Evaluating Germline Testing Panels in Southern African Males With Advanced Prostate Cancer

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ABSTRACT

Background: Germline testing for prostate cancer is on the increase, with clinical implications for risk assessment, treatment, and management. Regardless of family history, NCCN recommends germline testing for patients with metastatic, regional, very-high-risk localized, and high-risk localized prostate cancer. Although African ancestry is a significant risk factor for aggressive prostate cancer, due to a lack of available data no testing criteria have been established for ethnic minorities. **Patients and Methods:** Through deep sequencing, we interrogated the 20 most common germline testing panel genes in 113 Black South African males presenting with largely advanced prostate cancer. Bioinformatic tools were then used to identify the pathogenicity of the variants. **Results:** After we identified 39 predicted deleterious variants (16 genes), further computational annotation classified 17 variants as potentially oncogenic (12 genes; 17.7% of patients). Rare pathogenic variants included *CHEK2* Arg95Ter, *BRCA2* Trp31Arg, *ATM* Arg3047Ter (2 patients), and *TP53* Arg282Trp. Notable oncogenic variants of unknown pathogenicity included novel *BRCA2* Leu3038Ile in a patient with early-onset disease, whereas patients with *FANCA* Arg504Cys and *RAD51C* Arg260Gln reported a family history of prostate cancer. Overall, rare pathogenic and early-onset or familial-associated oncogenic variants were identified in 6.9% (5/72) and 9.2% (8/87) of patients presenting with a Gleason score ≥ 8 or $\geq 4 + 3$ prostate cancer, respectively. **Conclusions:** In this first-of-its-kind study of southern African males, we provide support of African inclusion for advanced, early-onset, and familial prostate cancer genetic testing, indicating clinical value for 30% of current gene panels. Recognizing current panel limitations highlights an urgent need to establish testing guidelines for men of African ancestry. We provide a rationale for considering lowering the pathologic diagnostic inclusion criteria and call for further genome-wide interrogation to ensure the best possible African-relevant prostate cancer gene panel.

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Background

Genetic testing for germline pathogenic variants is fast becoming routine practice for men presenting with high-risk prostate cancer (PCa) in Western countries.[1] Rare pathogenic variants in medium- to high-penetrance genes not only have therapeutic implications but independently or together with common variants predict disease susceptibility and adverse outcomes for the patient and family.[2] Studies of PCa germline are generally focused on European ancestral populations and have linked PCa risk and advanced disease to rare or low-frequency pathogenic variants within DNA repair and cancer predisposition genes.[3,4] Consequently, commercially available panels for PCa germline genetic testing include a combination of up to 20 genes, namely *BRCA1*, *BRCA2*, *ATM*, *CHEK2*, *PALB2*, *TP53*, *MLH1*, *MSH2*, *MSH6*, *PMS2*, *MUTYH*, *RAD50*, *RAD51C*, *RAD51D*, *APC*, *EPCAM*, *HOXB13*, *NBN*, *BRIP*, and *FANCA*.

It is well established that African ancestry is a significant risk factor for advanced PCa, with the lifetime risk of dying from PCa reported to increase by 2.3- to 5-fold for African Americans compared with all other ethnic groups within the United States.[5] For sub-Saharan Africa, PCa mortality rates are almost 2.7-fold greater than global estimates.[6] Along with a lack of germline genetic screening, studies focused on rare/low-frequency pathogenic variants within Africa have been lacking. According to the Philadelphia PCa Consensus Conference, no agreement could be reached about germline testing of people with African ancestry due to a lack of data.[7] A single study merging data from African American males with data from males from East Africa, specifically Uganda, associated rare *BRCA2*, *ATM*, *PALB2*, and *NBN* pathogenic variants with aggressive PCa, identifying novel African-specific predicted deleterious variants (PDVs).[8] The latter study highlighted the need for further evaluation of these European-biased panels across different regions and populations represented within sub-Saharan Africa.

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Home to the most genetically diverse population,[9] southern Africa has PCa mortality rates that are 1.4-fold greater than those in eastern Africa and 2.7-fold greater than those in the United States, at 22 per 100,000,[6] whereas Black South African males are at a 2.1-fold increased risk for advanced PCa at presentation compared with African American males (adjusted for age).[10] Our first-of-its-kind study aimed to determine whether current PCa germline screening panels have clinical benefit for males from southern Africa.

Patients and Methods

The study cohort included 113 South African males diagnosed with predominantly advanced PCa with a Gleason score or an International Society of Urological Pathology Grade Group (ISUP GG) biased toward high-risk ISUP GG ≥4 PCa (72/113, 63.7%), with an almost even distribution of intermediate-risk (ISUP GG 2/3, 18.6%) and low-risk (ISUP GG 1, 17.7%) disease; a mean age of 67 years (range, 45–99 years); a prostate-specific antigen (PSA) level of 370 ng/mL (range, 8–4,841 ng/mL) at diagnosis; and a family history of prostate or any cancer (Table 1). The elevated PSA levels observed within our study cohort have previously been reported for Black South African males.[10] Patients provided informed consent to participate in the study and were recruited as part of the Southern African Prostate Cancer Study (SAPCS), with approval granted by the University of Pretoria Faculty of Human Research Ethics Committee (HREC #43/2010, including US Federalwide Assurance FWA00002567 and IRB00002235 IORG0001762) in South Africa. Molecular genetic research for patients from the SAPCS bioresource was approved by the St. Vincent's Hospital Human Research Ethics Committee in Sydney, Australia (#SVH15/227).

Table 1. Patient Characteristics

Characteristic	n
Patients, N	113
Mean [SD] age, y	67.0 [8.3] (range, 45–99)
Mean [SD] PSA	370 [959] (range, 8–4,841)
ISUP grade	
1	20 (17.7%)
2	6 (5.3%)
3	15 (13.3%)
4, 5	72 (63.7%)
Cancer family history	
Prostate	5
Breast	5
Other	4
Unknown	101

Abbreviations: ISUP, International Society of Urological Pathology; PCa, prostate cancer; PSA, prostate-specific antigen.

Through genome-wide interrogation of 7,472,833 biallelic single-nucleotide variants (SNVs), all patients were classified genetically as being of African ancestry. As previously described[11] and in brief, DNA was extracted from whole blood and 2 × 150 cycle paired-end whole genomes were sequenced (Illumina HiSeq X Ten or NovaSeq) to an average of 46× coverage (range, 30–97×) and aligned to a GCRh38 reference, SNVs and small insertions and deletions (indels; <50 base pairs) were called using the Genome Analysis Toolkit (Broad Institute),[12] and larger high-confidence structural variants (SVs) were called using Manta (version 1.6.0, Illumina).[13] The 20 genes included in this study were selected based on the latest NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines) for Prostate Cancer (Version 4.2022),[14] multigene prostate cancer-specific panels,[7] and the recent suggestion for *APC*, *MUTYH*, and *RAD50* as PCa germline gene panel candidates.[15] Variant data for the 20-gene panel (including 2,000 bases upstream and downstream) were made available for this study through the SAPCS Data Access Committee and sequence data were deposited in the European Genome-Phenome Archive (https://ega-archive.org) under study accession number EGAS00001006425 and dataset accession number EGAD00001009067. The variant data are available via the European Variation Archive under project number PRJEB54721.

After the removal of common variants (minor allele frequency [MAF] ≥0.05), rare and low-frequency variations were further defined as a PDV if they were classified using ≥1 of 2 prediction tools as deleterious (Sorting Intolerant From Tolerant [SIFT]) or probably/possibly damaging (PolyPhen-2 [Polymorphism Phenotyping v2]), and/or they resulted in a stop-gain, missense, or splice-site donor variant, as previously described.[16] Variants identified as benign or likely benign in ClinVar were then excluded from the study. Further refinement for pathogenicity, based on the guidelines of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP)[17] using InterVar (Keck School of Medicine, USC), led to further exclusion and PDV refinement. Finally, the Cancer Genome Interpreter[18] tool was used to establish potential oncogenic status (high-risk gene status), specifically for PDVs with either undetermined pathogenicity or a lack of pathogenic evaluation, with further oncogenic potential (likely pathogenic) determined as a result of family history and/or early-onset disease presentation. Furthermore, SVs leading to major gene disruption were further defined as a predicted deleterious SV. All variations were manually observed and inspected through the Integrative Genomics Viewer[19] and patients' clinical records were assessed for each PDV; patients presenting

with germline potentially oncogenic *MUTYH* variants were interrogated for loss of heterozygosity (LoH) within the matched tumor using a TitanCNA[20] inferred copy number data generated previously.[11]

Results

Interrogating a total of 1,503,374 bases across the 20 genes from 113 patients of African ancestry with PCa, we identified 21,899 SNVs, 4,626 indels, and 73 SVs. SNVs and indels were assessed as PDVs and for pathogenic relevance using a 4-phase identifier system (Figure 1). Excluding for common variants (MAF ≥0.05) based on National Center for Biotechnology Information–derived African and European population identifiers, 78 SNVs were identified using SIFT and/or PolyPhen-2 as potentially deleterious (supplemental eTable 1, available with this article at JNCCN.org), of which *BRCA2* p.Ile2944Phe (c.8830A>T; MAF, 0.0619) and *APC* p.Ser26Arg (c.78C>A; MAF, 0.0663) were common within our study population. Although their potential contribution as low-penetrance susceptibility alleles could not be assessed, notably, *APC* p.Ser26Arg occurred in 13 patients, of whom 12 (92.3%) presented with ISUP GG ≥4 and 1 presented with ISUP GG 3, which was greater than expected for the study distribution (Table 1). *HOXB13* was the only gene with no SNVs identified during phase I analysis, which included absence for the most recently identified *HOXB13* p.Ter285Lys (c.853delA; rs77179853) associated with advanced PCa in West African men[21] and the well-established *HOXB13* p.Gly84Gluz.[22]

During phases II and III we excluded for variants determined not to be pathogenic. Specifically, 26 variants reported to be "benign" or "likely benign" in ClinVar (phase II) and 12 variants categorized as likely benign using the ACMG/AMP criteria (phase III) were removed. The 38 remaining PDVs found in 52 of the 113 patients were distributed across 16 of the selected genes; 3 were confirmed as pathogenic—*CHEK2* p.Arg95Ter (c.283G>A; ISUP GG 3), *ATM* p.Arg3047Ter (c.9139C>T; 2 patients, ISUP GG 4/5), and *TP53* p.Arg282Trp (c.844C>T; ISUP GG 4)—and 1 was likely pathogenic: *BRCA2* p.Trp31Arg (c.91T>C; ISUP GG 5 and family history of breast cancer), leaving 34 with undetermined pathogenicity.

Considering that a lack of African-relevant data would drive a higher proportion of PDVs of unknown or unconfirmed pathogenicity, we used the power of the Cancer Genome Interpreter (phase IV) to further interrogate the 34 undefined PDVs. We identified 12 predicted oncogenic variants spanning *APC*, *ATM*, *BRCA2*, *CHEK2*, *MUTYH*, *FANCA*, *MSH6*, *PALB2*, *PMS2*, *RAD50*, and

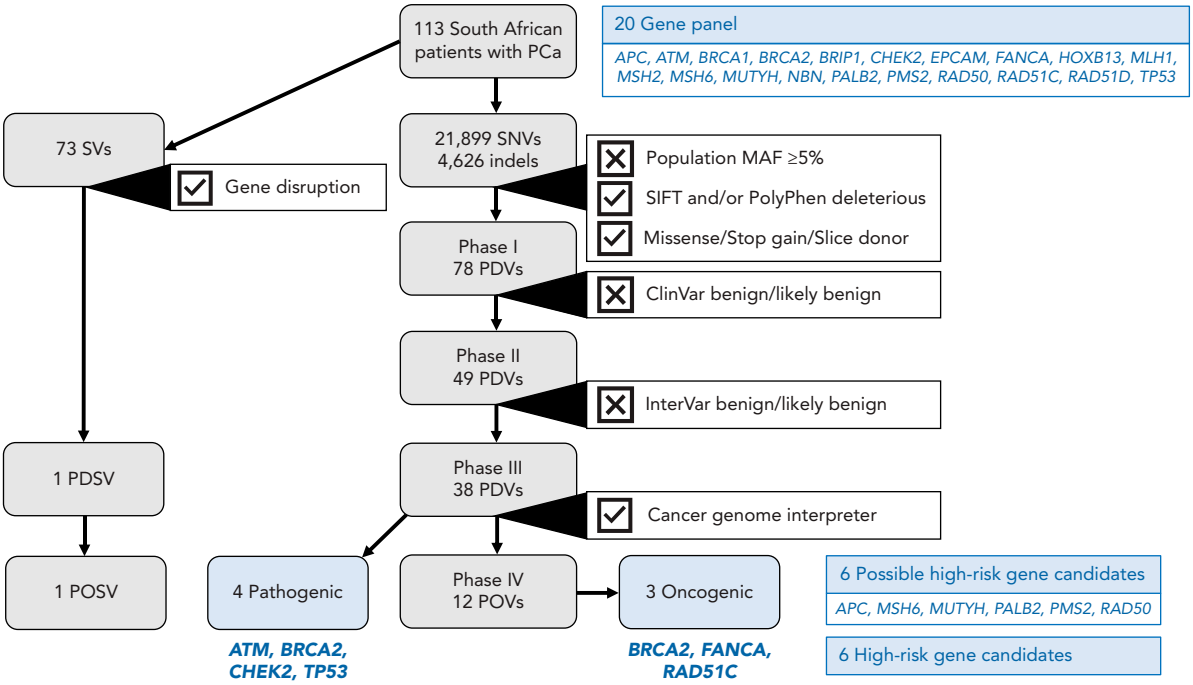

Figure 1. Phased workflow for identifying PDVs, including SNVs, small indels, and SVs/PDSVs, and filtered for MAF <0.05 and variant annotation including known pathogenic, predicted oncogenic, and high-risk POV/SVs in a cohort of 113 African patients with PCa for establishing gene-inclusion criteria for African-specific 20-gene panel (blue) germline testing, resulting in 12 candidate genes: 6 with high risk (pathogenic and/or oncogenic: *ATM*, *BRCA2*, *CHEK2*, *TP53*, *FANCA*, and *RAD51C*) and 6 with possible high risk (potentially oncogenic: *APC*, *MSH6*, *MUTYH*, *PALB2*, *PMS2*, and *RAD50*).

Abbreviations: indel, insertion and deletion; MAF, minor allele frequency; PCa, prostate cancer; PDSV, predicted deleterious structural variant; PDV, predicted deleterious variant; POSV, potentially oncogenic structural variant; POV, potentially oncogenic variant; SNV, single-nucleotide variant; SV, structural variant.

RAD51C, of which 2—*BRCA2* p.Leu3038Ile (c.9112C>A; ISUP GG 3) and *RAD50* p.Leu34Phe (c.102G>C; ISUP GG 4)—were novel (Table 2). Although age-related clonal hematopoiesis of intermediate potential (CHIP) was recently reported not to be a risk factor for PCa in males of European ancestry and not significantly associated with potentially pathogenic/deleterious DNA repair gene variants,[23] to ensure that pathogenic/oncogenic variants were inherited rather than CHIP-derived, read counts were used to determine variant allele frequencies, with all well above the 10% CHIP upper threshold (average, 48.6%; range, 20.9%–66.7%). Through further interrogation of SV break points, we observed an additional novel variant that disrupted the 3′ end of *MUTYH* via translocation with chromosome 2 (chr2:203987190), supported by split-read evidence (16/74 reads; variant allele frequency = 21.6%; ISUP GG 4). Predicting that RNA transcript breakage would result in defective base excision repair, we assumed oncogenic potential. Although studies suggest that *MUTYH* requires biallelic germline presentation for pathogenicity,[24] a large study of 10,389 patients with monoallelic *MUTYH* pathogenic cancer (33 tumor types) compared with >100,000 healthy individuals showed monoallelic variants that not only were increased in patients with cancer but also, together with a second somatic hit through LoH, promoted tumorigenesis.[25] Although somatic LoH was not detected in the patient with the *MUTYH* translocation, of the 2 patients presenting with the splice donor *MUTYH* variant (rs140288388 G>A), 1 (ISUP GG 2) presented with and 1 (ISUP GG 5) presented without matched tumor *MUTYH* LoH.

Overall, the 4 pathogenic and 13 potentially oncogenic variants were identified in 12 of the 20 genes (60%), which included 2 each in *ATM*, *BRCA2*, *APC*, *CHEK2*, and *MUTYH*, and 1 each in *FANCA*, *MSH6*, *PALB2*, *PMS2*, *RAD50*, *RAD51C*, and *TP53*. Further clinical observations for the pathogenic and potentially oncogenic variants in 20 (17.7%) patients showed that 75% presented with advanced PCa (ISUP GG ≥4), which increased to 90% when we included patients with ISUP GG 3. All but a single

Table 2. Pathogenic and Potentially Oncogenic Germline Variants Identified

Gene	Position (hg38)	Codon Change	dbSNP	Protein Change	Variant Type	Pathogenic/ Oncogenic[a]	No. of Patients (prevalence)	ALT/Total Reads[b]	VAF[b]
ATM	chr11:108365476	c.9139C>T	rs121434219	Arg3047Ter	Stop-gain	Pathogenic	2 (1.7%)	15/31, 17/36	47.2%, 48.4%
ATM	chr11:108227834	c.131A>G	rs150143957	Asp44Gly	Missense	Oncogenic	4 (3.5%)	8/24, 13/23, 22/38, 20/44	33.3%, 56.5%, 57.9%, 45.5%
BRCA2	chr13:32319100	c.91T>C	rs80359182	Trp31Arg	Missense	Pathogenic	1 (0.9%)	18/41	43.9%
BRCA2	chr13:32379908	c.9112C>A	Novel variant	Leu3038Ile	Missense	EO oncogenic	1 (0.9%)	26/43	60.5%
CHEK2	chr22:28734439	c.283G>A	rs587781269	Arg95Ter	Stop-gain	Pathogenic	1 (0.9%)	11/23	47.8%
CHEK2	chr22:28734558	c.164G>A	rs765799649	Ser55Phe	Missense	Oncogenic	1 (0.9%)	19/29	65.5%
MUTYH	chr1:45330036	NA	Novel variant	Translocation	Structural	Oncogenic	1 (0.9%)	16/74	21.6%
MUTYH	chr1:45331180	G>A	rs140288388	Splice site	Splice donor	Oncogenic	2 (1.7%)	34/55, 26/39	61.8%, 66.7%
APC	chr5:112827986	c.1606G>A	rs138098808	Glu536Lys	Missense	Oncogenic	1 (0.9%)	15/42	35.7%
APC	chr5:112827984	c.1604C>T	rs75870842	Ser535Phe	Missense	Oncogenic	1 (0.9%)	15/34	44.1%
FANCA	chr16:89783063	c.1510G>A	rs200291237	Arg504Cys	Missense	FH oncogenic	1 (0.9%)	23/41	56.1%
MSH6	chr2:47806344	c.3787C>T	rs367912290	Arg1263Cys	Missense	Oncogenic	1 (0.9%)	19/44	43.2%
PALB2	chr16:23626343	c.2641C>T	rs766315705	Gly881Ser	Missense	Oncogenic	1 (0.9%)	20/35	57.1%
PMS2	chr7:6003981	c.241C>T	rs730881919	Glu81Lys	Missense	Oncogenic	1 (0.9%)	15/26	57.7%
RAD51C	chr17:58709932	c.779G>A	rs730881926	Arg260Gln	Missense	FH oncogenic	1 (0.9%)	11/27	40.7%
RAD50	chr5:132557426	c.102G>C	Novel variant	Leu34Phe	Missense	Oncogenic	1 (0.9%)	20/40	50%
TP53	chr17:7673776	c.844G>A	rs28934574	Arg282Trp	Missense	Pathogenic	1 (0.9%)	9/43	20.9%

Abbreviations: ALT, alternative; CGI, Cancer Genome Interpreter; dbSNP, Single Nucleotide Polymorphism Database; EO, early-onset; FH, family history; NA, not applicable; PCa, prostate cancer; PDV, potentially deleterious variant; VAF, variant allele frequency.
[a]Pathogenic defined by ClinVar as pathogenic/likely pathogenic; oncogenic defined as PDVs further identified by CGI as being oncogenic, with 3 showing additional pathogenicity defined as family FH or EO.
[b]Number of ALT or variant reads against the total number of reads (including reference predicted) per genome used to determine the VAF for each pathogenic/oncogenic variant.

oncogenic variant were rare; specifically, *ATM* p.Asp44Gly (c.131A>G) was found in 4 patients (MAF, 0.017) all diagnosed with ISUP GG 4 PCa and lay within the telomere-length maintenance and DNA damage repair domain, which is critical for telomere maintenance function and cell viability[26] (Figure 2A). Notably, the patient with the novel *BRCA2* p.Leu3038Ile variant that lies within the central oligonucleotide/oligosaccharide single-stranded DNA binding fold of the highly conserved DNA binding domain, critical for mediating homologous recombination and maintaining genome stability[27] (Figure 2B), presented at an age 13 years younger at diagnosis than the study mean (54 vs 67 years of age). In addition, patients presenting with *FANCA* Arg504Cys (c.1510G>A; ISUP GG 4; age 65 years) and *RAD51C* Arg260Gln (c.779G>A; ISUP GG 3; age 64 years) reported a positive family history of PCa.

Discussion

Although PCa germline testing gene panels have almost exclusively been identified through studies focused on non-African populations, African ancestry is a significant risk factor for PCa and adverse outcomes.[5,6,8] Aside from a single East African study,[8] further inclusion across sub-Saharan Africa has been lacking. In addition, studies are recurrently reporting germline variations in genes associated with risk for aggressive PCa.[2,8] However, identifying high-penetrance genes that mediate the genetic pathways and influence the risk and course of the disease is challenging given the high numbers of sporadic cases of disease and the rarity of pathogenic variations.[2] In this first-of-its-kind study for southern Africa, we focused on Black South African males presenting with largely advanced disease, identifying 38 PDVs and one predicted deleterious SV in 16 of the 20 most common genes included in PCa screening panels. Noting that 10.2% of the PDVs/SVs were novel, we find that the bias toward variants of uncertain pathogenic significance, together with the identification of a single known PCa pathogenic variant, *CHEK2* p.Arg95Ter,[28] further emphasizes the need for further African-specific investigation to establish tailored PCa screening panels.

Overall, 5 patients presented with a known rare pathogenic variant. Except for *CHEK2* p.Arg95Ter, pathogenic variants were novel to PCa and included *ATM* p.Arg3047Ter, which is located in the FATC domain, was previously reported to block lymphocyte development (Figure 2A), and is considered pathogenic for hereditary cancer-predisposing and ataxia-telangiectasia syndromes[29]; *TP53* p.Arg282Trp, most predominantly associated with Li-Fraumeni and hereditary cancer-predisposing syndrome; and *BRCA2* p.Trp31Arg, shown to be likely pathogenic in hereditary breast and ovarian cancer. Notably, the African patient presenting with the pathogenic *BRCA2* variant reported a family history of breast cancer. The presence of the *ATM* pathogenic variant in 2 patients with advanced PCa calls for further consideration

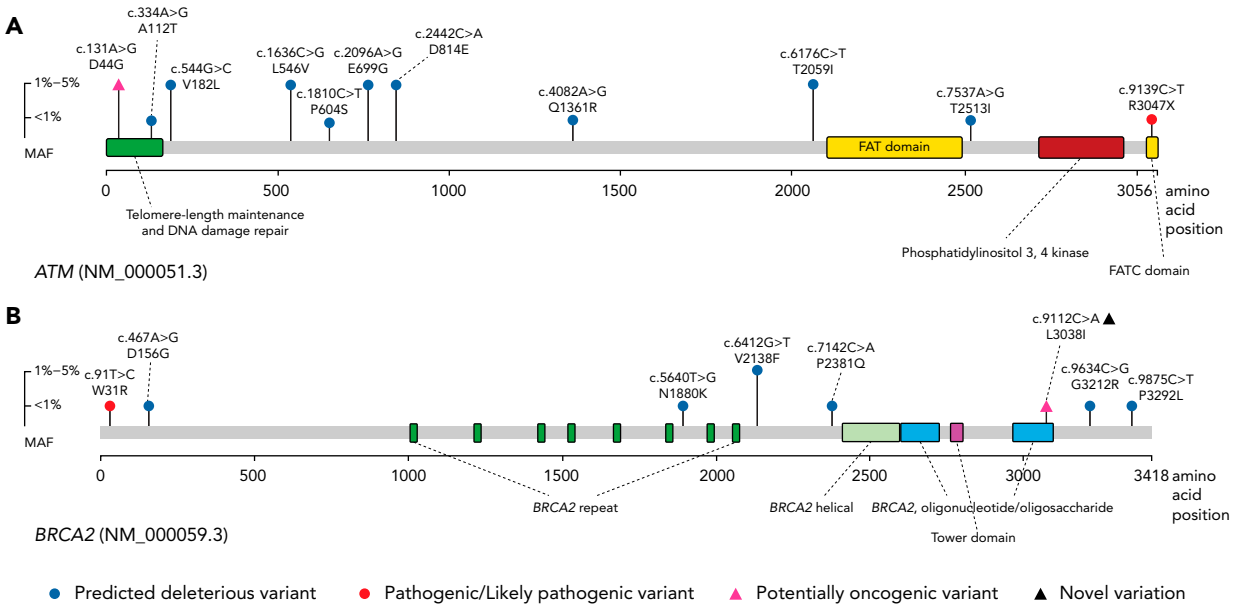

Figure 2. Amino acid position, MAF, and variant type, including pathogenic/likely pathogenic (ClinVar/InterVar), potentially oncogenic (CGI), predicted deleterious (SIFT/PolyPhen), splice variant, or novel, identified in 113 patients of African ancestry with PCa for the genes presenting with the highest number of potentially impactful germline variants in our study: **(A)** *ATM* and **(B)** *BRCA2*.
Abbreviations: CGI, Cancer Genome Interpreter; MAF, minor allele frequency; PCa, prostate cancer; PolyPhen, Polymorphism Phenotyping v2; SIFT, Sorting Intolerant From Tolerant.

for population-specific relevance. Overall, we observed a lower prevalence of rare pathogenic variants among our cohort (5.6%; 4/72 patients with ISUP GG 4/5) compared with the 11.8% reported for non-African patients with confirmed metastatic PCa,[30] whereas our data are comparable to those reported for East African patients (5.7%, unknown metastatic status) and African American patients (3.4%), including *ATM* and *BRCA2* presenting as African-relevant contributing pathogenic candidates.[8]

To address the lack of African-relevant data and bias toward PDVs of unconfirmed pathogenicity, we further annotated for oncogenic potential, identifying together with the pathogenic variants 13 additional potentially oncogenic variants, which taken together impacted 12 genes and 17.7% of the patients. Compared with the overall study, patients presenting with pathogenic and potentially oncogenic variants were biased toward advanced PCa, defined as ISUP GG ≥4 (63.7% vs 75%) or ISUP GG ≥3 (77.0% vs 90%), respectively. Although the true pathogenicity of the oncogenic variants is yet to be determined, the observed early-onset novel *BRCA2* and PCa familial *RAD51C* and *FANCA* variants provide additional merit for their pathogenic potential. Irrespective of pathogenicity, a recent US study suggested that the *RAD* family of genes (although excluding for *RAD51C*) is significantly more likely to harbor a germline variant in African (n=259) versus European (n=272) ancestral patients presenting with a bias toward low-risk disease (ISUP GG <3, 79.6% vs 83.5%, respectively).[31] As the most recent addition to the PCa gene panels, our study raises additional considerations for the inclusion of *FANCA*, at least when considering men of African ancestry. When we considered the early-onset and familial PCa associating rare oncogenic variants with known pathogenic variants, the prevalence increased to 6.9% (5/72) of patients presenting with high-risk or very-high-risk PCa, defined using the current NCCN Guidelines (ISUP GG ≥4)[14] or 9.2% (8/87) using the expanded criteria (ISUP GG ≥3).

Appreciating the limitation of the study size (113 patients) yet the bias toward advanced disease, notable exclusions included the lack of pathogenic/oncogenic *BRCA1*, *MSH2*, and *HOXB13* variants. Unlike *BRCA2*, an association between PCa risk and *BRCA1* mutation has been less consistent[32,33]; however, the NCCN Guidelines state that eligible patients should be evaluated for both *BRCA2* and *BRCA1* status.[14] More recently, the relative risk for *BRCA1* PCa-associated pathogenic variants has been associated with younger age of diagnosis.[32] Notably, our cohort was biased toward older age at presentation (mean age, 67 years; range, 45–99 years). In contrast to our study, pathogenic *BRCA1* variants in African Americans have been associated with early-onset[34] and metastatic PCa,[35] whereas both pathogenic and *BRCA1* variants of unknown significance are reportedly more frequent in African versus European ancestral Americans with PCa.[31,36,37] Compared with our study, the latter were overall biased toward patients presenting with lower-risk disease. *MSH2* is another gene that is known to frequently harbor pathogenic variants associated with advanced PCa[38]; however, only one PDV was found in the southern African population in our study and was identified as likely benign according to the ACMG/AMP classification. Although the 2017 Philadelphia Prostate Cancer Consensus Conference recommended testing for *HOXB13* variants, especially for suspected hereditary PCa,[39] we observed a high conservation of *HOXB13* in our limited study.

Comparing our data with the larger East African study (n=651),[8] although notable differences included the lack of pathogenic (or oncogenic) *NBN* variants in southern African patients and conversely *TP53* for East African patients, similarities included a notable exclusion for *MLH1* and *MSH2* across 764 sub-Saharan African patients. One should further appreciate that in addition to geographic and ethnic differences, there were notable differences in patient age (mean age, 67 vs 70 years) and tumor pathology (ISUP GG ≥4, 63.7% vs 47.2%) at presentation between the southern and East African studies (the latter of 441 patients with known pathology), respectively. Although the East African study was supported by African American data (n=1,447; ISUP GG ≥4, 24.7%),[8] the recent African American study[36] with a larger representation of patients with advanced PCa (n=237; ISUP GG ≥4, 32.9%) and interrogating a 14-gene panel concurred with the pathogenic relevance for *BRCA2* and *ATM* while further confirming the East African–identified *PALB2*, denoted as a high-risk gene candidate in our study. Additional overlaps with African American data most recently reported for 276 DNA damage repair genes[31] include the potential relevance for *PMS2* and another *RAD* family member, *RAD50*, as high-risk gene candidates. We concur that men of African ancestry present with a narrower spectrum of pathogenic variants compared with non-African men, highlighting the limitations for African-relevant translation. Conversely, after observing 12 of the 20 most-common tested genes presented with potentially oncogenic variants in southern African patients with PCa, we call for further clarification for pathogenicity. Notably, more studies need to be conducted within southern Africa and across the African diaspora to validate current findings and develop germline testing panels that are more aligned to the genetic profile of this population.

Conclusions

The high PCa mortality rates reported for southern African males, and in turn the high number, novelty, and expanse of oncogenic variants identified in our study, including pathogenic variants spanning *ATM*,

BRCA2, *CHEK2*, and *TP53*, with a further familial link to *FANCA* and *RAD51C* and an additional early-onset link to *BRCA2* (although the pathogenicity of variants in *APC*, *MSH6*, *MUTYH*, *PALB2*, *PMS2*, and *RAD50* cannot be excluded), highlight the immediate benefits for including African patients in largely routine Westernized germline carrier screening programs. Conversely, we highlight the limitations of the current 20-gene panel approach for men of African ancestry. Our data support the notion that alternative unknown gene targets could be playing a significant role for males of African ancestry, and as such, we call for additional African inclusion and genome-wide interrogation. Ultimately, guidelines focused on African inclusion need to be established to ensure that the clinical benefit for PCa screening through prevention or targeted therapy is available to all globally.

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eTable 1: Potentially Deleterious Variations and the Justification for Deleteriousness as Described by Each Dataset

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Chrom	Position (hg38)	Ref Allele	Alt Allele	dbSNP	Gene	Codon Change	Protein Change	Exonic Function	ACMG/AMP Classification	Hetero	Homo	MAF (Africans)	ClinVar	PolyPhen	SIFT	CGI	Ref to PCa	Freq of Minor Allele in Normal African Pop (NCBI)	Freq of Minor Allele in Normal European Pop (NCBI)
13	32379392	A	T	rs4987047	*BRCA2*	c.8830	Ile2944Phe	Missense variant	Likely benign	14 (12.2%)	—	0.0619	Benign	Possibly damaging	Deleterious	predicted driver: tier 1		0.04	0.00007
11	108251865	C	G	rs2227924	*ATM*	c.1636	Leu546Val	Missense variant	Likely benign	7 (6.1%)	2 (1.7%)	0.0487	Likely benign		Deleterious			0.019000	0.000170
16	89762787	T	C	rs147184552	*FANCA*	c.193	Met65Val	Missense variant		11 (9.6%)	—	0.0487	—		Deleterious			0.009300	0.000190
17	7676230	G	A	rs1800371	*TP53*	c.139	Pro47Ser	Missense variant	Likely benign	10 (8.7%)	—	0.0442	Benign		Deleterious			0.007800	0.000000
2	47369555	C	A	rs116429842	*EPCAM*	c.50	Thr17Lys	Missense variant	Likely benign	10 (8.7%)	—	0.0442	Benign		Deleterious			0.008600	0.000820
5	132618197	C	T	rs750947088	*RAD50*	c.3292	Arg1098Trp	Missense variant	Likely benign	10 (8.7%)	—	0.0442	Uncertain significance	Possibly damaging	Deleterious			0.000000	0.000000
8	89943291	T	C	rs72563785	*NBN*	c.2146	Asn716Asp	Missense variant	Likely benign	9 (8.0%)	—	0.0398	Likely benign		Deleterious			0.029200	0.000050
1	45329412	G	A	rs140118273	*MUTYH*	c.1460	Ser512Phe	Missense variant	Likely benign	8 (7.1%)		0.0353	Benign		Deleterious			0.004100	0.011438
11	108259051	C	A	rs3218695	*ATM*	c.2442	Asp814Glu	Missense variant	Likely benign	8 (7.1%)	—	0.0353	Benign		Deleterious			0.015500	0.000196
13	32340767	G	T	rs11571659	*BRCA2*	c.6412	Val2138Phe	Missense variant	Likely benign	7 (6.1%)	—	0.0309	Benign		Deleterious			0.010900	0.000054
16	23638125	T	C	rs138789658	*PALB2*	c.59	Lys20Arg	Missense variant	Likely benign	7 (6.1%)	—	0.0309	Conflicting interpretation	Probably damaging			1	0.014500	0.000086
7	6002607	G	A	rs116373169	*PMS2*	c.383	Ser128Leu	Missense variant	Uncertain significance	5 (4.4%)	1 (0.9%)	0.0309	Conflicting interpretation	Possibly damaging	Deleterious			0.005200	0.000065
8	89970463	G	A	rs769420	*NBN*	c.797	Pro266Leu	Missense variant	Likely benign	5 (4.4%)	—	0.0221	Benign	Probably damaging	Deleterious	predicted driver: tier 1		0.031200	0.000070
2	47369727	C	G	rs373746049	*EPCAM*	c.222	Cys74Trp	Missense variant		5 (4.4%)	—	0.0221	—		Deleterious			0.001100	0.000030
22	28734468	G	A	rs17883862	*CHEK2*	c.254	Pro85Leu	Missense variant	Likely benign	5 (4.4%)	—	0.0221	Likely benign	Possibly damaging	Deleterious			0.003000	0.000767
16	89771808	G	A	rs17232973	*FANCA*	c.2021	Ser674Leu	Missense variant	Likely benign	4 (3.5%)	—	0.0177	Benign		Deleterious			0.004700	0.000051
16	89816297	G	C	rs566370312	*FANCA*	c.319	Pro107Ala	Missense variant/ intronic variant		4 (3.5%)	—	0.0177			Deleterious			0.000000	0.000000
11	108227834	A	G	rs150143957	*ATM*	c.131	Asp44Gly	Missense variant	Uncertain significance	4 (3.5%)	—	0.0177	Uncertain significance	Possibly damaging	Deleterious	predicted driver: tier 1		0.000700	0.000006
17	35117023	G	A	rs200538950	*RAD51D*	c.159	Thr54Ile	Missense variant	Likely benign	4 (3.5%)	—	0.0177	Likely benign		Deleterious			0.001000	0.000000
5	132579882	C	T	rs2230017	*RAD50*	c.572	Thr191Ile	Missense variant	Likely benign	4 (3.5%)	—	0.0177	Likely benign		Deleterious	predicted driver: tier 1	2	0.016000	0.000476
11	108316091	C	T	rs144761622	*ATM*	c.6176	Thr2059Ile	Missense variant	Uncertain significance	3 (2.6%)	—	0.0132	Conflicting interpretations		Deleterious			0.004900	0.000006
11	108254011	A	G	rs147934285	*ATM*	c.2096	Glu699Gly	Missense variant	Uncertain significance	3 (2.6%)	—	0.0132	Conflicting interpretations		Deleterious			0.000800	0.000000
11	108244000	G	C	rs3218707	*ATM*	c.544	Val182Leu	Missense variant	Likely benign	3 (2.6%)	—	0.0132	Benign		Deleterious			0.024600	0.000690
17	43071232	G	A	rs56158747	*BRCA1*	c.4682	Thr1561Ile	Missense variant	Likely benign	3 (2.6%)	—	0.0132	Benign		Deleterious			0.004600	0.000008
5	132618158	A	G	rs143189763	*RAD50*	c.3253	Ile1085Val	Missense variant	Uncertain significance	3 (2.6%)	—	0.0132	Conflicting interpretations	Possibly damaging	Deleterious			0.003700	0.000024
17	35106394	C	T	rs80116829	*RAD51D*	c.628	Ala210Thr	Missense variant	Uncertain significance	3 (2.6%)	—	0.0132	Conflicting interpretations		Deleterious			0.004100	0.000007
17	35116931	A	T	rs376472075	*RAD51D*	c.251	Leu84His	Missense variant	Likely benign	3 (2.6%)	—	0.0132	Benign	Possibly damaging	Deleterious			0.002300	0.000000
11	108365476	C	T	rs121434219	*ATM*	c.9139	Arg3047Ter	Stop gained	Uncertain significance	2 (1.7%)	—	0.0088	Pathogenic		Deleterious	known in: CANCER-PR		0.000000	0.000000
17	43097280	G	T	rs55688530	*BRCA1*	c.557	Ser186Tyr	Missense variant	Likely benign	2 (1.7%)	—	0.0088	Benign		Deleterious			0.007600	0.000067
13	32326142	A	G	rs68071147	*BRCA2*	c.467	Asp156Gly	Missense variant	Likely benign	2 (1.7%)	—	0.0088	Conflicting interpretations		Deleterious			0.000900	0.000018

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eTable 1. Potentially Deleterious Variations and the Justification for Deleteriousness as Described by Each Dataset (cont.)

Chrom	Position (hg38)	Ref Allele	Alt Allele	dbSNP	Gene	Codon Change	Protein Change	Exonic Function	ACMG/AMP Classification	Hetero	Homo	MAF (Africans)	ClinVar	PolyPhen	SIFT	CGI	Ref to PCa	Freq of Minor Allele in Normal African Pop (NCBI)	Freq of Minor Allele in Normal European Pop (NCBI)
13	32398388	C	T	rs56121817	*BRCA2*	c.9875	Pro3292Leu	Missense variant	Likely benign	2 (1.7%)	—	0.0088	Benign		Deleterious	predicted driver: tier 1		0.000500	0.000060
13	32339995	T	G	rs11571657	*BRCA2*	c.5640	Asn1880Lys	Missense variant	Likely benign	2 (1.7%)	—	0.0088	Benign		Deleterious			0.009600	0.000017
16	89740840	G	A	rs141128234	*FANCA*	c.3792	Pro1264Leu	Missense variant	Likely benign	2 (1.7%)	—	0.0088	Likely benign	Probably damaging	Deleterious			0.000300	0.000040
16	89805288	A	G	rs145869646	*FANCA*	c.701	Met234Thr	Missense variant	Uncertain significance	2 (1.7%)	—	0.0088	Uncertain significance		Deleterious			0.000300	0.000000
16	89770570	G	A	rs45441106	*FANCA*	c.2216	Pro739Leu	Missense variant	Likely benign	2 (1.7%)	—	0.0088	Likely benign		Deleterious	predicted driver: tier 1		0.005100	0.000477
1	45331180	G	A	rs140288388	*MUTYH*		Splice site	Splice donor		2 (1.7%)	—	0.0088	Conflicting interpretations			predicted driver: tier 1		0.000900	0.000000
16	23641135	G	A	rs150390726	*PALB2*	c.23	Pro8Leu	Missense variant	Likely benign	2 (1.7%)	—	0.0088	Conflicting interpretations			predicted driver: tier 1		0.000900	0.000160
7	5992037	C	G	rs114185660	*PMS2*	c.924	Glu308Asp	Missense variant	Likely benign	2 (1.7%)	—	0.0088	Uncertain significance		Deleterious			0.000000	0.000100
17	35119712	A	G	rs533209845	*RAD51D*	c.76	Ser26Pro	Missense variant/ intronic variant	Likely benign	2 (1.7%)	—	0.0088	Likely benign		Deleterious			0.005900	0.000000
5	112827986	G	A	rs138098808	*APC*	c.1606	Glu536Lys	Missense variant	Uncertain significance	1 (0.9%)	—	0.0044	Conflicting interpretations		Deleterious	predicted driver: tier 1		0.001000	0.000000
5	112827984	C	T	rs75870842	*APC*	c.1604	Ser535Phe	Missense variant	Uncertain significance	1 (0.9%)	—	0.0044	Conflicting interpretations	Probably damaging	Deleterious	predicted driver: tier 1		0.001000	0.000006
5	112707847	G	A	rs367773779	*APC*	c.130	Ala44Thr	Missense variant	Likely benign	1 (0.9%)	—	0.0044	Conflicting interpretations		Deleterious			0.000300	0.000000
11	108235672	G	A	rs146382972	*ATM*	c.334	Ala112Thr	Missense variant	Uncertain significance	1 (0.9%)	—	0.0044	Conflicting interpretations		Deleterious			0.002000	0.000013
11	108287688	A	G	rs141921797	*ATM*	c.4082	Gln1361Arg	Missense variant	Uncertain significance	1 (0.9%)	—	0.0044	Uncertain significance		Deleterious			0.001200	0.000027
11	108331465	A	G	rs1480066803	*ATM*	c.7537	Thr2513Ile	Missense variant		1 (0.9%)	—	0.0044	—		Deleterious			0.000000	0.000000
17	43091931	C	G	rs56214134	*BRCA1*	c.3600	Gln1200His	Missense variant	Likely benign	1 (0.9%)	—	0.0044	Benign		Deleterious		3	0.000000	0.000000
13	32379908	C	A	Novel variant	*BRCA2*	c.9112	Leu3038Ile	Missense variant	Likely benign	1 (0.9%)	—	0.0044	—		Deleterious	predicted driver: tier 1		NR	NR
13	32319100	T	C	rs80359182	*BRCA2*	c.91	Trp31Arg	Missense variant	Uncertain significance	1 (0.9%)	—	0.0044	Likely pathogenic		Deleterious	predicted driver: tier 1		0.000000	0.000000
13	32354995	C	A	rs746751519	*BRCA2*	c.7142	Pro2381Gln	Missense variant	Uncertain significance	1 (0.9%)	—	0.0044	Uncertain significance	Probably damaging	Deleterious			0.000000	0.000000
13	32397030	G	C	rs55775473	*BRCA2*	c.9634	Gly3212Arg	Missense variant	Likely benign	1 (0.9%)	—	0.0044	Benign		Deleterious			0.002700	0.000000
17	61808531	T	C	rs141055990	*BRIP1*	c.854	His285Arg	Missense variant	Uncertain significance	1 (0.9%)	—	0.0044	Conflicting interpretations	Probably damaging	Deleterious			0.000600	0.000000
22	28734558	G	A	rs765799649	*CHEK2*	c.164	Ser55Phe	Missense variant	Uncertain significance	1 (0.9%)	—	0.0044	Uncertain significance		Deleterious	predicted driver: tier 1		0.000000	0.000000
22	28734439	G	A	rs587781269	*CHEK2*	c.283	Arg95Ter	Stop gained	Pathogenic	1 (0.9%)	—	0.0044	Pathogenic			known in: BRCA/OV-PR	4	0.000000	0.000000
2	47379832	G	C	rs1333948891	*EPCAM*	c.721	Asp241His	Missense variant		1 (0.9%)	—	0.0044	—	Possibly damaging				0.000000	0.000000
2	47378956	G	A	rs891924989	*EPCAM*	c.559	Glu187Leu	Missense variant		1 (0.9%)	—	0.0044	—		Deleterious			0.000000	0.000000
2	47373927	A	G	rs34474955	*EPCAM*	c.304	Ser102Gly	Missense variant	Likely benign	1 (0.9%)	—	0.0044	Likely benign		Deleterious			0.001400	0.000040
16	89739285	G	A	rs149775657	*FANCA*	c.4015	Leu1339Phe	Missense variant	Uncertain significance	1 (0.9%)	—	0.0044	Uncertain significance		Deleterious			0.000000	0.000000
16	89738666	C	T	rs74977201	*FANCA*	c.4303	Ala1435Thr	Missense variant	Uncertain significance	1 (0.9%)	—	0.0044	Conflicting interpretations		Deleterious			0.006300	0.000130
16	89799202	T	C	rs13336566	*FANCA*	c.857	Gln286Arg	Missense variant	Likely benign	1 (0.9%)	—	0.0044	Conflicting interpretations	Probably damaging	Deleterious			0.009900	0.000010
16	89773357	G	C	rs34592408	*FANCA*	c.1928	Pro643Arg	Missense variant	Likely benign	1 (0.9%)	—	0.0044	Benign	Possibly damaging	Deleterious			0.015800	0.000030
16	89783063	G	A	rs200291237	*FANCA*	c.1510	Arg504Cys	Missense variant	Uncertain significance	1 (0.9%)	—	0.0044	Uncertain significance		Deleterious	predicted driver: tier 1		0.000000	0.000040

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Chrom	Position (hg38)	Ref Allele	Alt Allele	dbSNP	Gene	Codon Change	Protein Change	Exonic Function	ACMG/AMP Classification	Hetero	Homo	MAF (Africans)	ClinVar	PolyPhen	SIFT	CGI	Ref to PCa	Freq of Minor Allele in Normal African Pop (NCBI)	Freq of Minor Allele in Normal European Pop (NCBI)
16	89816374	G	T	rs1598203859	*FANCA*	c.241	Pro81His	Missense variant		1 (0.9%)	—	0.0044	—		Deleterious			0.000000	0.000000
16	89758609	A	C	rs188695241	*FANCA*	c.2949	Ile983Met	Missense variant	Likely benign	1 (0.9%)	—	0.0044	Uncertain significance	Possibly damaging				0.000600	0.000000
3	37025725	A	T	rs1060500697	*MLH1*	c.1127	Asp376Val	Missense variant		1 (0.9%)	—	0.0044	Uncertain significance		Deleterious			NR	NR
2	47783357	C	T	rs34014629	*MSH2*	c.124	Pro42Ser	Missense variant	Likely benign	1 (0.9%)	—	0.0044	Conflicting interpretations		Deleterious			0.000900	0.000000
2	47806561	G	A	rs34625968	*MSH6*	c.3911	Arg1304Lys	Missense variant	Likely benign	1 (0.9%)	—	0.0044	Likely benign	Possibly damaging				0.007100	0.000011
2	47800238	G	T	Novel variant	*MSH6*	c.2255	Gly752Val	Missense variant	Uncertain significance	1 (0.9%)	—	0.0044	—		Deleterious			NR	NR
2	47806344	C	T	rs367912290	*MSH6*	c.3787	Arg1263Cys	Missense variant	Uncertain significance	1 (0.9%)	—	0.0044	Uncertain significance		Deleterious	predicted driver: tier 1		0.000000	0.000070
1	45330036			Novel variant	*MUTYH*	Translocation	Translocation with chr2:203987190	Structural variant		1 (0.9%)	—	0.0044	—					NR	NR
16	23626343	C	T	rs766315705	*PALB2*	c.2641	Gly881Ser	Missense variant	Uncertain significance	1 (0.9%)	—	0.0044	Uncertain significance	Probably damaging	Deleterious	predicted driver: tier 1		NR	NR
7	6003981	C	T	rs730881919	*PMS2*	c.241	Glu81Lys	Missense variant	Uncertain significance	1 (0.9%)	—	0.0044	Uncertain significance		Deleterious	predicted driver: tier 1		0.000000	0.000000
17	58709932	G	A	rs730881926	*RAD51C*	c.779	Arg260Gln	Missense variant	Uncertain significance	1 (0.9%)	—	0.0044	Uncertain significance		Deleterious	predicted driver: tier 1		0.000000	0.000000
17	35118618	G	A	rs140317560	*RAD51D*	c.146	Ala49Val	Missense variant		1 (0.9%)	—	0.0044	Conflicting interpretations	Probably damaging				0.002700	0.000000
5	132557426	G	C	Novel variant	*RAD50*	c.102	Leu34Phe	Missense variant	Uncertain significance	1 (0.9%)	—	0.0044	—		Deleterious	predicted driver: tier 1		NR	NR
17	7667318	C	G	rs2072731249	*TP53*	c.1082	Ser361Thr	Missense variant		1 (0.9%)	—	0.0044	—		Deleterious			NR	NR
17	7673776	G	A	rs28934574	*TP53*	c.844	Arg282Trp	Missense variant	Uncertain significance	1 (0.9%)	—	0.0044	Pathogenic		Deleterious	known in: CANCER-PR		0.000000	0.000040
1	45329412	G	A	rs140118273	*MUTYH*	c.1460	Ser512Phe	Missense variant	Likely benign			0.0353	Benign		Deleterious			0.004100	0.011438
11	108252824	C	T	rs2227922	*ATM*	*c.1810*	Pro604Ser	Missense variant	Likely benign			0.0044	Conflicting interpretations	Possibly damaging	Deleterious			0.009300	0.003466

Abbreviations: ACMG/AMP, American College of Medical Genetics and Genomics/Association for Molecular Pathology; Alt, alternate; CGI, Cancer Genome Interpreter; Chrom, chromosome; dbSNP, Single Nucleotide Polymorphism Database; Freq, frequency; Hetero, heterozygous; Homo, homozygous; MAF, minor allele frequency; NR, not reported; PCa, prostate cancer; PolyPhen, Polymorphism Phenotyping v2; Pop, population; ref, reference; SIFT, Sorting Intolerant From Tolerant.

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