights the value of large patient cohorts and more expansive genomic interrogations. Larger-scale analyses generate more comprehensive statistics that translate to more robust mPC preventative care and treatment.

In conclusion, our study provides supporting evidence that deleterious germline mutations in DR genes and *HOXB13* predispose men with European ancestry to mPC. Although the DR mutation frequency was lower than the initial report (11.8% by Pritchard et al[14]), it was consistent with other more recent studies (Appendix Table A4).[14,29,55,56] The combined carrier frequency in the 8 genes shared across these comparable studies (*ATM*, *BRCA1*, *BRCA2*, *MLH1*, *MSH2*, *MSH6*, *PALB2*, and *RAD51C*) is 6.3% (107 of 1,691), decreased from the initial report (8.7%). These findings have implications for the design of clinical trials in men with mPC. The TOPARP trial, a phase II clinical trial evaluating olaparib in men with mCRPC, showed that men with deleterious mutations in a DR gene had high response rates (14 [88%] of 16) to PARP inhibitors.[57] Currently, 4 separate phase II or III clinical trials are evaluating PARP inhibitors in men with mCRPC (ClinicalTrials.gov identifiers: NCT02854436, NCT02975934, NCT02952534, and NCT02952534). Most of the trials evaluating PARP inhibitors in mCRPC are designed using the frequency of DR PVs/LPVs published as observed in a hormone-sensitive mPC/mCRPC cohort.[14] In contrast, our cohort of predominately European descent had a lower frequency of PVs/LPVs at 5.7% in the same 20 genes evaluated by Pritchard et al.[14] If investigators designing a trial overestimate the frequency of deleterious DR mutations, it could lead to methodologic issues that may unexpectedly influence the results of the trial. Overestimation of enrollment could result in an underpowered study at time of closure or a trial that is unable to enroll a sufficient number of patients. Moving forward, our results suggest that investigators evaluating novel therapies in men with deleterious DR mutations should consider the population structure of study sites when designing clinical trials.

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APPENDIX

TABLE A1. QiaSeq V3 Custom Gene Panel With 38 DR Genes, *CDKN2A*, and *HOXB13*

Gene
ATM
ATR
BAP1
BARD1
BLM
BRCA1
BRCA2
BRIP1
CDH1
CDK12
CDKN2A[a]
CHEK1
CHEK2
ERCC5
FAM175A
FANCA
GEN1
HOXB13[b]
MLH1
MRE11A
MSH2
MSH6
MUTYH
NBN
PALB2
PARP3
PARP4
PMS2
POLD1
POLE
RAD18
RAD23B
RAD51B
RAD51C
RAD51D
RAD52
SLX4
TP53
WRN
XRCC2

Abbreviation: DR, DNA repair.
[a]Apoptosis gene.
[b]Gene associated with hereditary cancer-predisposing syndrome.

TABLE A2. Rare VOIs (n = 23) in Patients With mPC (n = 49)

Gene	dbSNP ID	Allele Change	Amino Acid Change	Consequence	ClinVar	BayesDel	BayesDel Cutoff	Cohort AF	Maximum AF
ATM	rs139552233	c.2932T>C	p.Ser978Pro	MS (0.87)	B, VUS	0.0639245	–0.0522746	1	0.005
ATM	rs138327406	c.4388T>G	p.Phe1463Cys	MS (0.76)	B, LB, VUS	0.0898925	–0.0522746	1	0.001
BARD1	rs376727038	c.1339C>G	p.Leu447Val	MS (0.82)	VUS	0.197258	–0.0610607	1	0.00017
BRCA2	rs11571833	c.9976A>T	p.Lys3326Ter	NS	B	0.0232392	–0.0990118	8	0.011
CDH1	rs786201999	c.2302G>A	p.Asp768Asn	MS (0.83)	VUS	–0.0165351	–0.0850884	1	6.5e–5
CDK12	rs138292741	c.1047-2A>G	—	SV	—	—	—	1	0.00044
CHD1	rs61759467	c.2006A>T	p.Glu669Val	MS (0.78)	—	–0.110621	—	9	0.014
CHEK1	rs756054805	c.1135C>T	p.Arg379Ter	NS	US	0.567317	—	1	0.00001
CHEK2	rs864622537	c.1162C>T	p.His388Tyr	MS (0.91)	VUS	0.423116	–0.0131521	1	9.0e–6
CHEK2	rs17879961	c.599T>C	p.Ile200Thr	MS (0.54)	P, LP, RF, US	–0.0906394	–0.0131521	4	0.016
CHEK2	rs72552323	c.608T>C	p.Ile203Thr	MS (0.89)	VUS	0.353499	–0.0131521	1	0.00002
ERCC5	rs756544378	c.13G>A	p.Gly5Arg	MS (0.82)	—	0.367422	—	1	0.00001
ERCC5	13_103519085_C/-	c.2423delC	p.Thr808MetfsTer25	FS	—	—	—	1	—
MLH1	rs35502531	c.1852_1853AA>GC	p.Lys618Ala	MS	—	—	0.20848	3	—
MLH1	3_37059042_T/G	c.836T>G	p.Val279Gly	MS (0.93)	—	0.409192	0.20848	1	0
MSH2	rs63749914	c.905T>C	p.Leu302Ser	MS (0.99)	VUS	0.569311	0.257734	1	0
PARP4	rs371092679	c.3285-2delCTT	—	SV	—	—	—	1	—
PARP4	13_25021149_CTTAC/-	c.3285delCTTAC	—	SV	—	—	—	1	—
PMS2	rs63750123	c.52A>G	p.Ile18Val	MS (0.64)	B, VUS	–0.0479661	–0.0781267	8	0.014
POLD1	19_947_ATATCGAG/ CCATCGCC	c.947_954ATATCGAG> CCATCGCC	p.AspIleGlu316_318AlaIleAla	MS	—	—	—	3	—
POLE	rs763031537	c.5480C>T	p.Ser1827Leu	MS (0.63)	—	0.126082	—	1	0.00002
WRN	rs150148567	c.1717A>G	p.Thr573Ala	MS (0.56)	VUS	–0.0233533	–0.0920501	1	0.0030
WRN	rs141563618	c.2114C>T	p.Thr705Ile	MS (0.56)	VUS	0.0500764	–0.0920501	1	0.00075

NOTE. Table lists rare variants observed in patients with metastatic prostate cancer (mPC; 3 patients carried multiple variants) that did not meet requirements for inclusion in pathogenic/likely pathogenic variants listed in Table 2 but were potentially deleterious. When available, BayesDel scores were compared against gene-specific predicted pathogenic cutoff values. BayesDel score higher than gene-specific cutoff was considered potentially deleterious.

Abbreviations: AF, allele frequency; B, benign; dbSNP, Single Nucleotide Polymorphism Database; FS, frameshift; LB, likely benign; LP, likely pathogenic; MS, missense (revel score); NS, nonsense; P, pathogenic; RF, risk factor; SV, splice variant; US, uncertain significance; VOI, variant of interest; VUS, variant of unknown significance.

TABLE A3. Comparison of Baseline Characteristics Between Clinically Actionable PV/LPV Carriers and Noncarrier Patients (N = 317)

Characteristic	Median (Q1-Q3)		*P*[b]
	PV/LPV Carriers (n = 27)[a]	Noncarrier Patients (n = 290)	
PSA at diagnosis, ng/mL	17 (7-32)	16 (7-89)	—
PSA at diagnosis, ng/mL			.890
Mean	170	189	
No.	21	258	
Age at initial diagnosis, years	63 (58-68)	65 (58-70)	.298
Age at BR diagnosis, years	66 (62-72)	67 (62-72)	.694
Age at mPC diagnosis, years	70 (66-76)	70 (63-77)	.570
Age at death, years	76 (71-79)	75 (68-82)	.934
Gleason score, No. (%)	26 (7.5)	267 (8.0)	—
≥ 8 (n = 188)	14 (53.4)	169 (63.3)	.398[c]
7 (n = 94)	7 (26.9)	67 (25.1)	.816[c]
≤ 6 (n = 44)	5 (19.2)	31 (11.6)	.341[c]

NOTE. Statistical analysis not adjusted for known first cousin *BRCA2* PV rs80359636 carriers.

Abbreviations: BR, biochemical recurrence; mPC, metastatic prostate cancer; PSA, prostate-specific antigen; PV/LPV, pathogenic/likely pathogenic germline variant; Q, quartile.

[a]Twenty-seven PV/LPV carriers with 28 total variants because of a double PV/LPV carrier.

[b]*P* values were calculated at a 95% CI.

[c]χ^2 $P = .475$.

TABLE A4. Comparison of DR Gene Mutations Among Studies With Comparable Cohort Demographics

Cohort Characteristics	Pritchard[14] (n = 692)	Annala[55] (n = 319)	Antonarakis[56] (n = 172)	Leongamornlert[29] (n = 191)	Boyle (n = 317)	Total
PC subcategory	mHSPC/mCRPC	mCRPC	mCRPC	fPC	mPC	
Race, %		Canadian cohort		UK cohort		
Non-Hispanic white	83.2		87.2		96.6	
Hispanic	1.6				1.4	
Non-Hispanic black	5.8				0.3	
Asian or Pacific Islander	1.7				1.4	
Prevalence of germline PVs in DR genes, No. (%)[a]	60/692 (8.7)	20/319 (6.3)	9/172 (5.2)	8/191 (4.2)	10/317 (3.2)	107/1,691 (6.3)

Abbreviation: DR, DNA repair; fPC, familial prostate cancer; mCRPC, metastatic castration-resistant prostate cancer; mHSPC, metastatic hormone-sensitive prostate cancer; mPC, metastatic prostate cancer (defined here as biochemical recurrence, mHSPC, or mCRPC); PV, pathogenic variant.
[a]Only DR genes analyzed across all studies were compared. Genes included *ATM*, *BRCA1*, *BRCA2*, *MLH1*, *MSH2*, *MSH6*, *PALB2*, and *RAD51C*.