Original Article

Germline Genetic Variants in Men With Prostate Cancer and One or More Additional Cancers

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BACKGROUND: Prostate cancer has a significant heritable component, and rare deleterious germline variants in certain genes can increase the risk of the disease. The aim of the current study was to describe the prevalence of pathogenic germline variants in cancer-predisposing genes in men with prostate cancer and at least 1 additional primary cancer. **METHODS:** Using a multigene panel, the authors sequenced germline DNA from 102 men with prostate cancer and at least 1 additional primary cancer who also met ≥1 of the following criteria: 1) age ≤55 years at the time of diagnosis of the first malignancy; 2) rare tumor type or atypical presentation of a common tumor; and/or 3) ≥3 primary malignancies. Cancer family history and clinicopathologic data were independently reviewed by a clinical genetic counselor to determine whether the patient met established criteria for testing for a hereditary cancer syndrome. **RESULTS:** Sequencing identified approximately 3500 variants. Nine protein-truncating deleterious mutations were found across 6 genes, including *BRCA2*, ataxia telangiectasia mutated (*ATM*), mutL homolog 1 (*MLH1*), BRCA1 interacting protein C-terminal helicase 1 (*BRIP1*), partner and localizer of BRCA2 (*PALB2*), and fibroblast growth factor receptor 3 (*FGFR3*). Likely pathogenic missense variants were identified in checkpoint kinase 2 (*CHEK2*) and homeobox protein Hox-B13 (*HOXB13*). In total, 11 of 102 patients (10.8%) were found to have pathogenic or likely pathogenic mutations in cancer-predisposing genes. The majority of these men (64%) did not meet current clinical criteria for germline testing. **CONCLUSIONS:** Men with prostate cancer and at least 1 additional primary cancer are enriched for harboring a germline deleterious mutation in a cancer-predisposing gene that may impact cancer prognosis and treatment, but the majority do not meet current criteria for clinical genetic testing. ***Cancer* 2017;123:3925-32**. *© 2017 American Cancer Society*.

KEYWORDS: gene panel, genetic testing, germline variants, multiple primary malignant neoplasms, prostate cancer.

INTRODUCTION

Prostate cancer has been shown to have a strong heritable component and to exhibit Mendelian inheritance patterns; however, the identification of highly penetrant genes accounting for hereditary prostate cancer has proven challenging. To our knowledge to date, there are a limited number of cancer predisposition genes that have been definitively shown to increase the risk of prostate cancer. In 2012, our laboratory identified a recurrent mutation in the homeobox protein Hox-B13 (*HOXB13*) gene on chromosome 17 through linkage analysis.[1] The *HOXB13* G84E mutation typically occurs on a common haplotype consistent with a founder allele and accounts for approximately 5% of all cases of hereditary prostate cancer in men of European descent.[2] Some studies have found evidence that this G84E mutation increases the risk of other cancers and is observed more frequently in individuals with prostate cancer plus an additional primary cancer.[3-5]

Prostate cancer is a potential phenotypic manifestation in individuals with germline mutations in homologous DNA damage repair (DDR) genes and individuals with Lynch syndrome (LS). Men in families with hereditary breast and ovarian cancer (HBOC) syndrome and who carry deleterious mutations in DDR genes, including *BRCA2*, have been observed to have an increased risk of prostate cancer and are more likely to have prostate cancer with a clinically aggressive phenotype.[6-8] Multiple recent studies of men with metastatic prostate cancer who were unselected for family history have shown that a significant minority of these individuals harbor pathogenic or likely pathogenic variants in DDR genes.[9-11] Studies also have found that prostate cancer is increased in individuals with LS, which classically presents as multiple individuals in a family presenting with ≥1 primary cancers including colorectal, small bowel, endometrial, and bladder/ureteral cancers and is due to germline mutations in mismatch repair genes.[12,13]

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Known cancer susceptibility syndromes now number >100, although mutations in high-penetrance genes explain only a fraction of heritable cancers.[14] Common features of hereditary cancer syndromes include early age of onset, multiple affected generations, rare tumor types, and/or multiple primary malignancies. However, hereditary cancers, similar to sporadic cancers, can be heterogeneous with regard to their presentation, pathology, and outcomes. Identifying individuals for genetic testing of cancer susceptibility genes is based primarily on family and personal cancer history with a goal of the prevention and early detection of cancers in these high-risk populations.

Multiple primary malignant neoplasms (MPMNs) (defined as tumors of different histology arising in distinct anatomic locations in a single individual) are relatively rare, reportedly comprising 6.3% of tumor registry cases.[15] MPMNs may be synchronous, occurring at the same time, or metachronous, occurring >6 months apart.[16] Individuals with certain cancer syndromes, such as Li-Fraumeni syndrome (LFS), are well known to carry a particularly high risk of developing MPMNs. For example, a study of unselected individuals with sarcoma demonstrated that sarcoma populations overall have a high incidence of pathogenic germline mutations, and that those germline carriers in the study were significantly more likely to have an MPMN phenotype.[17,18] In addition, a retrospective study of individuals with multiple primary malignancies who were referred for clinical genetic testing found that 44 of 111 individuals (39.6%) carried a variant in ≥1 cancer predisposition genes, with DNA mismatch repair genes among the most frequently mutated.[19] Although the presence of certain constellations of MPMNs in a single individual is considered to be one indication for referral for genetic risk assessment, to the best of our knowledge the percentage of individuals with MPMNs who are referred for genetic assessment and the outcomes of clinical genetics referrals in these patients with multiple primary cancers has not been extensively described.

Given the evidence that rare deleterious mutations in cancer predisposition genes contribute to prostate cancer, we set out to determine the frequency of germline mutations in men with prostate cancer and at least 1 additional primary neoplasm. We hypothesized that by using a rigorous clinical definition including an MPMN phenotype and early-onset cancers, we would increase the likelihood of detecting those individuals with deleterious germline mutations, which are able to be passed on and confer a cancer risk to subsequent generations. We used a multigene panel approach, which provides the opportunity to sequence the coding regions of multiple genes simultaneously via next-generation sequencing.

MATERIALS AND METHODS

Patient Selection

Patients were selected from the University of Michigan's Prostate Cancer Genetics Project and Cancer Genetics Clinic registry. Both are approved by the local Institutional Review Board and obtained informed consent from each participant. The Prostate Cancer Genetics Project enrolls men with prostate cancer who have at least 1 living first-degree or second-degree relative with prostate cancer, and/or who were diagnosed with prostate cancer before age 55 years (>4000 consented individuals from 1792 families). The Cancer Genetics Clinic registry recruits patients with a personal or family history suggestive of hereditary cancer risk (approximately 5000 consented individuals from 3800 families). Initial queries of these 2 registries identified 414 men diagnosed with early-onset and/or familial prostate cancer who had been diagnosed with at least 1 additional primary malignancy (excluding nonmelanoma skin cancer). From these cases, we used the following criteria to further select patients for this study: 1) early age of onset of first malignancy (age ≤55 years); 2) diagnosed with rare cancers (eg, pancreatic cancer, testicular cancer, sarcoma, brain cancer, parathyroid cancer, or Hodgkin lymphoma); and/or 3) ≥3 primary malignancies diagnosed in a single individual. Each individual patient provided a cancer family history, which was pathologically confirmed when possible and was used to construct a 3-generation pedigree. Individuals who were known carriers of pathogenic germline mutations associated with hereditary cancer syndromes were excluded. Medical records pertaining to prostate cancer diagnoses were reviewed, and prostate cancers were categorized as clinically aggressive if they exhibited ≥1 the following features: a Gleason score >7, tumors classified as T3b or T4, a prediagnosis prostate-specific antigen (PSA) level >15 ng/mL, Gleason score of 7 and a prediagnosis PSA level >10 ng/mL, or N1 or M1 disease at the time of diagnosis.

The personal and family histories for each subject were reviewed by a certified genetic counselor to determine whether these were suggestive of a hereditary cancer syndrome and whether they met published criteria for clinical genetic testing (as defined by the National Comprehensive Cancer Network [NCCN] using 2015 guidelines for hereditary breast ovarian cancer [HBOC], Li-Fraumeni syndrome (LFS), Lynch syndrome (LS),

phosphatase and tensin homolog [PTEN] hamartoma tumor syndrome, or familial adenomatous polyposis).

Gene Mutational Analysis

Gene mutation profiling was performed on DNA extracted from peripheral blood using the Qiagen GeneRead DNAseq Comprehensive Cancer Panel (CCP) (Qiagen Inc, Germantown, Maryland) consisting of multiplex polymerase chain reaction primer sets that amplify >95% of the exonic regions of a panel of genes, including genes associated with hereditary cancer syndromes with high and moderate penetrance as well as genes mutated in pathways involved in the carcinogenesis of prostate cancer and additional tumor types. The majority of samples (94 samples) were typed using version 2 of the Qiagen GeneRead DNAseq CCP, which included 160 genes. The remaining samples (8 samples) were typed using version 1 of the Qiagen GeneRead DNAseq CCP, which included 124 genes. A list of genes included in each panel is found in Supporting Information Table 1. Sequencing was performed on an Illumina HiSeq (Illumina Inc, San Diego, California), and analysis of data was performed using the GeneRead Targeted Exon Enrichment Panel Data Analysis Portal (Qiagen Inc) (http://ngsdataanalysis.sabiosciences.com/NGS2/). In addition, Sanger sequencing for the *HOXB13* G84E allele was performed on 93 of 102 patients in this cohort for whom DNA was available, because *HOXB13* was not included in either Qiagen gene panel.

Called variants were annotated with Annovar.[20] Deleterious, protein-truncating variants were identified, with putative functional importance preferentially given to stop/loss, frameshift insertions/deletions, and splice variants. All deleterious and missense variants were referenced for pathogenicity using the publically available databases ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) and Breast Cancer Information Core (BIC) (https://research.nhgri.nih.gov/bic/), and established consensus guidelines.[21-23] Pathogenic and likely pathogenic variants were confirmed via Sanger sequencing.

Statistical Analysis

Clinicopathological characteristics including age at the time of diagnosis of the first primary tumor, age at the time of diagnosis of prostate cancer, and PSA level at the time of prostate cancer diagnosis were compared between pathogenic germline mutation carriers and noncarriers via a 2-sided Student *t* test. Gleason score, race, presence of ≥3 primary malignancies, whether or not the patient met NCCN criteria for genetic testing of any kind, and the presence of clinically aggressive prostate cancer were compared via the Fisher exact test. P values <.05 were deemed statistically significantly different.

RESULTS

Patients

A total of 102 men with prostate cancer, at least 1 additional primary cancer, and who met ≥1 of 3 additional inclusion criteria were selected for germline mutation profiling (Fig. 1). The clinical characteristics of this study population are described in Table 1. The mean age at the time of diagnosis of the first primary cancer was 51 years and the mean age at the time of prostate cancer diagnosis was 53 years. The majority of patients (76 of 102 patients) had 2 primary cancers, 22 had 3 primary cancers, and 4 patients had 4 primary cancers. Melanoma was the most common additional primary cancer (see Supporting Information Table 2). Greater than one-half of the men had prostate cancer of Gleason score ≥7, and 30% had clinically aggressive prostate cancer. Forty patients (39% of the current study cohort) met the criteria for clinical genetic testing of any syndrome based on review of personal and family histories, with the majority of patients (38 of 40 patients) meeting criteria for HBOC.

Germline Mutational Events

In total, >3500 variants were identified among the 102 individuals tested, including 2 nonsense, 7 frameshift, 5 in-frame coding insertions or deletions, and 525 missense variants. Eleven of the 102 men in the current study

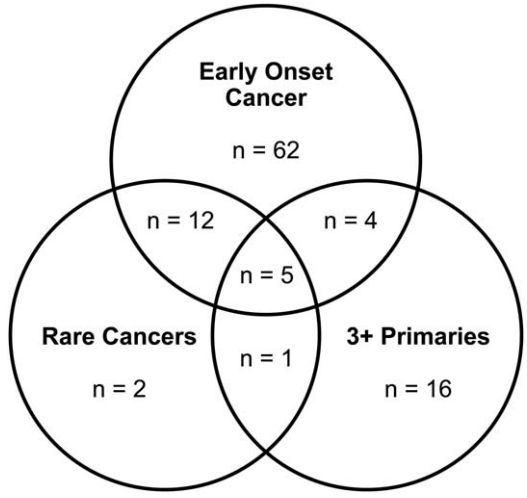

Figure 1. Venn diagram summarizing the qualifying inclusion criteria of the final cohort of 102 men. Criteria included: 1) early age of onset of first malignancy (age ≤55 years); 2) diagnosed with rare cancers, including pancreatic cancer, sarcoma, and male breast cancer; and/or 3) ≥3 primary malignancies diagnosed in a single individual.

TABLE 1. Clinical and Pedigree Features of the Cohort of 102 Men With Prostate Cancer and ≥1 or More Additional Primary Cancers

Median age at diagnosis (range), y	First cancer: 51 (5-76) Prostate cancer: 53 (31-84)
Race, no. (%)	White: 96 (94.1) African American: 6 (5.9)
Median PSA at diagnosis (range), ng/mL	5.6 (1.0-75.5)
Gleason score, no. (%)	Score <7: 38 (43.2) Score ≥7: 50 (56.8)
Total no. of multiple primary tumors (including prostate cancer), no. (%)	2 primary malignancies: 76 (74.5) 3 primary malignancies: 22 (21.6) 4 primary malignancies: 4 (3.9)
Cancer syndrome criteria, no (%)[a]	None: 62 (60.8) Any: 40 (39.2) HBOC: 38 (37.3) LS: 6 (5.9) LF: 2 (2.0)
Clinically aggressive prostate cancer, no. (%)[b]	31 (30.4)

Abbreviations: HBOC, hereditary breast and ovarian cancer; LI, Li-Fraumeni syndrome; LS, Lynch syndrome; PSA, prostate-specific antigen.
[a] National Comprehensive Cancer Network guidelines for clinical genetic testing for HBOC, LS, and LF.
[b] Clinically aggressive prostate cancer was defined as meeting ≥1 of the following criteria: Gleason score>7, tumor classification of T3b or T4, prediagnosis PSA level >15 ng/mL, Gleason score of 7 and a prediagnosis PSA level of >10 ng/mL, or N1 or M1 classification at diagnosis.

(10.8%) harbored pathogenic or likely pathogenic mutations in cancer-predisposing genes. Eight men were found to harbor protein-truncating germline variants in 1 of 6 cancer predisposition genes: *BRCA2* (3 cases), ataxia telangiectasia mutated (*ATM*; 2 cases), mutL homolog 1 (*MLH1*; 1 case), BRCA1 interacting protein C-terminal helicase 1 (*BRIP1*; 1 case), partner and localizer of BRCA2 (*PALB2*; 1 case), and fibroblast growth factor receptor 3 (*FGFR3*; 1 case), with 1 man harboring deleterious variants in both *BRCA2* and *MLH1* (Table 2). This patient had 3 primary malignancies (prostate cancer, kidney cancer, and bladder cancer). Review of the 525 missense mutations using ClinVar resulted in the identification of 2 likely pathogenic missense mutations in 2 men who had the same likely pathogenic missense variant in checkpoint kinase 2 (*CHEK2*). Additional sequencing of the *HOXB13* prostate cancer-predisposing gene in 93 of 102 men identified 2 carriers of the known prostate cancer risk-associated G84E allele. One of these G84E carriers also harbored a pathogenic *BRCA2* splice variant and had 3 primary malignancies: prostate cancer, liver cancer, and bladder cancer.

Men who harbored a germline mutation did not differ with respect to age of onset, family history, number of primary malignancies, or tumor phenotypes compared with those men who were not found to have a deleterious or pathogenic germline mutation from our panel of genes (see Supporting Information Table 3). Based on expert review of pedigrees using 2015 NCCN cancer genetics guidelines, only 4 of the 11 individuals with a pathogenic germline variant (36%) met the criteria for a hereditary cancer syndrome and would have qualified for clinical genetic testing based on their personal and/or family history. Three of these 4 individuals met the criteria for HBOC testing and harbored pathogenic variants in *ATM*, *BRIP1*, and *CHEK2*, respectively; the fourth individual met the criteria for HBOC and LS testing and harbored a pathogenic variant in both *BRCA2* and *MLH1*. The aforementioned *HOXB13* G84E allele and *BRCA2* splice variant carrier with prostate cancer, liver cancer, and bladder cancer did not meet any criteria for testing.

DISCUSSION

We identified deleterious or likely pathogenic germline mutations in 10.8% of men with prostate cancer and ≥1 additional primary cancers. Protein-truncating variants were found in 6 genes (*BRCA2*, *ATM*, *MLH1*, *BRIP1*, *PALB2*, and *FGFR3*) and a likely pathogenic missense mutation was noted in 1 gene (*CHEK2*) from a multigene panel of 160 selected cancer genes, with the majority of these variants found in genes whose function is important for DDR. In addition, the prostate cancer risk-associated *HOXB13* G84E allele, which recently has been shown to be associated with an increased risk of multiple cancers in a single individual, was found in 2 individuals with an MPMN phenotype.[4] The most frequently mutated gene in the current study was the HBOC gene, *BRCA2*. The majority of the individuals with pathogenic or likely pathogenic germline variants (7 of 11 patients) did not meet current criteria for clinical genetic testing and thus would

TABLE 2. Pathogenic Variants in Men With Prostate Cancer and Multiple Primary Malignancies

Gene	Chromosomal Location	Variant Type	Allele Change	Amino Acid Change	dbSNP ID[a]	No. of Carriers
BRCA2	13	Frame shift	A->AT	p.Q1429fs	Rs80359440	1
	13	Frame shift	T->TA	p.Y2215fs	Rs80359615	1
	13	Splice variant	A->T	p.T3085fs	Rs61757642	1
ATM	11	Frame shift	ACT->A	p.T761fs	Rs587781658	1
	11	Stop-gain	T->G	p.L1457X	Rs373226793	1
PALB2	16	Frame shift	GAACAA->G	p.Q60fs	Rs180177143	1
BRIP1	17	Frame shift	AT->A	p.N541fs		1
MLH1	3	Frame shift	TAGCC->T	p.A661fs		1
FGFR3	4	Frame shift	CAG->C	p.D787fs	Rs759113408	1
CHEK2	22	Missense	T->C	p.I157T	Rs17879961	2
HOXB13	17	Missense	A->G	p.G84E	Rs138213197	2

Abbreviations: *ATM*, ataxia telangiectasia mutated; *BRIP1*, BRCA1 interacting protein C-terminal helicase 1; *CHEK2*, checkpoint kinase 2; dbSNP ID, database of single-nucleotide polymorphisms identification; *FGFR3*, fibroblast growth factor receptor 3; *HOXB13*, homeobox protein Hox-B13; *MLH1*, mutL homolog 1; *PALB2*, partner and localizer of BRCA2.
[a] http://www.ncbi.nlm.nih.gov/projects/SNP/.

likely not have been identified as being at risk of a hereditary cancer syndrome otherwise. In this pilot study, there was no difference noted with regard to carriers versus noncarriers in terms of prostate cancer metastatic disease, aggressiveness, or age of onset of prostate cancer. However, because the current study selected for early age of onset of malignancy as one of the inclusion criteria, it would be difficult to ascertain a difference with regard to the age of onset in carriers versus noncarriers from the current study population.

The prevalence of germline mutations in this selected population of men with prostate cancer is similar to the rates of 8% to 17% found in recent studies focusing on the identification of germline mutations in men with metastatic prostate cancer who were unselected for family history.[9-11,24] Also similarly, the majority of deleterious variants in the current study were in DDR pathway genes.[9-11,24] Unique to the current study population is that there was no statistical difference with regard to the presence of metastatic or aggressive disease noted in mutation carriers, suggesting that patients with multiple primary malignancies including prostate cancer may be at an increased risk of harboring deleterious germline mutations in DDR genes regardless of metastatic disease or Gleason score (Fig. 2). Identifying these men with DDR mutations now is not only important for risk assessment but also for treatment given the sensitivity of DDR-deficient tumors to platinum-based chemotherapeutics and adenosine diphosphate (ADP) ribose polymerases (PARP) inhibitors. A phase 2 study of olaparib in previously treated patients with metastatic prostate cancer found that 6 of 50 patients harbored deleterious variants in the DDR-related genes *ATM* and *BRCA2*, with all 6 demonstrating a response to PARP inhibition.[9] In the era of targeted therapies, the early identification of a DDR germline mutation in men with prostate cancer and the MPMN phenotype could significantly alter the treatment course and outcomes for the multiple cancers in these patients.

The identification of a risk allele within an individual with cancer also has enormous impact for that patient's family members with regard to risk assessment, cancer screening, and cancer prevention. For example, men with *BRCA2* germline mutations are known to be at an increased risk of prostate cancer, and typically demonstrate an earlier age of onset of disease and aggressive clinical phenotypes.[6,7,25,26] These high-risk prostate cancer features have led to guideline recommendations for prostate cancer screening beginning at age 40 years in unaffected *BRCA2* mutation carriers. The results of the current study also suggest that the use of multigene panel genetic tests may be particularly useful in this population given the varied tumor phenotypes, genes mutated, and the finding that a majority of the mutation carriers did not meet current NCCN guidelines for clinical genetic testing for hereditary cancer syndromes. For example, as seen in the pedigree shown in Figure 2, a patient with prostate cancer and melanoma was found to harbor a deleterious *BRCA2* mutation; however, this proband did not meet current clinical criteria for germline genetic testing. At the time of subsequent testing, this patient's unaffected brother also was found to have this same deleterious *BRCA2* mutation. This exemplary finding will alter recommendations for cancer screening and treatment not only for the proband but also for his at-risk relatives, not only for prostate cancer but other HBOC-associated malignancies as well.

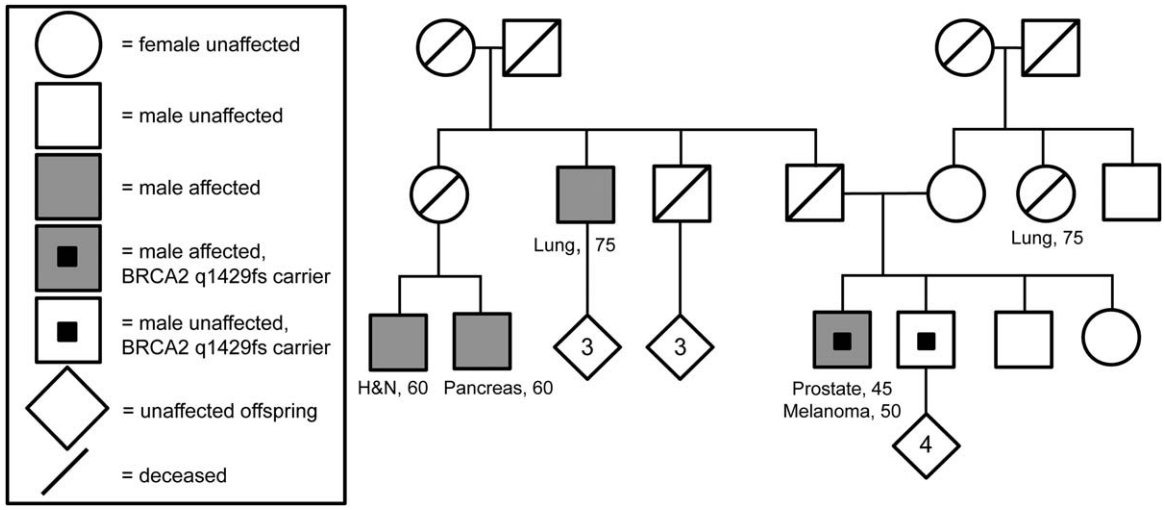

Figure 2. Pedigree analysis of a proband with the BRCA2 q1429fs germline mutation and multiple primary malignant neoplasm phenotype. H & N indicates head and neck cancer.

Large-scale tumor sequencing via comprehensive panels focused on actionable mutations is quickly becoming ubiquitous at most comprehensive cancer centers, and the identification of germline variants of undetermined significance are an increasing concern. The results of the current study are in keeping with multiple recent studies of germline sequencing in patients with cancer demonstrating that germline aberrations in general are more frequent than previously believed and can be found in patients across all age groups and tumor types regardless of family history.[17,27-30] These studies highlight potential shortcomings in current clinical genetic testing practices, which rely primarily on constellations of specific personal and family cancer histories to determine whether a patient should pursue germline mutation testing. Additional parameters independent of family history, such as multiple primary cancers, early age of disease onset, and/or rare and/or aggressive histologies may be beneficial to add to the decision algorithm for germline testing in patients with prostate cancer.

Although the findings of the current study are novel, there are limitations, including the small sample size and the lack of paired somatic sequencing to better determine a pathogenic variant's impact on the phenotype of the tumor. We rely on the putative functional changes of a germline mutation to aid in determining its clinical pathogenic impact, which does not always align across tumor types. For example, a K3326X stop-gain variant in *BRCA2* was found in 2 individuals in the current study; however, although this variant has been shown to increase the risk of developing breast and/or ovarian cancer, its pathogenicity in prostate cancer is less clear and is categorized as benign in ClinVar and thus was not included in our pathogenic carrier rate for the current study.[31] In addition, as with the majority of large-panel whole-exome sequencing studies, there is a high rate of variants of unknown significance, including missense variants of unknown clinical impact. Given the stringent criteria used in selecting for deleterious functional mutations, including restricting missense variants to only those referenced with supporting evidence as cancer-associated pathogenic or likely pathogenic in ClinVar, the pathogenic or likely pathogenic germline variant prevalence in the population in the current study may be underestimated. The reported prevalence also does not reflect any pathogenic variants harbored in genes not tested in this panel. It also should be noted that the vast majority of the selected patient population in the current study (approximately 90%) were negative for pathogenic or likely pathogenic mutations in the panel of cancer-associated genes; in addition, there were individuals who were discovered to have novel mutations or mutations in moderately penetrant genes. However, these individuals and their family members still may have an increased risk of prostate or other cancers and warrant longitudinal cancer screening. These findings highlight the potential clinical and ethical dilemmas regarding how to best inform patients and their families of cancer risk and highlight the necessity of a

multidisciplinary approach to genetic screening and testing in patients with cancer that incorporates genetic counselors, physicians, molecular pathology, and psychosocial care for discussing, consenting, performing, and interpreting these genetic tests.

Quantifying and qualifying the prevalence and penetrance of pathogenic germline variants in unique subgroups of men with prostate cancer and multiple primary malignancies will provide a better understanding of the underlying molecular aberrations involved in the pathogenesis of different tumor types, allow for targeted therapeutic approaches, and better define high-risk groups of patients who would benefit from early screening and intervention. The results of the current study, along with those of other recent germline studies, have shown that certain clinical populations such as those with an MPMN phenotype, early-onset cancer, and/or metastatic/aggressive prostate cancer are enriched for germline variants and thus warrant consideration for genetic testing regardless of whether they meet current clinical criteria for hereditary cancer syndromes. However, health insurance does not typically cover genetic testing for patients outside of guideline criteria. It is particularly important for patients with prostate cancer and their families to identify heritable pathogenic variants that could prompt prostate screening in unaffected carriers, screening that otherwise is not currently recommended in the general US population.[32] Future larger studies to better define risk and outcomes in this population of men with prostate cancer and MPMNs who harbor deleterious germline variants is warranted.

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CONFLICT OF INTEREST DISCLOSURES

The authors made no disclosures.

AUTHOR CONTRIBUTIONS

Patrick G. Pilié: Conceptualization, methodology, formal analysis, investigation, data curation, writing–original draft, and visualization. **Anna M. Johnson**: Conceptualization, methodology, validation, formal analysis, investigation, data curation, writing–review and editing, and visualization. **Kristen L. Hanson**: Methodology, validation, investigation, data curation, and writing–review and editing. **Megan E. Dayno**: Methodology, validation, investigation, data curation, and writing–review and editing. **Ashley L. Kapron**: Methodology, formal analysis, data curation, writing–review and editing, and visualization. **Elena M. Stoffel**: Conceptualization, methodology, investigation, writing–review and editing, supervision, and project administration. **Kathleen A. Cooney:** Conceptualization, methodology, validation, formal analysis, resources, writing–review and editing, supervision, project administration, and funding acquisition.

REFERENCES

1. Ewing CM, Ray AM, Lange EM, et al. Germline mutations in HOXB13 and prostate-cancer risk. *N Engl J Med.* 2012;366:141-149.
2. Xu J, Lange EM, Lu L, et al; International Consortium for Prostate Cancer Genetics. HOXB13 is a susceptibility gene for prostate cancer: results from the International Consortium for Prostate Cancer Genetics (ICPCG). *Hum Genet.* 2013;132:5-14.
3. Beebe-Dimmer JL, Hathcock M, Yee C, et al. The HOXB13 G84E mutation is associated with an increased risk for prostate cancer and other malignancies. *Cancer Epidemiol Biomarkers Prev.* 2015;24:1366-1372.
4. Hoffmann TJ, Sakoda LC, Shen L, et al. Imputation of the rare HOXB13 G84E mutation and cancer risk in a large population-based cohort. *PLoS Genet.* 2015;11:e1004930.
5. Laitinen VH, Wahlfors T, Saaristo L, et al. HOXB13 G84E mutation in Finland: population-based analysis of prostate, breast, and colorectal cancer risk. *Cancer Epidemiol Biomarkers Prev.* 2013;22:452-460.
6. Castro E, Goh C, Leongamornlert D, et al. Effect of BRCA mutations on metastatic relapse and cause-specific survival after radical treatment for localised prostate cancer. *Eur Urol.* 2015;68:186-193.
7. Castro E, Goh C, Olmos D, et al. Germline BRCA mutations are associated with higher risk of nodal involvement, distant metastasis, and poor survival outcomes in prostate cancer. *J Clin Oncol.* 2013;31:1748-1757.
8. Gleicher S, Kauffman EC, Kotula L, Bratslavsky G, Vourganti S. Implications of high rates of metastatic prostate cancer in BRCA2 mutation carriers. *Prostate.* 2016;76:1135-1145.
9. Mateo J, Carreira S, Sandhu S, et al. DNA-repair defects and olaparib in metastatic prostate cancer. *N Engl J Med.* 2015;373:1697-1708.
10. Pritchard CC, Mateo J, Walsh MF, et al. Inherited DNA-repair gene mutations in men with metastatic prostate cancer. *N Engl J Med.* 2016;375:443-453.
11. Robinson D, Van Allen EM, Wu YM, et al. Integrative clinical genomics of advanced prostate cancer. *Cell.* 2015;161:1215-1228.
12. Raymond VM, Mukherjee B, Wang F, et al. Elevated risk of prostate cancer among men with Lynch syndrome. *J Clin Oncol.* 2013;31:1713-1718.
13. Haraldsdottir S, Hampel H, Wei L, et al. Prostate cancer incidence in males with Lynch syndrome. *Genet Med.* 2014;16:553-557.
14. Stadler ZK, Schrader KA, Vijai J, Robson ME, Offit K. Cancer genomics and inherited risk. *J Clin Oncol.* 2014;32:687-698.
15. Rosso S, De Angelis R, Ciccolallo L, et al; EUROCARE Working Group. Multiple tumours in survival estimates. *Eur J Cancer.* 2009;45:1080-1094.
16. Xu LL, Gu KS. Clinical retrospective analysis of cases with multiple primary malignant neoplasms. *Genet Mol Res.* 2014;13:9271-9284.
17. Mitchell G, Ballinger ML, Wong S, et al; International Sarcoma Kindred Study. High frequency of germline TP53 mutations in a prospective adult-onset sarcoma cohort. *PLoS One.* 2013;8:e69026.
18. Ballinger ML, Goode DL, Ray-Coquard I, et al; International Sarcoma Kindred Study. Monogenic and polygenic determinants of sarcoma risk: an international genetic study. *Lancet Oncol.* 2016;17:1261-1271.
19. Whitworth J, Hoffman J, Chapman C, et al. A clinical and genetic analysis of multiple primary cancer referrals to genetics services. *Eur J Hum Genet.* 2015;23:581-587.
20. Chang X, Wang K. wANNOVAR: annotating genetic variants for personal genomes via the web. *J Med Genet.* 2012;49:433-436.
21. Harrison SM, Riggs ER, Maglott DR, et al. Using ClinVar as a resource to support variant interpretation. *Curr Protoc Hum Genet.* 2016;89:8.16.1-8.16.23.

22. Rehm HL, Berg JS, Brooks LD, et al; ClinGen. ClinGen–the clinical genome resource. *N Engl J Med.* 2015;372:2235-2242.
23. Richards S, Aziz N, Bale S, et al; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med.* 2015;17:405-424.
24. Hart SN, Ellingson MS, Schahl K, et al. Determining the frequency of pathogenic germline variants from exome sequencing in patients with castrate-resistant prostate cancer. *BMJ Open.* 2016;6:e010332.
25. Maier C, Herkommer K, Luedeke M, Rinckleb A, Schrader M, Vogel W. Subgroups of familial and aggressive prostate cancer with considerable frequencies of BRCA2 mutations. *Prostate.* 2014;74:1444-1451.
26. Na R, Zheng SL, Han M, et al. Germline mutations in ATM and BRCA1/2 distinguish risk for lethal and indolent prostate cancer and are associated with early age at death. *Eur Urol.* 2017;71:740-747.
27. Meric-Bernstam F, Brusco L, Daniels M, et al. Incidental germline variants in 1000 advanced cancers on a prospective somatic genomic profiling protocol. *Ann Oncol.* 2016;27:795-800.
28. Mork ME, You YN, Ying J, et al. High prevalence of hereditary cancer syndromes in adolescents and young adults with colorectal cancer. *J Clin Oncol.* 2015;33:3544-3549.
29. Schrader KA, Cheng DT, Joseph V, et al. Germline variants in targeted tumor sequencing using matched normal DNA. *JAMA Oncol.* 2016;2:104-111.
30. Zhang J, Walsh MF, Wu G, et al. Germline mutations in predisposition genes in pediatric cancer. *N Engl J Med.* 2015;373:2336-2346.
31. Meeks HD, Song H, Michailidou K, et al. BRCA2 polymorphic stop codon K3326X and the risk of breast, prostate, and ovarian cancers. *J Natl Cancer Inst.* 2016;108(2). pii: djv315.
32. Jemal A, Fedewa SA, Ma J, et al. Prostate cancer incidence and PSA testing patterns in relation to USPSTF screening recommendations. *JAMA.* 2015;314:2054-2061.