original reports

Pathogenic Variants in Cancer Predisposition Genes and Prostate Cancer Risk in Men of African Ancestry

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abstract

PURPOSE In studies of men of European ancestry, rare pathogenic variants in DNA repair pathway genes have been shown to be associated with risk of aggressive prostate cancer. The contribution of rare coding variation to prostate cancer risk in men of African ancestry has not been established.

METHODS We sequenced a panel of 19 DNA repair and cancer predisposition genes in 2,453 African American and 1,151 Ugandan cases and controls with prostate cancer. Rare variants were classified as pathogenic or putatively functionally disruptive and examined in association with prostate cancer risk and disease aggressiveness in gene and pathway-level association analyses.

RESULTS Pathogenic variants were found in 75 of 2,098 cases (3.6%) and 31 of 1,481 controls (2.1%; odds ratio [OR], 1.82; 95% CI, 1.19 to 2.79; $P = .0044$), with the association being stronger for more aggressive disease phenotypes (OR, 3.10; 95% CI, 1.54 to 6.23; $P = .0022$). The highest risks for aggressive disease were observed with pathogenic variants in the *ATM*, *BRCA2*, *PALB2*, and *NBN* genes, with ORs ranging from approximately 4 to 15 in the combined study sample of African American and Ugandan men. Rare, nonpathogenic, nonsynonymous variants did not have a major impact on risk of overall prostate cancer or disease aggressiveness.

CONCLUSION Rare pathogenic variants in DNA repair genes have appreciable effects on risk of aggressive prostate cancer in men of African ancestry. These findings have potential implications for panel testing and risk stratification in this high-risk population.

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INTRODUCTION

African American men have a 70% higher incidence rate and a 130% higher mortality rate of prostate cancer (PCa) than white men.[1] Incidence rates in Uganda are among the highest of all African countries,[2] and men in sub-Saharan Africa have been reported to have the highest mortality rates compared with men of other races or ethnicities,[3] which is likely a consequence of the high percentage of men with aggressive disease at diagnosis.[4] Inherited susceptibility is an important contributor to PCa risk,[5] with studies identifying 175 common variants that explain approximately 37% of familial PCa risk.[6-8] There is growing support for the role of rare or low-frequency coding variation in PCa risk from studies in men of European ancestry.[9] In *HOXB13*, a missense variant has been associated with risk, with effects being greater among men with early-onset and familial PCa.[10] Studies in whites have shown pathogenic variants in *BRCA1* and *BRCA2* to confer increased risk and to be more strongly associated with aggressive disease.[11] Studies in whites have also provided strong evidence supporting the role of pathogenic variants in other DNA repair genes (eg, *ATM*, *CHEK2*, *PALB2*, *RAD51D*) in aggressive PCa.[12,13]

The PCa relevance of rare variants in DNA repair pathway genes has not been established for men of African ancestry. In this study, we sequenced 19 DNA repair and known cancer predisposition genes in 2,121 cases and 1,483 controls to investigate the aggregate contribution of rare pathogenic and nonpathogenic coding variation to risk of PCa and aggressive disease in African American and Ugandan men.

METHODS

Study Subjects

The men in this study are from the Multiethnic Cohort (MEC), the Los Angeles Study of Aggressive Prostate Cancer (LAAPC), and the Uganda Prostate Cancer Study (UGPCS). Men with higher Gleason and stage from the following studies were overselected to

ASSOCIATED CONTENT

Data Supplement

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

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CONTEXT

Key Objective

To our knowledge, this is the first study aimed to investigate the contribution of rare pathogenic and nonpathogenic coding variation in DNA repair genes to risk of prostate cancer and aggressive disease specifically in men of African ancestry.

Knowledge Generated

Rare pathogenic variants in *BRCA2, ATM, PALB2*, and *NBN* were significantly associated with a high risk of aggressive disease. In addition, we found a substantial number of pathogenic variants that had not been previously reported in major public databases, which highlights the importance of sequencing efforts in genetically diverse populations.

Relevance

These findings underscore the clinical relevance of rare pathogenic variants in DNA repair genes in men of African ancestry, which could be used to build better predictive and prognostic gene-based tests to identify men at higher risk of aggressive prostate cancer in this population.

increase power to identify rare variants associated with more clinically aggressive disease. Participants all signed a written consent, and the study protocol was approved by the Institutional Review Boards of the University of Southern California and Makerere University. The human investigations were performed after approval by a local Human Investigations Committee and in accordance with an assurance filed with and approved by the Department of Health and Human Services, where appropriate.

Multiethnic Cohort. MEC is a prospective cohort study of men and women recruited from Hawaii and California between 1993 and 1996.[14] Incident patients with PCa were identified through linkage to cancer SEER registries in Hawaii and California. Information on histologic status of disease (eg, Gleason score, stage) was also obtained from the SEER registries. The MEC includes > 1,200 African American patients with PCa with biospecimens. In the current study, 760 African American PCa cases, overselected for high stage and Gleason score, and 996 controls were included. Also included were 620 African American PCa cases recruited in Los Angeles County between 2007 and 2015.

Los Angeles Study of Aggressive Prostate Cancer. In LAAPC, incident African American PCa cases were identified through the Los Angeles County SEER cancer registry between 1999 and 2003.[15] A total of 458 African American PCa cases were recruited, of whom 77 with nonlocalized disease or Gleason score > 7 were included in the current study.

Uganda Prostate Cancer Study. UGPCS is a case-control study of PCa in Uganda that began in 2010. As of July 2016, 664 incident PCa cases and 487 controls have been recruited.[16] Cases were enrolled from 13 hospitals/clinics across the country, whereas controls were enrolled from nonurologic clinics (eg, surgery). All controls had a prostate-specific antigen (PSA) level < 4 ng/mL, and a saliva spit kit was used to collect a biospecimen at recruitment. Gleason score was available for 441 cases (68%), and PSA level at diagnosis was available for 434 (67%) cases. Information regarding family history of PCa was obtained by questionnaire at cohort entry in the MEC and at case-control enrollment in LAAPC and UGPCS.

Disease Aggressiveness

Aggressive and nonaggressive PCa was defined based on stage, Gleason score, death from PCa, and PSA level at diagnosis. African American men with high-risk PCa (n = 645; 44.6%) were defined as those with nonlocalized disease or Gleason score > 7 or those who died as a result of PCa. African American men with low-/intermediate-risk PCa (n = 802; 55.4%) were those with localized disease and Gleason score ≤ 7. In UGPCS, high-risk PCa (n = 414; 63.6%) was defined as Gleason score > 7 or PSA level > 50 ng/mL, whereas low-/intermediate-risk PCa (n = 53; 8.1%) was defined as Gleason score ≤ 7 and PSA level ≤ 50 ng/mL. The 50-ng/mL cut point for PSA level was chosen based on the advanced disease state at which most Ugandan patients are diagnosed because we had no patients with a PSA level of ≤ 20 ng/mL in our study. Because stage information was not available in UGPCS, metastatic disease was defined as a PSA level > 100 ng/mL.

Targeted Gene Sequencing

Genomic DNA libraries were prepared using a custom QIAseq amplicon-based targeted panel (Qiagen, Germantown, MD)[17] of coding regions and essential splice sites for 19 cancer predisposition genes with a core function in DNA damage repair: *ATM*, *BARD1*, *BRCA1*, *BRCA2*, *BRIP1*, *CHEK2*, *MLH1*, *MRE11A*, *MSH2*, *MSH6*, *NBN*, *PALB2*, *PMS2*, *PTEN*, *RAD50*, *RAD51C*, *RAD51D*, *TP53*, and *XRCC2*.[12,13] The panel was designed to capture all exonic regions of the investigated genes.

Of the 3,604 samples, 3,588 were successfully sequenced with 99.6% of target bases covered ≥ 20×, whereas 25 samples were removed because of insufficient coverage (< 90% of bases covered ≥ 20×), high degree of relationship with other samples (relatedness score > 0.5), or excessive homozygosity. A total of 2,098 cases and 1,481 controls were retained for analysis.

Variant Annotation

CAVA (Clinical Annotation of Variants) v.1.1.1 was used to annotate variants according to functional effect.[18] Given the focus of the study on variants that might alter protein structure and function, variants in introns (n = 215), 5′-UTR (n = 15), 3′-UTR (n = 7), in-frame insertions/deletions (n = 14), synonymous variants (n = 537), and those located > 2 bp outside of the consensus splice site (n = 147) according to CAVA were excluded. We also removed 148 variants having frequency ≥ 1% in controls or in gnomAD. The remaining variants (n = 1,163) with frequency < 1% were sorted into 2 groups based on predicted functional and pathogenic annotation (Data Supplement). Variants predicted to result in protein truncation or to significantly alter the protein sequence (frameshift insertion/deletions, gain of stop codon or loss of essential splice site donor/acceptor) and missense variants (nonsynonymous codon change, exon start/end codon change) that were reported as pathogenic or likely pathogenic in ClinVar[19] by 1 or more clinical laboratories (Ambry, SCRP, InVitae, GeneDX, Emory, and InSiGHT) were classified as pathogenic (tier 1; n = 91). Missense variants not identified as being pathogenic or likely pathogenic in ClinVar were annotated with dbNSFP version 3.3a[20] using 5 *in silico* algorithms (Polyphen2-HumDiv, PolyPhen2-HumVar, LRT, Mutation Taster, and SIFT) to predict potential functional effects.[21] Tier 2 variants (n = 566) were those predicted by ≥ 1 of the 5 dbNSFP algorithms to be putatively functionally altered, which included a small number of missense variants (n = 10) in tier 1. We subsequently annotated variants at the gene set level, with genes grouped by the DNA repair process.[22]

Statistical Analysis

Principal components (PCs) were calculated using 311 weakly correlated (mean r^2, 0.01) common single nucleotide polymorphisms (frequency ≥ 1% and call rate ≥ 0.98) using EIGENSTRAT.[23] Tier 1 and tier 2 variants were evaluated in gene burden testing, with the minor alleles summed at the gene level in each individual and counts compared between cases and controls. In addition, we assessed the association across all genes (polygenic burden test) and gene sets grouped by DNA repair process (pathway-level burden test). We report odds ratios (ORs) and 95% CIs from logistic regression and *P* values from a likelihood ratio test, adjusting for age, ethnicity (Ugandan *v* African American), and the first 2 PCs, primarily capturing variation in African ancestry. Statistical significance was set at $P < .05$ in 2-sided tests, because many of these genes have been associated with PCa risk in men of European ancestry.[12,13]

RESULTS

The mean age at diagnosis of PCa was 66.71 years for African American cases and 70.77 for Ugandan cases (Table 1). Based on the overselection of cases with high stage and high Gleason score at diagnosis (see Methods), 44.6% of African American cases and 63.6% of Ugandan cases were classified as having high-risk PCa.

In total, 647 rare (< 1%) putatively disruptive variants (tier 1 plus tier 2; see Methods) were identified in the 19 genes (Data Supplement). Variants were annotated as nonsynonymous (84.4%), frameshift insertions/deletions (6.5%), stop-gain (4.5%), exon start/end codon changes (3.1%), and essential splice sites (1.5%; Table 2). We identified 111 variants (41 in African Americans and 71 in Ugandans), all with frequency ≤ 0.1%, not previously reported in gnomAD or ClinVar (Table 2). Sixty-four variants (9.9%) were shared across the 2 groups, whereas 419 (64.8%) and 164 (25.3%) variants were observed exclusively in African Americans and Ugandans, respectively.

We identified 91 tier 1 pathogenic variants in 16 of the 19 genes. Ten variants in 7 genes (*ATM, BRCA2, CHEK2, MLH1, MSH6, PMS2, NBN*) were observed in more than 1 sample (Data Supplement), with the most common being *c.1100delC* (rs555607708) in *CHEK2* that was present in 6 African American men (OR, 0.16; 95% CI, 0.02 to 1.51; $P = .066$). *BRCA2* had the largest number of pathogenic variants (n = 23; 25.3% of tier 1) followed by *ATM* (n = 16; 17.6%), with the remaining genes having ≤ 8 variants each (Table 3).

In gene burden testing, pathogenic variants in *BRCA2* (OR, 3.92; 95% CI, 1.34 to 11.47; $P = .0045$), *ATM* (OR, 3.83; 95% CI, 1.09 to 13.41; $P = .018$), and *PALB2* (4 cases and 0 controls; $P = .034$) were significantly associated with risk, whereas an association with *NBN* was borderline significant (OR, 3.50; 95% CI, 0.75 to 16.35; $P = .074$; Table 3; Fig 1). Positive associations with these genes were observed in both African Americans and Ugandans, although the associations were only statistically significant in Ugandans. Variants in *CHEK2*, which included *c.1100delC*, were under-represented in cases versus controls (OR, 0.47; 95% CI, 0.15 to 1.45; $P = .18$). However, exclusion of *c.1100delC* altered the risk estimate for *CHEK2* toward the null (OR, 0.99; 95% CI, 0.22 to 4.48; $P = .99$; data not shown).

The positive associations observed with pathogenic variants were limited primarily to high-risk disease (*BRCA2*: OR, 5.29; 95% CI, 1.74 to 16.06; $P = .0014$; *ATM*: OR, 5.15; 95% CI, 1.40 to 18.93; $P = .0067$; *PALB2*: 3 cases and 0 controls, $P = .021$) and Gleason score > 7 tumors (*BRCA2*: OR, 7.70; 95% CI, 2.43 to 24.43; $P = .00017$; *ATM*: OR, 7.11; 95% CI, 1.81 to 27.94; $P = .0029$; *PALB2*: 2 cases and 0 controls, $P = .025$; Data Supplement; Fig 2a). In African Americans, the association with *BRCA2* was statistically significant for Gleason score > 7 (OR, 5.23; 95% CI, 1.23 to 22.30; $P = .021$; Data Supplement; Fig 2b). In Ugandans, *BRCA2* and *ATM* were significantly associated with Gleason score > 7 (*BRCA2*: OR, 11.99; 95% CI, 1.35 to 106.71; $P = .0078$; *ATM*: 3 cases and 0 controls; $P = .015$) and with metastatic disease defined by PSA level > 100 ng/mL at diagnosis (*BRCA2*: OR, 14.94; 95% CI, 1.69 to 132.44; $P = .0027$; *ATM*: 4 cases and 0 controls; $P = .007$;

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TABLE 1. Descriptive Characteristics of African American and Ugandan Prostate Cancer Cases and Controls

	African Americans		Ugandans	
Characteristic	**Cases**	**Controls**	**Cases**	**Controls**
Total	1,447	995	651	486
Mean age (± SD), years	66.71 (8.5)	71.52 (5.4)	70.77 (9.4)	65.04 (8.9)
Age, years[a]				
≤ 65	607 (41.9)	153 (15.4)	187 (28.7)	273 (56.2)
> 65	840 (58.1)	842 (84.6)	464 (71.3)	213 (43.8)
First-degree family history of PCa				
No	1,026 (70.9)	794 (79.8)	424 (65.1)	443 (91.1)
Yes	319 (22.0)	101 (10.2)	61 (9.4)	12 (2.5)
NA[b]	102 (7.1)	100 (10.1)	166 (25.5)	31 (6.4)
Gleason score				
≤ 7	1,075 (74.3)		233 (35.8)	
> 7	352 (24.3)		208 (31.9)	
NA	20 (1.4)		210 (32.4)	
Stage[c]				
Localized	1,061 (73.3)			
Regional	265 (18.3)			
Metastatic	73 (5.1)			
NA	48 (3.3)			
PSA level, ng/mL[d]				
< 50			127 (19.5)	
50-100			101 (15.5)	
> 100			206 (31.6)	
NA			217 (33.3)	
Aggressiveness[e]				
Low/intermediate risk	802 (55.4)		53 (8.1)	
High risk	645 (44.6)		414 (63.6)	
NA[f]	0 (0)		184 (28.3)	

NOTE. All data are No. (%) unless otherwise indicated.

Abbreviations: NA, not available; PCa, prostate cancer; PSA, prostate-specific antigen; SD, standard deviation.

[a]Age of diagnosis for cases; age of recruitment for controls.

[b]Not available because of missing data.

[c]Stage of prostate cancer only available for African Americans.

[d]PSA level at diagnosis only available for Ugandans.

[e]Low-/intermediate-risk disease was defined as Gleason score ≤ 7 and localized disease (for African Americans) or PSA level ≤ 50 ng/mL (for Ugandans); high-risk disease was defined as Gleason score > 7 or nonlocalized disease (regional or metastatic) or PSA level > 50 ng/mL or death from prostate cancer.

[f]Men with missing Gleason score and PSA level at diagnosis.

Data Supplement; Fig 2c). Other genes were not strongly associated with aggressive phenotypes, except for a significantly higher number of *CHEK2* variants in controls compared with cases with high Gleason score (0 cases and 8 controls, $P = .036$). The risk estimates for *BRCA2* and *ATM* remained statistically significant when including cases with a Gleason score of 7 in the high-risk category (data not shown).

In aggregating all pathogenic variants, 75 of 2,098 cases (3.6%) and 31 of 1,481 controls (2.1%) were carriers (OR, 1.82; 95% CI, 1.19 to 2.79; $P = .0044$; Table 4; Data Supplement), with the risk being higher for high-risk disease (OR, 2.27; 95% CI, 1.43 to 3.61; $P = .00046$) and metastatic PCa (OR, 3.10; 95% CI, 1.54 to 6.23; $P = .0022$). Associations were even larger when restricting the analyses to *ATM*+*BRCA2*+*PALB2*+*NBN* or the 2 most significantly associated genes, *BRCA2* and *ATM*, with ORs of approximately 4 for overall PCa and 5.3 to 9.2 for aggressive disease phenotypes, and with the higher risks

TABLE 2. Annotation of 647 Rare Putatively Disruptive Variants in 3,579 Men of African Ancestry

		Variant Type (No.)[a]					gnomAD[b]		ClinVar Pathogenic[c]		Unreported[d]	
Variants Identified	Total (No.)	EE	ESS	FS	NSY	SG	No.	%	No.	%	No.	%
Tier 1[e]	91	2	10	42	8	29	54	59.3	69	75.8	17	18.7
Tier 2[f]	566	20	0	0	546	0	401	70.8	10	1.8	94	16.6
Tier 2 high pathogenic score[g]	220	10	0	0	210	0	148	67.3	10	4.5	46	20.9
Frequency, %												
≤ 0.1	600	18	10	42	501	29	401	66.8	69	11.5	111	18.5
> 0.1, ≤ 0.5	38	2	0	0	36	0	37	97.4	0	0	0	0
> 0.5, ≤ 1	9	0	0	0	9	0	9	100	0	0	0	0
Total	647	20	10	42	546	29	447	69.1	69	10.7	111	17.2

NOTE. Information on publicly available datasets retrieved from ANNOVAR and based on human genome build 37.

Abbreviations: EE, exon start/stop codon change; ESS, essential splice site donor/acceptor; FS, frameshift insertion/deletion; NSY, nonsynonymous coding; SG, stop codon gained.

[a]Functional annotation of variants as predicted by CAVA (Clinical Annotation of Variants).

[b]No. and fraction (%) of variants in gnomAD exome sequence data from 123,136 individuals and whole-genome sequence data from 15,496 individuals across 8 ethnicities (AFR, AMR, ASJ, EAS, FIN, NFE, SAS, OTH). AFR, African/African American; AMR, Admixed American; ASJ, Ashkenazi Jewish; EAS, East Asian; FIN, Finnish; NFE, Non-Finnish European; SAS, South Asian; OTH, Other (population not assigned).

[c]No. and fraction (%) of variants reported pathogenic or likely pathogenic in ClinVar by 1 or more clinical laboratories (Ambry, SCRP, InVitae, GeneDX, Emory, and InSiGHT).

[d]No. and fraction (%) of variants not reported in gnomAD exome/genome sequence data or ClinVar.

[e]Tier 1 includes protein-truncating variants (ESS/FS/SG) and pathogenic/likely pathogenic missense variants in ClinVar (EE/NSY); the 2 EE and 8 NSY variants in tier 1 are also in tier 2.

[f]Tier 2 includes missense variants (EE/NSY) pathogenic/likely pathogenic in ClinVar or predicted deleterious by ≥ 1 algorithm (LRT, MutationTaster, PolyPhen2 HDIV, PolyPhen2 HVAR, SIFT).

[g]Tier 2 variants reported pathogenic/likely pathogenic in ClinVar or predicted deleterious by all 5 algorithms.

observed in Ugandans (ORs, 9.1 to 22.2; Table 4; Data Supplement). The aggregation of pathogenic variants by pathway showed higher risk with genes linked with Fanconi anemia (OR, 3.92; 95% CI, 1.50 to 10.26; $P = .0015$) and homologous recombination (OR, 2.02; 95% CI, 1.25 to 3.26; $P = .0026$; Data Supplement). However, these associations were no longer significant ($P > .05$) after removing *BRCA2* (Data Supplement), except for a borderline increased risk of high-risk disease (OR, 1.77; 95% CI, 1.05 to 2.99; $P = .031$; Data Supplement).

Of the 566 tier 2 nonsynonymous variants (see Methods) identified, 360 (63.6%) were unique to 1 sample. As for the tier 1 pathogenic variants, 2 of the largest genes harbored the largest number of tier 2 variants: *ATM* (n = 78; 13.8%) and *BRCA2* (n = 68; 12.0%; Data Supplement).

In gene burden testing, tier 2 variants in *NBN* were inversely associated with overall risk (OR, 0.61; 95% CI, 0.39 to 0.94; $P = .027$; Data Supplement) and with aggressive phenotypes (Gleason score > 7: OR, 0.37; 95% CI, 0.15 to 0.87; $P = .011$; high risk: OR, 0.54; 95% CI, 0.30 to 0.95; $P = .026$; Data Supplement). Variants in *PTEN* were only found in African American cases (4 *v* 0 controls; $P = .041$), whereas *ATM* variants were associated with risk in Ugandans (OR, 1.52; $P = .032$), but not in African Americans (OR, 0.91; $P = .52$). Suggestive associations in subgroup analyses were also observed, including with *XRCC2* with Gleason score > 7 in African Americans (OR, 3.63; 95% CI, 1.52 to 8.65; $P = .0042$; Data Supplement).

Restricting tier 2 variants to those reported as pathogenic or likely pathogenic in ClinVar or predicted to be functionally relevant by all 5 algorithms (n = 220; see Methods) did not affect the results; a positive association with *MLH1* was noted in African Americans (OR, 6.68; 95% CI, 0.85 to 52.80; $P = .023$; Data Supplement). There was limited evidence of association with tier 2 variants across the full set of genes and gene sets grouped by DNA repair process (Data Supplement).

DISCUSSION

In men of African ancestry, rare pathogenic variants in *BRCA2, ATM, PALB2*, and *NBN* were significantly associated with a high risk of aggressive PCa, with effect sizes ranging from approximately 4 to 15 in the combined study sample of African American and Ugandan men. Sequencing at high coverage in men of African ancestry revealed a substantial number of pathogenic variants that had not been previously reported in major public databases (18.5% with frequency ≤ 0.1%), highlighting the importance of risk allele discovery efforts in genetically diverse populations.

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TABLE 3. Gene Burden Results for 91 Tier 1 Variants and Prostate Cancer Risk

	Overall (2,098 cases/1,481 controls)					African Americans (1,447 cases/995 controls)				Ugandans (651 cases/486 controls)			
Gene[a]	Sites[b]	Samples (case/control)[c]	Frequency (case/control)[d]	OR (95% CI)[e]	*P*[f]	Samples (case/control)	Frequency (case/control)	OR (95% CI)	*P*	Samples (case/control)	Frequency (case/control)	OR (95% CI)	*P*
BRCA2	23	21/4	0.01/0.0027	3.92 (1.34 to 11.47)	.0045	9/3	0.0062/0.003	1.91 (0.48 to 7.59)	.34	12/1	0.018/0.0021	10.30 (1.28 to 82.58)	.0036
ATM	16	14/3	0.0067/0.002	3.83 (1.09 to 13.41)	.018	7/3	0.0048/0.003	2.04 (0.50 to 8.28)	.3	7/0	0.011/0		.012
PALB2	4	4/0	0.0019/0		.034	3/0	0.0021/0		.092	1/0	0.0015/0		.47
NBN	8	9/2	0.0042/0.0013	3.50 (0.75 to 16.35)	.074	4/1	0.0028/0.001	2.86 (0.30 to 26.91)	.32	5/1	0.0077/0.002	3.50 (0.39 to 31.90)	.21
CHEK2	7	5/8	0.0023/0.0054	0.47 (0.15 to 1.45)	.18	4/7	0.0028/0.0070	0.37 (0.10 to 1.36)	.13	1/1	0.0015/0.0021	0.36 (0.02 to 5.96)	.48
RAD51C	1	0/1	0/0.00067		.18	0/1	0/0.001		.16				
PMS2	1	2/0	0.00095/0		.18	2/0	0.0014/0		.23				
MRE11A	4	1/3	0.00048/0.002	0.27 (0.03 to 2.60)	.22	0/3	0/0.003		.083	1/0	0.0015/0		.1
TP53	1	0/1	0/0.00067		.22	0/1	0/0.001		.33				
BRIP1	1	1/0	0.00048/0		.25					1/0	0.0015/0		.49
XRCC2	1	1/0	0.00048/0		.29	1/0	0.00069/0		.3				
BARD1	3	1/2	0.00048/0.0013	0.32 (0.03 to 3.55)	.34	1/2	0.00069/0.002	0.27 (0.02 to 2.96)	.26				
BRCA1	7	5/2	0.0024/0.0013	1.83 (0.35 to 9.51)	.46	3/1	0.0021/0.001	2.84 (0.26 to 30.59)	.36	2/1	0.0031/0.0021	1.11 (0.09 to 13.54)	.93
MLH1	3	3/1	0.0014/0.00067	1.96 (0.20 to 19.10)	.54	3/1	0.0021/0.001	2.21 (0.22 to 22.61)	.48				
RAD50	4	3/1	0.0014/0.00067	1.87 (0.19 to 18.19)	.57	3/1	0.0021/0.001	1.82 (0.16 to 21.16)	.62				
MSH6	7	5/3	0.0024/0.002	1.09 (0.26 to 4.61)	.9	4/0	0.0028/0		.073	1/3	0.0015/0.0062	0.26 (0.03 to 2.66)	.22

[a]Genes ordered by increasing *P* value for association in the overall sample.
[b]No. of variants identified in each gene.
[c]No. of carriers in cases/controls.
[d]Burden allele frequency in cases/controls.
[e]Odds ratio and 95% CI from the Wald test; risk estimates not computed when no carriers in cases or controls.
[f]*P* value from the likelihood ratio test.

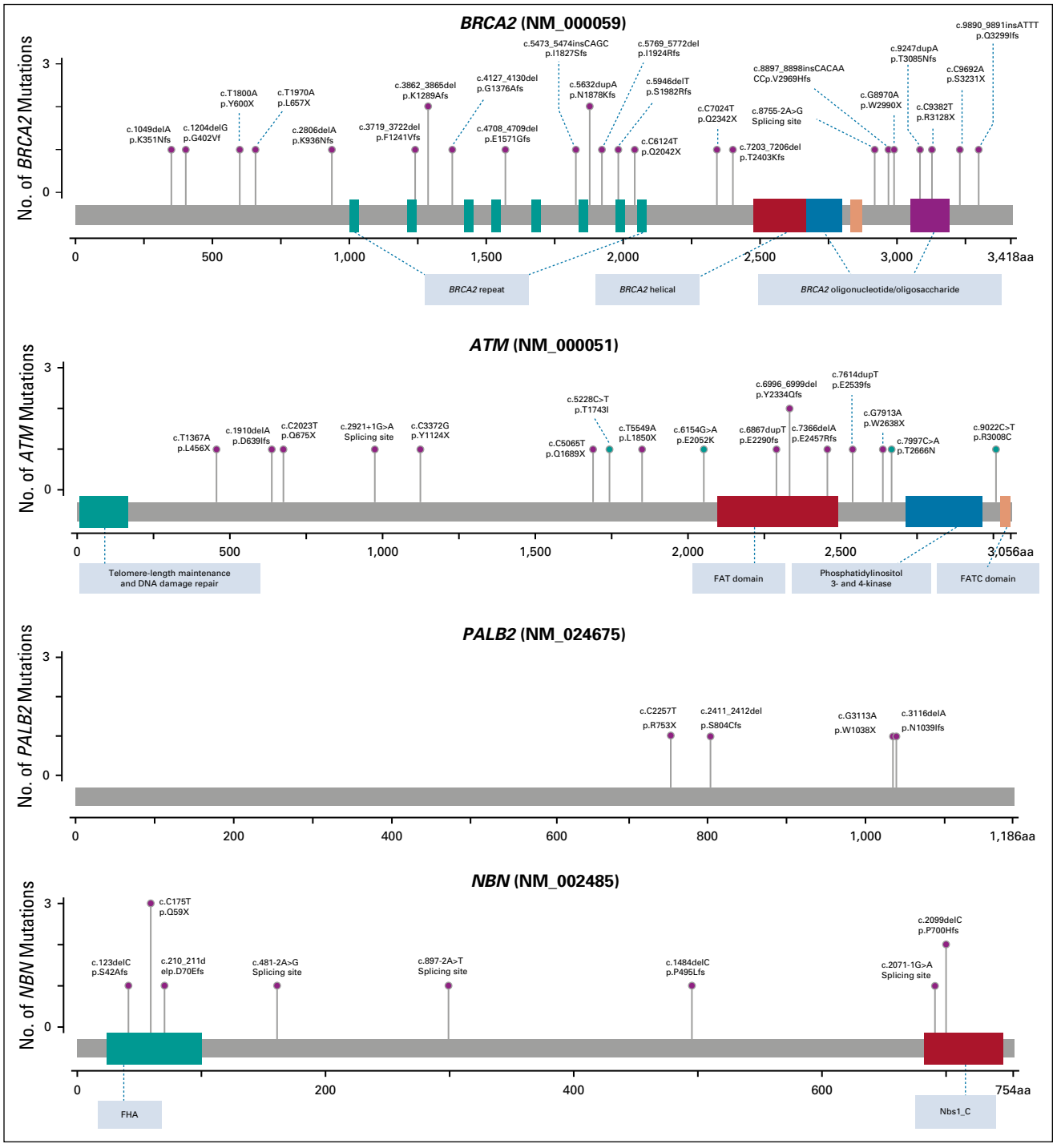

FIG 1. Tier 1 variants in genes significantly associated with prostate cancer risk. Each variant (DNA position and resulting amino acid change) in these genes and protein domains is presented by lollipop plots, with the variant type indicated by color. Variants predicted to result in protein truncation or significantly alter the protein sequence (frameshift insertion/deletions, stop codon gained or essential splice site donor/acceptor) and missense variants (nonsynonymous coding, exon start/end codon change) that were reported as pathogenic or likely pathogenic in ClinVar are coded in violet and green, respectively. On the graph of each gene, the *x*-axis represents the number of amino acid residues, and the *y*-axis represents the total number of variants identified. Protein domains are also distinguished by color. FAT domain, focal adhesion targeting domain; FATC domain, focal adhesion targeting carboxyterminal domain; FHA, forkhead-associated domain; Nbs1_C, Nijmegen breakage syndrome C-terminal domain.

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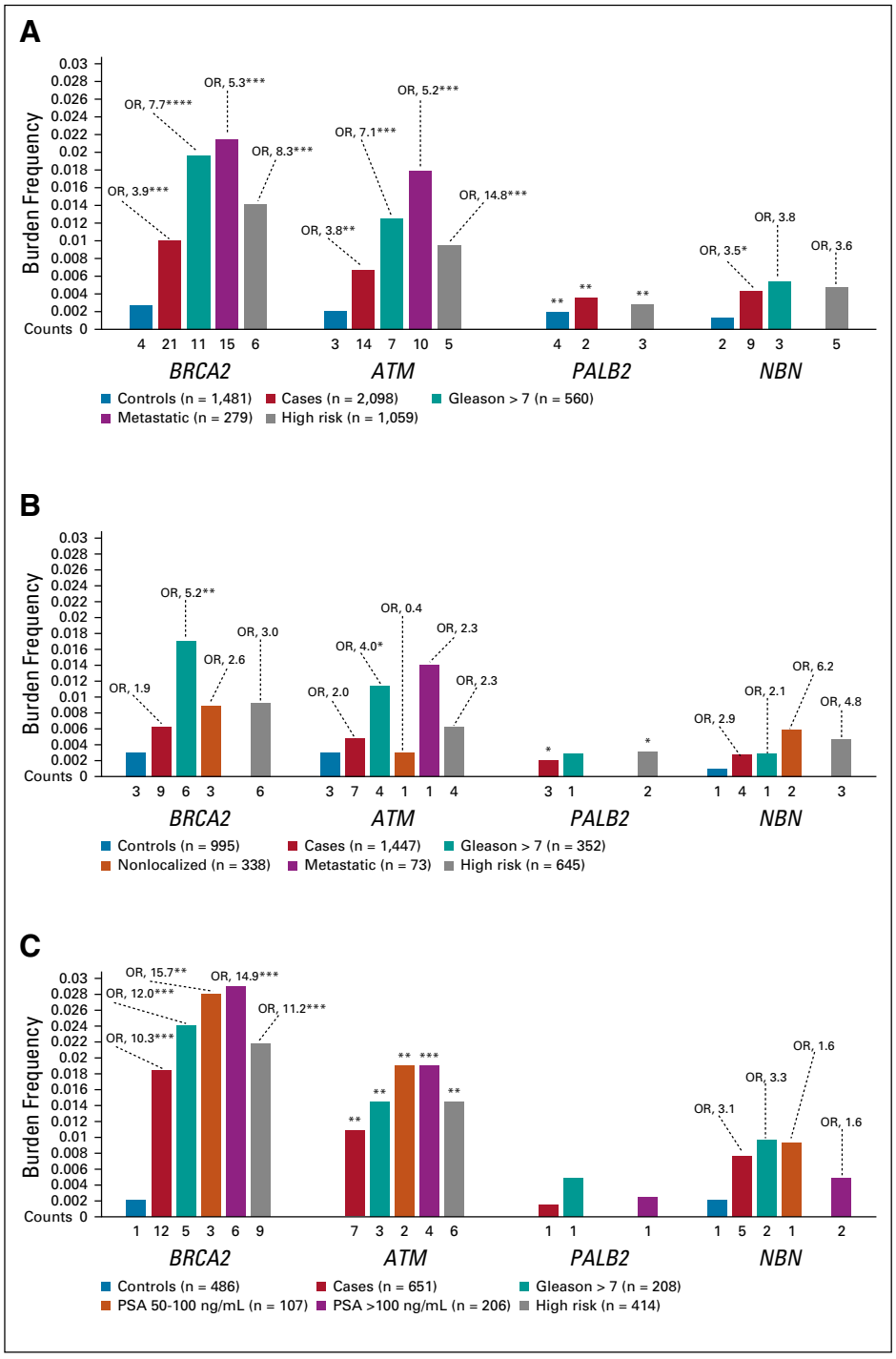

FIG 2. Gene burden associations of tier 1 variants with prostate cancer risk by case-control status and strata of disease phenotype. Histograms are used to represent associations of rare pathogenic variants in *ATM*, *BRCA2*, *PALB2*, and *NBN* genes with prostate cancer risk and aggressiveness in (A) African Americans and Ugandans, (B) African Americans only, and (C) Ugandans only. In each plot, the *x*-axis reflects the genes and counts of individuals by subgroup, and the *y*-axis reflects the burden frequency for each subgroup. Numbers above the bars represent risk estimates for cases and aggressive phenotypes compared with controls, with stars indicating the strength of association (*P* value). Case-control status and strata of disease phenotype are distinguished by color. (*) $.05 < P \leq .1$; (**) $.01 < P \leq .05$; (***) $.001 < P \leq .01$; (****) $P \leq .001$.

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TABLE 4. Polygenic Association Analysis of 91 Tier 1 Variants and Prostate Cancer Risk by Strata of Disease Phenotype

		Overall (2,098 cases/1,481 controls)				African Americans (1,447 cases/995 controls)			Ugandans (651 cases/486 controls)		
Group	Comparison	Sites[a]	Frequency (cases/controls)[b]	OR (95% CI)[c]	*P*[d]	Frequency (cases/controls)	OR (95% CI)	*P*	Frequency (cases/controls)	OR (95% CI)	*P*
All genes	Cases *v* controls	91	0.036/0.021	1.82 (1.19 to 2.79)	.0044	0.03/0.024	1.31 (0.77 to 2.22)	.32	0.047/0.014	3.24 (1.37 to 7.68)	.0034
All genes	High risk *v* controls[e]	70	0.044/0.021	2.27 (1.43 to 3.61)	.00046	0.037/0.024	1.68 (0.91 to 3.10)	.1	0.056/0.014	3.60 (1.47 to 8.78)	.0024
All genes	Metastatic *v* controls[f]	41	0.057/0.021	3.10 (1.54 to 6.23)	.0022	0.027/0.024	0.92 (0.17 to 4.84)	.92	0.068/0.014	4.64 (1.74 to 12.32)	.0014
BRCA2+ATM+PALB2+NBN	Cases *v* controls	51	0.023/0.0061	4.20 (2.05 to 8.63)	.0000069	0.016/0.007	2.42 (1.00 to 5.85)	.037	0.038/0.0041	9.40 (2.16 to 40.93)	.000086
BRCA2+ATM+PALB2+NBN	High risk *v* controls	40	0.031/0.0061	5.47 (2.59 to 11.54)	5.8^{E-07}	0.023/0.007	3.47 (1.34 to 9.00)	.0078	0.043/0.0041	9.10 (2.05 to 40.34)	.00022
BRCA2+ATM+PALB2+NBN	Metastatic *v* controls	20	0.039/0.0061	6.72 (2.40 to 18.87)	.00028	0.014/0.007	0.96 (0.07 to 13.33)	.97	0.048/0.0041	11.10 (2.32 to 53.22)	.00036
BRCA2+ATM	Cases *v* controls	39	0.017/0.0047	3.91 (1.73 to 8.86)	.00021	0.011/0.006	1.98 (0.74 to 5.31)	.16	0.029/0.0021	14.88 (1.93 to 114.47)	.00017
BRCA2+ATM	High risk *v* controls	31	0.024/0.0047	5.29 (2.27 to 12.36)	.000017	0.015/0.006	2.64 (0.89 to 7.82)	.074	0.036/0.0021	16.22 (2.09 to 126.00)	.00014
BRCA2+ATM	Metastatic *v* controls	18	0.039/0.0047	9.21 (3.03 to 27.95)	.000062	0.014/0.006	1.00 (0.07 to 14.30)	.1	0.048/0.0021	22.24 (2.72 to 181.69)	.000073

[a]No. of variants identified in each group.

[b]Burden allele frequency in cases/controls.

[c]Odds ratio and 95% CI from the Wald test.

[d]*P* value from the likelihood ratio test.

[e]High-risk disease was defined as Gleason score > 7 or nonlocalized disease or prostate-specific antigen level > 50 ng/mL or death from prostate cancer.

[f]Metastatic disease for Ugandans was defined as prostate-specific antigen level > 100 ng/mL. Prostate-specific antigen level at diagnosis not available for African Americans; stage of prostate cancer not available for Ugandans.

Overall, 5.7% of men of African ancestry defined as having metastatic PCa were carriers of pathogenic variants in the 19 DNA repair genes analyzed, which is lower than the 11.8% prevalence observed among white men with metastatic disease sequenced using a similar 20-gene panel.[13] The strongest evidence of association was observed with *BRCA2*, which is consistent with previous studies in men of European ancestry that found *BRCA2* germline variants to be associated with aggressive PCa.[13,24] However, we observed a lower frequency of *BRCA2* variants in metastatic cases (2.1%) compared with those reported in white men (5.3%).[13] Our findings are also in agreement with a prior study in men of European ancestry that identified deleterious variants in *ATM* associated with aggressive PCa.[13,25] More comparable frequencies were found for metastatic cases with *ATM* variants (1.8%) compared with white men (1.6%).[13] We also observed an enrichment of pathogenic variants in *PALB2* and *NBN* among cases versus controls, which also supports a number of previous studies in men of European ancestry.[13,25,26] However, overall pathogenic variants in *ATM*, *BRCA2*, *PALB2*, and *NBN* genes collectively accounted for only 3.9% of metastatic cases in our study versus 7.7% of metastatic cases in white men.[13]

Although pathogenic variants were associated with risks of aggressive disease in both African American and Ugandan men, differences in effect size were noted. For African American men, 3.4% of cases with Gleason score > 7 (*v* 1.0% with Gleason score ≤ 7) carried pathogenic variants in these genes. These percentages were greater for Ugandan men, where 5.2% of cases with Gleason score > 7 (*v* 1.3% with Gleason score ≤ 7) and 4.8% of cases with PSA level > 100 ng/mL (*v* 1.6% with PSA level < 50 ng/mL) were pathogenic variant carriers. These population differences in rare variation frequency and the larger effect size in gene burden testing observed in Ugandans reflects the higher proportion of metastatic disease at diagnosis and PSA screening used in the selection of controls in the Ugandan study (see Methods).

To our knowledge, this study represents the first large investigation aimed at identifying and testing rare pathogenic variants in DNA repair genes in association with PCa risk for men of African ancestry. This empirical evidence highlights the clinical relevance of pathogenic variants in some of the genes previously associated with aggressive disease in white men as major risk factors for aggressive PCa in men of African ancestry. Thus, guidelines regarding gene panel testing for men at high risk for metastatic disease that have been based primarily on studies among white men are appropriate to consider for men of African ancestry. Additional large studies of aggressive PCa phenotypes and more extensive gene panels will help to build better predictive and prognostic gene-based tests of aggressive PCa and to better guide patient management for men of African ancestry.

The study has a few limitations, including the small number of clinically defined cases with metastatic PCa and the lack of complete clinical data for cases (eg, stage for Ugandans). Grade migration over time may have resulted in misclassification of men with lower Gleason scores being categorized into higher Gleason score categories. If this is the case, the associations between pathogenic variants and aggressive PCa would likely be biased toward the null, with true effect sizes being larger. However, the positive associations observed in aggressive disease groups defined by Gleason score, stage, and PSA suggest that grade migration did not bias the results. Another limitation of this study is that we did not investigate large indels that could result in loss of function of the entire protein.[27] Whole-genome sequencing will be needed to explore a more complete spectrum of pathogenic variants, including noncoding variation and large indels, in PCa in men of African ancestry.

In conclusion, this study provides strong support for the clinical importance of rare pathogenic variants in DNA repair genes in the development of aggressive PCa for men of African ancestry. Quantifying the prevalence and penetrance of pathogenic germline variants in these and other DNA repair pathway genes will be required to better define men at high risk for clinically severe disease who would benefit from more regular screening and targeted prevention or therapeutic approaches.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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