Manuscript Title: Germline Genetic Variants in Men with Prostate Cancer and One or More Additional Cancers

Running Title: Genetics of Men w/ PCa & Other Cancers

Authors: Patrick G. Pilié, MD[1]; Anna M. Johnson, MS[2]; Kristen Hanson, MS, CGC[2]; Megan E. Dayno, BS[2]; Ashley L. Kapron, PhD[3]; Elena M. Stoffel, MD[2,4]; Kathleen A. Cooney, MD[3]

Affiliations:
[1]Division of Cancer Medicine, University of Texas MD Anderson Cancer Center, Houston TX 77030
[2]Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, MI 48109
[3]Department of Internal Medicine, University of Utah School of Medicine, Salt Lake City, UT 84132
[4]University of Michigan Comprehensive Cancer Center, Ann Arbor, MI 48109

Corresponding Author:
Kathleen A. Cooney MD
Department of Internal Medicine
University of Utah Health System
Salt Lake City, UT 84112
Email: kathleen.cooney@hsc.utah.edu
Fax: 801-581-5393
Phone: 801-581-7606

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Precis for use in the Table of Contents: In this study, pathogenic germline mutations in cancer-predisposing genes, predominantly involved in the DNA damage repair pathway, were found in a significant minority of a selected population of men with prostate cancer and at least one other primary malignancy. The majority of the men with germline mutations would not have qualified for clinical genetic testing under current guidelines, highlighting the need for expanded inclusion criteria for genetic testing in prostate cancer, particularly given the impact of pathogenic germline mutations on not only a proband's own treatment but also cancer screening and prevention strategies for his entire family.

Author Contributions:

Conceptualization: Formulation of overarching research goals and aims.	Patrick G. Pilié, Anna M. Johnson, Elena M. Stoffel, Kathleen A. Cooney
Methodology: Development or design of methodology; creation of models.	Patrick G. Pilié, Anna M. Johnson, Kristen Hanson, Megan E. Dayno, Ashley L. Kapron, Elena M. Stoffel, Kathleen A. Cooney
Software: Programming, software development; designing computer programs; implementation of the computer code and supporting algorithms; testing of existing code components.	n/a
Validation: Verification, whether as a part of the activity or separate, of the overall replication/reproducibility of results/experiments and other research outputs.	Anna M. Johnson, Kristen Hanson, Megan E. Dayno, Kathleen A. Cooney
Formal analysis: Application of statistical, mathematical, computational, or other formal techniques to analyze or synthesize study data.	Patrick G. Pilié, Anna M. Johnson, Ashley L. Kapron, Kathleen A. Cooney
Investigation: Research and investigation process, specifically performing the experiments, or data/evidence collection.	Patrick G. Pilié, Anna M. Johnson, Kristen Hanson, Megan E. Dayno, Elena M. Stoffel
Resources: Provision of study materials, reagents, materials, patients, laboratory samples, animals, instrumentation, computing resources, or other analysis tools.	Kathleen A. Cooney
Data curation: Management activities to annotate (produce metadata), scrub data and maintain research data (including software code, where it is necessary for interpreting the data itself) for initial use and later re-use.	Patrick G. Pilié, Anna M. Johnson, Kristen Hanson, Megan E. Dayno, Ashley L. Kapron
Writing – original draft: Preparation, creation and/or presentation of the published work, specifically writing the initial draft (including substantive translation).	Patrick G. Pilié
Writing – review and editing: Preparation, creation and/or presentation of the published work by those from the original research group, specifically critical review, commentary or revision – including pre- or post-publication stages.	Anna M. Johnson, Kristen Hanson, Megan E. Dayno, Ashley L. Kapron, Elena M. Stoffel, Kathleen A. Cooney
Visualization: Preparation, creation and/or presentation of the published work, specifically visualization/data presentation.	Patrick G. Pilié, Anna M. Johnson, Ashley L. Kapron
Supervision: Oversight and leadership responsibility for the research activity planning and execution, including mentorship external to the core team.	Elena M. Stoffel, Kathleen A. Cooney
Project administration: Management and coordination responsibility for the research activity planning and execution.	Elena M. Stoffel, Kathleen A. Cooney
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Abstract

Background: Prostate cancer has a significant heritable component, and rare deleterious germline variants in certain genes can increase the risk of prostate cancer. Our aim was to describe the prevalence of pathogenic germline variants in cancer predisposing genes in men with prostate cancer and at least one additional primary cancer.

Methods: Using a multi-gene panel, we sequenced germline DNA from 102 men with prostate cancer and at least one additional primary cancer who also met one or more of the following criteria: 1) age ≤ 55 at diagnosis of first malignancy, 2) rare tumor type or atypical presentation of a common tumor, and/or 3) three or more primary malignancies. Cancer family history and clinicopathologic data were independently reviewed by a clinical genetic counselor to determine if the patient met established criteria for testing for a hereditary cancer syndrome.

Results: Sequencing identified ~3500 variants. Nine protein truncating deleterious mutations were found across six genes including *BRCA2*, *ATM, MLH1*, *BRIP1*, *PALB2*, and *FGFR3*. Likely pathogenic missense variants were identified in *CHEK2* and *HOXB13*. In total, 11/102 (10.8%) subjects 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 one additional primary cancer are enriched for harboring a germline deleterious mutation in a cancer predisposing gene that may impact cancer prognosis and treatment, but most do not meet current criteria for clinical genetic testing.

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

Introduction

Prostate cancer has been shown to have a strong heritable component and exhibit Mendelian inheritance patterns; however, identification of highly penetrant genes accounting for hereditary prostate cancer has proven challenging. 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 *HOXB13* gene on chromosome 17 through linkage analysis.[1] The *HOXB13* G84E mutation is typically 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 seen 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 genes and individuals with Lynch Syndrome. Men in families with hereditary breast and ovarian cancer (HBOC) syndrome and who carry deleterious mutations in DNA damage repair 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 unselected for family history have shown a significant minority of these individuals harbor pathogenic or likely pathogenic variants in DNA damage repair genes.[9-11] Studies have also found prostate cancer is increased in individuals with Lynch Syndrome (LS), which classically presents as multiple individuals in a family presenting with one or more primary cancers including colorectal, small bowel, endometrial, and bladder/ureteral cancers and is due to germline mutations in mismatch repair genes.[12,13]

Known cancer susceptibility syndromes now number >100, though 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, like sporadic cancers, can be heterogeneous in their presentation, pathology, and outcomes. Identifying individuals for genetic testing of cancer-susceptibility genes is primarily based on family and personal cancer history with a goal of 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 greater than six months apart.[16] Individuals with certain cancer syndromes, such as Li-Fraumeni (LF), are well known to carry a particularly high risk of developing MPMNs. For example, a study of unselected individuals with sarcoma has shown 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 MPMN phenotype.[17,18] In addition, a retrospective study of individuals with multiple primary malignancies who were referred for clinical genetic testing found 44/111 (39.6%) carried a variant in one or more cancer predisposition genes, with DNA mismatch repair genes among the most frequently mutated.[19] While the presence of certain constellations of MPMNs in a single individual is considered as one indication for referral for genetic risk assessment, the percentage of individuals with MPMNs referred for genetic assessment and the outcomes of clinical genetics referrals in these persons 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 one 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 cancer risk to subsequent generations. We used a multi-gene panel approach which provides the opportunity to sequence the coding regions of multiple genes simultaneously via next generation sequencing.

Patients & Methods

Patient Selection

Subjects were selected from the University of Michigan's Prostate Cancer Genetics Project (UM PCGP) and the University of Michigan's Cancer Genetics Clinic registry (UM CGC). Both are approved by the local Institutional Review Board and obtain informed consent from each participant. The UM PCGP enrolls men with prostate cancer who have at least one living first or second degree relative with prostate cancer, and/or who were diagnosed with prostate cancer before age 55 (more than 4,000 consented individuals from 1792 families). The UM CGC recruits patients with personal or family history suggestive of hereditary cancer risk (approximately 5000 consented individuals from 3800 families). Initial queries of these two registries identified 414 men diagnosed with early-onset and/or familial prostate cancer who had been diagnosed with at least one additional primary malignancy (excluding non-melanoma 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 (≤55 years old), 2) diagnosed with rare cancers (e.g.,

pancreatic cancer, testicular cancer, sarcoma, brain cancer, parathyroid cancer, Hodgkin's lymphoma) and/or 3) three or more primary malignancies in a single individual. Each individual patient provided a cancer-family history, which was pathologically confirmed when possible; and 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 one of more the following features: Gleason sum >7, stage T3b or T4 tumor, pre-diagnosis PSA>15 ng/mL, Gleason score=7 and pre-diagnosis PSA >10ng/mL, N1 or M1 at diagnosis.

Personal and family history for each subject was 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 or NCCN using 2015 guidelines for Hereditary Breast Ovarian Cancer (HBOC), Li Fraumeni Syndrome (LFS), Lynch Syndrome (LS), PTEN Hamartoma Tumor Syndrome (PHTS) or Familial Adenomatous Polyposis (FAP).

Gene Mutational Analysis

Gene mutation profiling was performed on DNA extracted from peripheral blood using the Qiagen GeneRead DNAseq Comprehensive Cancer Panel (CCP) consisting of multiplex PCR primer sets which amplify >95% of the exonic regions of a panel of genes including genes associated with high and moderate penetrance hereditary cancer syndromes as well as genes mutated in pathways involved in carcinogenesis of prostate cancer and additional tumor types. The majority of samples (94) were typed using the Qiagen GeneRead DNAseq CCP version 2, which included 160 genes. The remaining samples (8) were typed using the Qiagen GeneRead

DNAseq CCP version 1, 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, and analysis of data was performed using the GeneRead Targeted Exon Enrichment Panel Data Analysis Portal (http://ngsdataanalysis.sabiosciences.com/NGS2/). In addition, Sanger sequencing for the *HOXB13* G84E allele was performed on 93/102 subjects in this cohort for whom DNA was available, as *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, 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 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

Clinicopathogical characteristics including age at diagnosis of first primary, age at diagnosis of prostate cancer, PSA at prostate cancer diagnosis were compared between pathogenic germline mutation carriers and non-carriers via two-sided T test. Gleason score, race, presence of 3 or more primary malignancies, whether or not patient met NCCN criteria for genetic testing of any kind, and the presence of clinically aggressive prostate cancer were compared via Fisher's exact test. P values <0.05 were deemed significantly different.

Results

Patients

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

Germline Mutational Events

In total, over 3500 variants were identified among 102 individuals tested, including 2 nonsense, 7 frameshift, 5 in-frame coding insertions or deletions, and 525 missense variants. Eleven out of 102 (10.8%) men in this study harbored pathogenic or likely pathogenic mutations in cancer-predisposing genes. Eight men were found to harbor protein truncating germline variants in one of six cancer predisposition genes: *BRCA2* (3 cases), *ATM* (2), *MLH1* (1), *BRIP1* (1), *PALB2* (1), and *FGFR3* (1), with one man harboring deleterious variants in both *BRCA2* and *MLH1* (Table 2). This man had three primary malignancies (prostate cancer, kidney cancer, and bladder cancer). Review of 525 missense mutations using Clinvar resulted in the identification of two likely pathogenic missense mutations in two men who had the same likely pathogenic missense variant in *CHEK2*. Additional sequencing of the *HOXB13* prostate cancer predisposing gene in 93/102 men identified two carriers of the known prostate cancer-risk associated G84E

allele. One of these G84E carriers also harbored a pathogenic *BRCA2* splice variant and had three 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 to those men who were not found to have a deleterious or pathogenic germline mutation from our panel of genes (Supporting Information Table 3). Based on expert review of pedigrees using 2015 NCCN cancer genetics guidelines, only 4/11 (36%) of the individuals with a pathogenic germline variant met 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 four individuals met criteria for HBOC testing and harbored pathogenic variants in *ATM, BRIP1,* and *CHEK2* respectively; the fourth individual met 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

Among men with prostate cancer and one or more additional primary cancers, we identified deleterious or likely pathogenic germline mutations in 10.8% of this selected population. Protein truncating variants were found in six genes (*BRCA2, ATM, MLH1*, *BRIP1*, *PALB2*, and *FGFR3)* and a likely pathogenic missense mutation in one gene (*CHEK2*) from a multi-gene panel of 160 selected cancer genes, with the majority of these variants found in genes whose function is important for DNA damage repair (DDR). In addition, the prostate cancer risk associated *HOXB13* G84E allele, which has recently been shown to be associated with an increased risk for multiple cancers in a single individual, was found in two individuals with a

MPMN phenotype.[4] The most frequently mutated gene in our study was the HBOC gene, *BRCA2*. The majority (7/11) of the individuals with pathogenic or likely pathogenic germline variants did not meet current criteria for clinical genetic testing and thus would likely not have been identified as at risk for a hereditary cancer syndrome otherwise. In this pilot study, there was no difference in carrier versus non carriers in terms of prostate cancer metastatic disease, aggressiveness, or age of onset of prostate cancer. However, as this study selected for early age of onset of malignancy as one of the inclusion criteria, it would be difficult to ascertain a difference in age of onset in carriers versus non carriers from the study population.

Prevalence of germline mutations in this selected population of men with prostate cancer is similar to rates of 8-17% found in recent studies focusing on the identification of germline mutations in men with metastatic prostate cancer unselected for family history[9-11,24]. Also similarly, the majority of deleterious variants in our study were in DDR pathway genes.[9-11,24] Unique to our study population is that there was no statistical difference in presence of metastatic or aggressive disease in mutation carriers, suggesting that patients with multiple primary malignancies including prostate cancer may be at increased risk of harboring deleterious germline mutations in DDR genes regardless of metastatic disease or gleason score (e.g., Figure 2). Identifying these men with DDR mutations is now not only important for risk assessment but also for treatment given DDR deficient tumors' sensitivity to platinum-based chemotherapeutics and PARP inhibitors. A phase II study of olaparib in previously treated metastatic prostate cancer patients found 6/50 subjects harbored deleterious variants in the DDR-related genes, *ATM* and *BRCA2*, with all six showing 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 MPMN

phenotype could significantly alter the treatment course and outcomes for these patients' multiple cancers.

The identification of a risk allele within an individual with cancer also has enormous impact for that patient's family members in regards to risk assessment, cancer screening, and cancer prevention. For example, men with *BRCA2* germline mutations are known to be at increased risk for prostate cancer, and typically display 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 in unaffected *BRCA2* mutation carriers. Our current study also suggests that 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 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. Upon subsequent testing, this patient's unaffected brother was also found to have this same deleterious *BRCA2* mutation. This exemplary finding will alter recommendations for cancer screening and treatment for the proband, but also for his at-risk relatives, not only for prostate cancer but also other HBOC-associated malignancies.

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. Our study is in line with multiple recent studies of germline sequencing in cancer patients showing germline aberrations are in general more frequent than previously thought and can be found in patients

across 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 decide whether or not 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/aggressive histologies may be beneficial to add to the decision algorithm for germline testing in prostate cancer.

While the findings of our 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 tumor(s)' phenotype. We rely on a germline mutation's putative functional changes 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 two individuals in this study; however, while 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 this study.[31] In addition, as with most 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 we used 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, our pathogenic or likely pathogenic germline variant prevalence in this population may be underestimated. The reported prevalence also does not reflect any pathogenic variants harbored in genes not tested in this panel. It should also be noted that the vast majority of this selected patient population in the study (~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 may still have an increased risk for prostate or other cancers and warrant longitudinal cancer screening. These findings highlight the potential clinical and ethical dilemmas for 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 cancer patients 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 that would benefit from early screening and intervention. Our study, along with other recent germline studies, have shown that certain clinical populations such as those with a 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 meeting 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 prostate cancer patients and their families to identify heritable pathogenic variants that could prompt prostate screening in unaffected carriers- screening that is otherwise 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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Figure Legends

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 (≤55 years old), 2) diagnosed with rare cancers including pancreatic cancer, sarcoma, male breast cancer and/or 3) three or more primary malignancies in a single individual.

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

Table 1. Clinical & pedigree features for the cohort of 102 men with prostate cancer and one or more additional primary cancers.

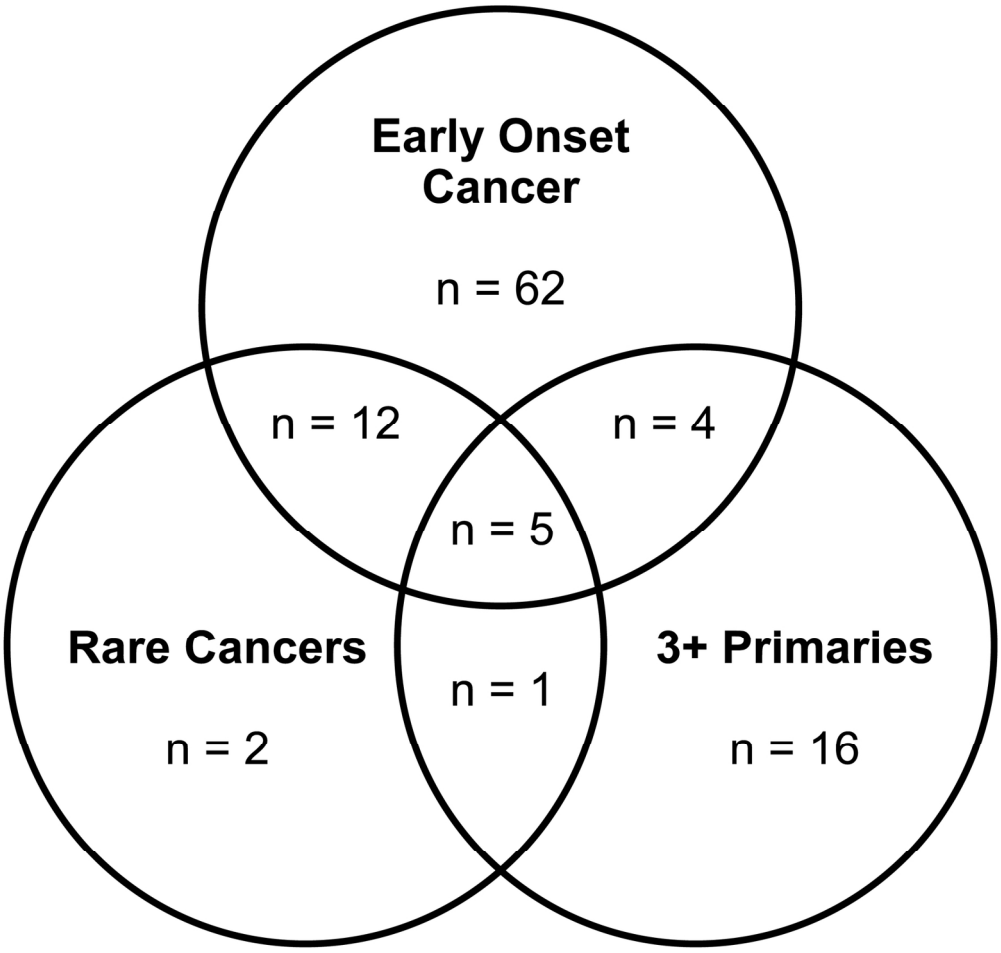
Age at diagnosis in years Median (range)	**First Cancer:** 51 (5-76) **Prostate Cancer:** 53 (31-84)
Race (percentage)	**Caucasian:** 96 (94.1) **African American:** 6 (5.9)
PSA at diagnosis[€] Median (range)	5.6 (1.0-75.5)
Gleason Score N (percentage)	**<7:** 38 (43.2) **≥7:** 50 (56.8)
Total number of multiple primaries (including prostate cancer) N (percentage)	**Two primary malignancies:** 76 (74.5) **Three primary malignancies:** 22 (21.6) **Four primary malignancies:** 4 (3.9)
Cancer Syndrome Criteria[¶] N (percentage)	**None:** 62 (60.8) **Any:** 40 (39.2) **HBOC:** 38 (37.3) **LS:** 6 (5.9) **LF:** 2 (2.0)
Clinically Aggressive Prostate Cancer[£] N (percentage)	31 (30.4)

[€]PSA=prostate specific antigen; [¶]National Comprehensive Cancer Network guidelines for clinical genetic testing for: HBOC (hereditary breast and ovarian cancer), LS (Lynch syndrome), LF(Li-Fraumeni); [£]Clinically aggressive prostate cancer defined as meeting one of the following criteria: Gleason sum>7, tumor stage T3b or T4, pre-diagnosis PSA>15ng/ml, Gleason sum=7 and pre-diagnosis PSA>10ng/ml, N1 or M1 at diagnosis.

Table 2. Pathogenic variants in men with prostate cancer and multiple primary malignancies

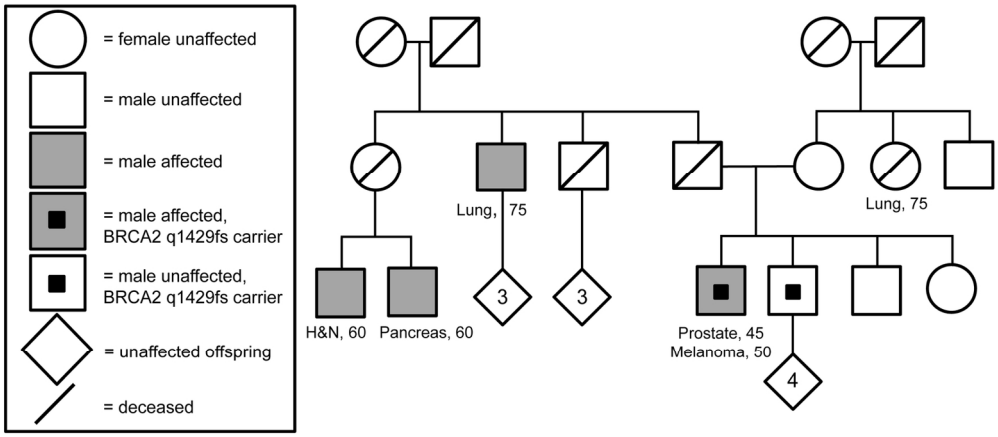
Gene	Location*	Variant Type	Allele Change	AA Change	dbSNP ID[£]	# Carriers
BRCA2	13	FS[1]	A->AT	p.Q1429fs	Rs80359440	1
	13	FS	T->TA	p.Y2215fs	Rs80359615	1
	13	SV[2]	A->T	p.T3085fs	Rs61757642	1
ATM	11	FS	ACT->A	p.T761fs	Rs587781658	1
	11	SG[3]	T->G	p.L1457X	Rs373226793	1
PALB2	16	FS	GAACAA->G	p.Q60fs	Rs180177143	1
BRIP1	17	FS	AT->A	p.N541fs		1
MLH1	3	FS	TAGCC->T	p.A661fs		1
FGFR3	4	FS	CAG->C	p.D787fs	Rs759113408	1
CHEK2	22	MS[4]	T->C	p.I157T	Rs17879961	2
HOXB13	17	MS	A->G	p.G84E	Rs138213197	2

*Chromosomal location; AA=amino acid; [£]http://www.ncbi.nlm.nih.gov/projects/SNP/; [1]frameshift; [2]splice variant; [3]stopgain; [4]missense.



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

78x75mm (600 x 600 DPI)



Pedigree analysis of proband with BRCA2 q1429fs germline mutation and multiple primary malignant neoplasm phenotype. H&N= head & neck cancer.

75x33mm (600 x 600 DPI)

Supporting Information Table 1.
Genes included in the Qiagen GeneRead DNAseq CCP versions 1 and 2.

Gene	Included in Version 1	Included in Version 2
ABCC1	yes	
ABL1	yes	yes
AKT1	yes	yes
AKT2		yes
AKT3	yes	
ALK	yes	yes
AMER1		yes
APC	yes	yes
AR		yes
ARID1A	yes	yes
ARID2		yes
ASXL1	yes	yes
ATM	yes	yes
ATRX		yes
BAP1		yes
BCL6		yes
BCOR		yes
BRAF	yes	yes
BRCA1	yes	yes
BRCA2	yes	yes
BRIP1		yes
BTK		yes
BUB1B		yes
CARD11	yes	yes
CASP8	yes	
CBL	yes	yes
CBLB		yes
CD79A		yes
CD79B		yes
CDC73	yes	yes
CDH1	yes	yes
CDK12		yes
CDK4		yes
CDKN2A	yes	yes
CEBPA	yes	
CHEK2		yes
CIC		yes
CREBBP	yes	yes
CRLF2	yes	yes
CSF1R	yes	yes
CTNNA1	yes	

CTNNB1	yes	yes
CTSL1	yes	
CYLD	yes	yes
DAXX		yes
DDB2		yes
DDR2		yes
DICER1		yes
DNMT3A	yes	yes
ECT2L		yes
EGFR	yes	yes
EP300	yes	yes
EPCAM		yes
ERBB2	yes	yes
ERBB3		yes
ERBB4	yes	yes
ERCC5		yes
ESR1		yes
EZH2	yes	yes
FAM123B	yes	
FAM46C		yes
FANCA		yes
FANCD2		yes
FANCE		yes
FAS		yes
FBXO11		yes
FBXW7	yes	yes
FGFR1	yes	
FGFR2	yes	yes
FGFR3	yes	yes
FH		yes
FIGF	yes	
FKBP9	yes	
FLCN		yes
FLT1	yes	
FLT3	yes	yes
FLT4	yes	
FOXL2	yes	
FUBP1		yes
GATA1	yes	yes
GATA2	yes	yes
GATA3		yes
GNA11	yes	yes
GNAQ	yes	yes
GNAS	yes	yes
GPC3		yes
GRIN2A	yes	yes
H3F3A		yes

HDAC4	yes	
HIST1H3B		yes
HNF1A	yes	yes
HRAS	yes	yes
HSP90B1	yes	
HSPH1		yes
IDH1	yes	yes
IDH2	yes	yes
IGF2R	yes	
IKZF1		yes
IL6ST		yes
IL7R	yes	yes
JAK1		yes
JAK2	yes	yes
JAK3	yes	yes
KDM6A	yes	yes
KDR	yes	yes
KIT	yes	yes
KLF6		yes
KMT2D		yes
KRAS	yes	yes
MAP2K1	yes	yes
MAP2K2		yes
MAP2K4	yes	yes
MAP3K1		yes
MAP4K3		yes
MDM2		yes
MED12		yes
MEN1	yes	yes
MET	yes	yes
MLH1	yes	yes
MPL	yes	
MSH2	yes	yes
MSH6	yes	yes
MTOR	yes	yes
MUTYH		yes
MYC		yes
MYD88	yes	yes
NF1	yes	yes
NF2	yes	yes
NFE2L2		yes
NFKBIA		yes
NOS1	yes	
NOTCH1	yes	yes
NOTCH2	yes	yes
NPM1	yes	yes
NRAS	yes	yes

NTN3	yes	
PALB2		yes
PARP1	yes	
PARP4	yes	
PAX5	yes	yes
PBRM1		yes
PDGFRA	yes	yes
PDGFRB	yes	
PHF6		yes
PIK3C2A	yes	
PIK3CA	yes	yes
PIK3R1	yes	yes
PIK3R5	yes	
PMS2		yes
POLR3A	yes	
PPP2R1A	yes	yes
PRDM1		yes
PRKAR1A	yes	yes
PRKCE	yes	
PTCH1	yes	yes
PTEN	yes	yes
PTGS2	yes	
PTPN11	yes	yes
PTPRC	yes	
RAC1		yes
RB1	yes	yes
RET	yes	yes
ROS1	yes	yes
RUNX1	yes	
SDHB		yes
SETD2	yes	yes
SF3B1		yes
SLC7A8		yes
SMAD2	yes	
SMAD4	yes	yes
SMARCA4	yes	yes
SMARCB1	yes	yes
SMO	yes	yes
SOCS1	yes	
SPOP		yes
SRC	yes	yes
STK11	yes	yes
SUFU		yes
TERT	yes	yes
TET2	yes	
TGFBR2	yes	
TNFAIP3	yes	yes

TNFRSF14		yes
TNKS	yes	
TOP1	yes	
TP53	yes	yes
TRRAP	yes	
TSC1		yes
TSC2		yes
TSHR	yes	yes
U2AF1		yes
VHL	yes	yes
WT1	yes	yes
XPC		yes
XPO1	yes	
ZNF2		yes
ZRSR2		yes

Supporting Information Table 2. Additional primary cancers in the cohort of 102 men with prostate cancer.

Additional Primary Cancers	Number of Subjects
Atypical fibrous histiocytoma	1
Bladder	6
Bladder & Colon	1
Bladder & Esophageal	1
Bladder & Kidney	3
Bladder & Liver	2
Bladder & Lung	2
Bladder & Lymphoma	1
Bladder & Melanoma	1
Bone	1
Brain	2
Brain-Glioblastoma & Melanoma	1
Carcinoid, small bowel	1
Colon	5
Colon & Vocal cord	1
Colon/rectum & Lung	1
Esophagus	3
Esophagus & Pancreas	1
Head and Neck	5
Head and Neck & Lung	1
Head and Neck (2 primaries) & Thyroid	1
Head and Neck & Sarcoma	1
Hodgkins	1
Kidney	5
Kidney & Melanoma	1
Liposarcoma	1
Lung	4
Lung & Melanoma & Sarcoma	1
Lung & Melanoma & Pancreas	1
Lymphoma	1
Lymphoma & Renal cancer	1
Mantle cell & Thyroid	1
Melanoma	28
Melanoma & Renal cancer	1
Pancreas	4
Parathyroid	1
Renal cancer & Sarcoma (2 primaries)	1
Renal cancer & Thyroid	1
Testicular	4
Thyroid	3

Supporting Information Table 3. Clinicopathologic characteristics of mutation carriers versus non-carriers.

	All Samples (N=102)	Carriers (N=11)	Non Carriers (N=91)	P Value
Age at Dx of Prostate Cancer (median/range)	53 (31-84)	54 (46-71)	53 (31-84)	0.8750*
Pre-Dx PSA* ** (median/range)	5.55 (1-75.56)	5.5 (3.47-21.4)	5.6 (1-75.56)	0.6498*
Gleason Score (N/%)****				
<7	38 (43.2)	6 (54.5)	31(40.2)	0.5158**
7	43 (48.9)	4 (36.4)	40 (51.9)	
>7	7 (8.0)	1 (9.1)	6 (7.8)	
Ancestry (N/%)				
European	96 (94.1)	10 (90.9)	86 (94.5)	0.5050**
African	6 (5.9)	1 (9.1)	5 (5.5)	
Clinically Aggressive PC (N/%)				
Yes	31 (30.4)	4 (36.4)	27 (29.7)	0.7316**
No	71 (69.6)	7 (63.6)	64 (70.3)	
Number of Cancers (N/%)				
2	76 (74.5)	7 (63.6)	69 (75.8)	0.4649**
3 or more	26 (25.5)	4 (36.4)	22 (24.2)	
Criteria for Clinical Testing (N/%)				
Yes	40 (39.2)	4 (36.4)	35 (38.5)	1**
No	62 (60.8)	7 (63.6)	56 (61.5)	

*Two sided t-test; **Fisher's exact test; ***N=83 total samples with available pre-diagnosis PSA; ****N=88 total samples with available Gleason score.