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ARTICLE

Genetics and Genomics

The *HOXB13* variant X285K is associated with clinical significance and early age at diagnosis in African American prostate cancer patients

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BACKGROUND: Recently, a novel *HOXB13* variant (X285K) was observed in men of African descent with prostate cancer (PCa) in Martinique. Little is known about this or other variants in *HOXB13* which may play a role in PCa susceptibility in African-American (AA) men.

METHODS: We sequenced *HOXB13* in an AA population of 1048 men undergoing surgical treatment for PCa at Johns Hopkins Hospital.

RESULTS: Seven non-synonymous germline variants were observed in the patient population. While six of these variants were seen only once, X285K was found in eight patients. In a case–case analysis, we find that carriers of this latter variant are at increased risk of clinically significant PCa (1.2% carrier rate in Gleason Score ≥7 PCa vs. 0% in Gleason Score <7 PCa, odds ratio, OR = inf; 95% Confidence Interval, 95%CI:1.05-inf, $P = 0.028$), as well as PCa with early age at diagnosis (2.4% carrier rate in patients <50 year vs. 0.5% carrier rate in patients ≥50 year, OR = 5.25, 95% CI:1.00–28.52, $P = 0.03$).

CONCLUSIONS: While this variant is rare in the AA population (~0.2% MAF), its ancestry-specific occurrence and apparent preferential association with risk for the more aggressive disease at an early age emphasizes its translational potential as an important, novel PCa susceptibility marker in the high-risk AA population.

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INTRODUCTION

African-American (AA) men are more likely to be diagnosed with advanced prostate cancer (PCa) and are nearly twice as likely to die from the disease than men of European ancestry [1]. While the reasons are complex and not fully understood, this disparity cannot be fully accounted for by differences in access to care or socio-economic status, indicating that there are biological contributions [2].

While numerous studies in predominantly European–American patients have leveraged large cohorts to identify the molecular drivers of PCa, few equivalent studies have been conducted in patients of African ancestry. For example, the Cancer Genome Atlas (TCGA) sequencing effort for PCa is minimally informative for AA, as it contains only 14% ($n = 43$) AA men, of whom only 17 have the intermediate or high-risk disease [3]. These low numbers, combined with extensive disease heterogeneity, have hampered discovery efforts to identify genomic drivers of PCa among AA populations. Thus, the biological determinants of PCa risk overall and aggressive disease in particular in AA men are still unclear and their discovery remains a critical and unmet need in cancer health disparities research. Our lack of knowledge about the molecular drivers of PCa among AA men remains a major barrier to the implementation of precision medicine in this high-risk population.

The identification of the first bona fide PCa-specific susceptibility gene, *HOXB13*, a prostate-specific transcription factor, was reported in 2012 [4]. Using positional information from linkage analyses in PCa families, a rare but recurrent missense change, G84E, was identified in the *HOXB13* gene on *17q21*. In an analysis of germline DNA from over 5000 PCa cases and controls, the frequency of G84E was significantly higher in cases (1.4%) than controls (0.1–0.4%). An enrichment of G84E was found in PCa patients who were diagnosed at an early age and with a positive family history of PCa. These findings have been consistently confirmed, with odds ratio (OR)s for PCa varying from 2- to 15-fold [5]. Combined analyses of different study populations in the International Consortium for Prostate Cancer Genetics demonstrated that the most common variant in *HOXB13* in US men, G84E, had the highest frequency in individuals of Nordic descent [6]. Indeed, as many as 8–10% of Swedish [7] and Finnish [8] men with family history-positive PCa diagnosed at an early age carry a G84E *HOXB13* variant, compared to ~1% or less in unaffected men in these populations. A critical additional finding was that all G84E variant

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carriers shared a common haplotype [6], i.e., they are all descended from a common founder, presumably of Swedish or Finnish origin.

Despite the established role of *HOXB13* G84E in PCa susceptibility in men of European descent, little is known about a possible role for *HOXB13* variants in PCa risk in men of African ancestry. After an initial observation in a single AA PCa case by Akbari et al. in 2012 [9], Marlin et al. recently reported observing *HOXB13* c.853delT (X285K) in multiple PCa cases of African descent in a small study from Martinique [10]. Among 46 early-onset PCa patients (age at diagnosis <51), three were observed to have X285K. Based upon these preliminary findings, we hypothesised that there may exist currently uncharacterised, recurrent, function-altering variants in the *HOXB13* gene that may play an important role in PCa susceptibility, particularly for early-onset disease, in African Americans. In this study, we examine an expanded number of AA PCa patients for the occurrence of X285K and other variants in *HOXB13* which may confer increased risk in this high-risk population.

METHODS

Study subjects

This is a retrospective study including 1048 PCa patients of African-American ancestry as determined by self-report. Study subjects were patients undergoing radical prostatectomy (RP) for clinically localised PCa at the Brady Urological Institute of Johns Hopkins Hospital Baltimore, Maryland, USA. Clinical and demographic information of these patients, including age at diagnosis, family history of PCa, surgical GS, and tumour staging (TMN) were obtained from an IRB approved, research database containing no PHI. The only criteria for selection, other than radical prostatectomy for PCa, was African-American ancestry and availability of discarded, deidentified, non-tumour involved tissue samples from surgery as a source of germline DNA. The institutional review board at Johns Hopkins University School of Medicine approved this study.

Sequencing of germline DNA and bioinformatics analysis

Whole-exome sequencing was performed on germline DNA derived from non-tumour involved seminal vesicle tissue from the cases using Novogene sequencing service. The Agilent SureSelect Human All Exon V5 was used to capture and enrich exomic sequences. Enriched libraries were sequenced using an Illumina HiSeq 2500 system. The mean sequencing depth of coverage was 71x.

Paired-end reads were aligned to the GRCh37 version of the human genome using Burrows-Wheeler Aligner v0.7 to generate BAM files. After sorting the BAM files using samtools, PCR duplicates marked using Picard and realignment around putative gaps was performed using the Genome Analysis Toolkit (GATK) v3.2-2. Variant calling was performed with the GATK Haplotype caller. ANNOVAR (http://annovar.openbioinformatics.org/en/latest) and snpEff were used for annotating variants. For retrieving information including population frequency estimates, population-based databases ExAC (http://exac.broadinstitute.org/), and gnomAD (https://gnomad.broadinstitute.org) were used, and the clinical database, ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/variation/) was used to assess the pathogenicity of variants [11]. SIFT (https://sift.bii.a-star.edu.sg) and Polyphen2 (http://genetics.bwh.harvard.edu/pph2/) were used to assess potential deleteriousness of non-synonymous changes.

Statistical analysis

The frequency of variants in *HOXB13* was compared and analysed using Fisher's exact test. A proportional trend test was used to estimate the significance of trends among multiple groups.

We used 2170 ancestral informative markers (AIMs) of the Illumina global screening array (GSA) to assess genetic background of self-reported African-American (AA) subjects. The analysis was performed using principal component analysis within PLINK and the top 20 principal components (eigens) were obtained [12]. We also plotted the top two eigens of AA subjects together with three anchored racial populations for 1KG (CEU, YRI and EAS). All AA subjects in our study are consistent with recent admixture of CEU and YRI [12].

A type I error of 0.05 (two-sided) was used to define statistical significance. All the statistical analyses were performed using R software (version 4.0.4). The OR and 95% confidence interval were estimated using the R software "fisher.test". All ORs were adjusted for principal component eigens to account for potential confounding by ancestry.

RESULTS

To search for germline coding sequence variants in *HOXB13* in a large, well-characterised African-American PCa population, we sequenced both exons of the gene in 1048 AA men who had

Table 1. Baseline demographic characteristics of the study population.

Variables	All	Clinical variables by Gleason Score		
		GS ≥ 7	GS ≤ 6	*P* value
No. of patients	1048	650 (62.0%)	397 (37.9%)	–
Age at diagnosis (year, median + IQR)	57 (52–62)	58 (52–63)	55 (51–60)	1.58×10^{-8}
PSA (mean ± SD)	7.64 ± 6.32	8.73 ± 7.31	5.87 ± 3.61	$<2.20 \times 10^{-16}$
Gleason Score (*n*, %)				
<=6	397 (37.9%)	0 (0.00%)	397 (100%)	–
7	542 (51.7%)	542 (83.4%)	0 (0.00%)	
>=8	108 (10.3%)	108 (16.6%)	0 (0.00%)	
Missing	1 (0.1%)	–	–	
Family history**				
Positive	493 (47.0%)	305 (46.9%)	187 (47.1%)	0.77
Negative	528 (50.4%)	333 (51.2%)	195 (49.1%)	
Missing	27 (2.6%)	12 (1.8%)	15 (3.8%)	
Pathological TMN stage				
$T_2N_0(N_x)M_x$	700 (66.8%)	364 (56.0%)	336 (84.6%)	$<2.20 \times 10^{-16}$*
$T_{3a}N_0(N_x)M_x$	208 (19.8%)	161 (24.8%)	47 (11.8%)	
$T_{3b}N_0(N_x)M_x$	76 (7.3%)	67 (10.3%)	4 (1.0%)	
All stages N_1	32 (3.1%)	32 (4.9%)	0 (0.0%)	
$T_xN_xM_x$/missing	32 (3.1%)	–	–	

*Proportion trend test.
**First or second-degree relative diagnosed with prostate cancer.

undergone radical prostatectomy (RP) for treatment of PCa at the Brady Urological Institute between 2006 and 2018. Demographic characteristics and baseline clinical information are summarised in Table 1. The average age at diagnosis was 56.8 years old. The total number of cases with pathologic GS ≥ 7 was 650, while 397 cases had GS ≤ 6. For the pathologic stage, 700 cases (66.8%) were $T_2N_0M_X$, with 284 cases (28.1%) $T_{3a}N_0M_X$ or T_{3b} N_0M_X; 32 cases (3.1%) were N_1.

We identified seven different rare non-synonymous changes in *HOXB13*: G84E, S93A, C100Y, L106R, P134Q, T242I and X285K. All variants except X285K were observed only once in the study population. With the exception of *S93A*, all missense variants are predicted to be damaging or possibly damaging by SIFT and/or Polyphen2. T242I affects a conserved amino acid at the beginning of the second alpha helix in the DNA-binding homeodomain. P134Q is adjacent to a conserved domain harbouring a putative binding site for MEIS homeobox cofactors. Table 2 lists a summary of these variants, and Fig. 1 shows the position of the variants.

X285K was the only recurrent non-synonymous change observed, seen in 8 AA cases (carrier frequency 0.76%). An examination of the pathologic variables in these cases revealed that all carriers of X285K had cancers with Gleason Score (GS) 7 (3 + 4, $n = 4$ and 4 + 3, $n = 3$), or GS 9 (4 + 5, $n = 1$), Table 3. In all cases with GS ≥ 7 ($n = 650$), the carrier frequency of X285K was 1.2% ($n = 8$), whereas in the 395 cases with GS ≤ 6, no carriers were observed; (odds ratio, OR = inf; 95% confidence interval, 95% CI:1.05-inf, $P = 0.028$, Table 4. In addition to the higher grade, X285K was significantly associated with earlier age at diagnosis of PCa. The median age of diagnosis in X285K carriers was 50.0 years (interquartile range, IQR: 42.0–63.0 years) vs. 57.0 years (IQR: 52.0–62.0 year) in non-carriers. The carrier rate of this variant in PCa cases with age at diagnosis <50 years was 2.4% (4 out of 170), which was significantly higher than the carrier rate (0.5%, 4 out of 878) in PCa with age at diagnosis ≥50 years (OR = 5.25, 95% CI: 1.00–28.52, $P = 0.03$, Table 5).

Frequency data available for X285K (rs77179853) from population databases demonstrated a consistently low minor allele fraction (MAF) in AA populations (Table 2, MAF 0.22% in gnomAD v.3 genomes, $n = 42{,}030$ alleles). Compared to this AA population data, the frequency of X285K was non-significantly higher in AA PCa cases overall (MAF 0.38%, OR = 1.74, 95% CI: 0.73–3.59, $P = 0.15$), but significantly higher in cases with GS ≥ 7 (MAF 0.63%, OR = 2.86, 95% CI: 1.20–5.90, $P = 0.01$).

DISCUSSION

To the best of our knowledge, this study is the largest *HOXB13* sequencing analysis in AA PCa patients published to date. We found that the X285K variant was: (1) the only recurrent *HOXB13* variant in the AA study cohort, (2) associated with earlier age at diagnosis of PCa; and (3) associated with GS ≥ 7 PCa in AA.

HOXB13 codes for a highly prostate-specific transcription factor that is necessary for normal prostate development [13]. Its expression is maintained throughout adulthood, and during the initiation and progression of most prostate cancers. HOXB13 interacts with AR to modulate the expression of various androgen-responsive genes, and this interaction with normal and variants of AR (eg ARv7) has been proposed to play a key role in reprograming the cistrome in both primary and metastatic PCa [14–16]. However, a comprehensive understanding of the role that *HOXB13* plays in prostate biology has not been described.

Despite extensive studies characterising *HOXB13* germline variants in PCa in men of European and Asian descent, little is known about possible associations of such variants in men of African descent. In this study, we sequenced the coding region of *HOXB13* in germline DNA from a well-characterised population of 1048 AA men undergoing surgical treatment for clinically localised PCa. This analysis revealed a set of six non-recurrent

Table 2. Non-synonymous variants in HOXB13 (chr17q21.32, Access No. NM_006361) in AA PCa cases.

CHR	POS (hg19)	Type	Exon	cDNA	Protein	MAF	SIFT	Polyphen2	ExAC			GnomAD exome			GnomAD genome		
									ALL	AFR	NFE	ALL	AFR	NFE	ALL	AFR	NFE
17	46804154	Stoploss	Exon 2	c.853delT	p.X285K	0.0038	na	na	0.0002	0.0021	0	0.0002	0.0024	0	0.0005	0.0018	0
17	46804282	Missense	Exon 2	c.C725T	p.T242I	0.0005	Deleterious	Possibly damaging	.	.	.	.	.	.	3.23E-05	0.0001	0
17	46805555	Missense	Exon 1	c.C401A	p.P134Q	0.0005	Tolerated	Possibly damaging	.	.	.	.	.	.	.	.	.
17	46805639	Missense	Exon 1	c.T317G	p.L106R	0.0005	Tolerated	Possibly damaging	8.30E-06	9.88E-05	0	1.63E-05	0.0003	0	.	.	.
17	46805657	Missense	Exon 1	c.G299A	p.C100Y	0.0005	Deleterious	Benign	.	.	.	.	.	.	.	.	.
17	46805679	Missense	Exon 1	c.T277G	p.S93A	0.0005	Tolerated	Benign	8.37E-06	0.0001	0	4.07E-06	6.60E-05	0	.	.	.
17	46805705	Missense	Exon 1	c.G251A	p.G84E	0.0005	Deleterious	Probably damaging	0.0022	0.0004	0.0031	0.0019	0.0004	0.0024	0.0021	0.0005	0.0025

MAF minor allele frequency in study population, *AFR* African American, *NFE* non-Finnish European.

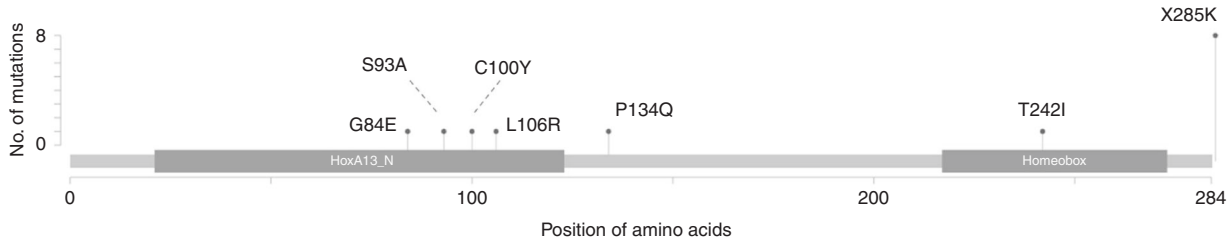

Fig. 1 Location of variants in *HOXB13*.

Table 3. Clinicopathologic information for X285K carriers.

ID	Pre-biopsy PSA (ng/ml)	Age at diagnosis	Ancestry	Pathology Gleason Score		FH	pr wt	T	FCF CP	E ECP	SM	OC	LN	SV
1	5.1	64	African-American	7	4 + 3	0	37.6	T3a	N	Y	N	N	N	N
2	11.6	41	African-American	7	3 + 4	0	35.7	T2	N	N	Y	Y	N	N
3	5.8	60	African-American	7	3 + 4	1	28.9	T2	N	N	N	Y	N	N
4	2.4	45	African-American	7	3 + 4	1	33.1	T2	N	N	N	Y	N	N
5	18	49	African-American	7	3 + 4	0	47.5	T3b	Y	N	N	N	Y	Y
6	5	51	African-American	9	4 + 5	0	33.9	T2	N	N	N	Y	N	N
7	13	65	African-American	7	4 + 3	0	73.7	T2	N	N	N	Y	N	N
8	7.4	39	African-American	7	4 + 3	0	41.2	T2	N	N	N	Y	N	N
9*	5.1	66	European–American	8	3 + 5	0	61.4	T3a	N	Y	Y	N	N	N

T surgical T stage, *FCP* focal capsular penetration, *ECP* extensive capsular penetration, *SM* surgical margins, *OC* organ confined, *LN* lymph node positive, *SV* seminal vesicle invasion, *FH* first or second-degree relative with PCa, *pr wt* prostate weight, *Y* yes/positive, *N* no/negative, *na* not available.
*This patient is a self-reported European American with African and European admixture in the ancestry analysis.

Table 4. Carrier rates of the HOXB13 mutation (c.853delT X285K) in AA population and in different GS groups.

Mutation	Carrier rates	Clinical significance based on Gleason Score			
		GS ≥ 7 ($n = 650$)	GS ≤ 6 ($n = 397$)	OR (95% CI)	*P* value
Negative	1034 (98.7%)	638 (98.2%)	395 (99.5%)	Ref	Ref
c.853delT X285K	8 (0.8%)	8 (1.2%)	0 (0%)	inf (1.05-inf)	0.028

missense variants, and one recurrent, non-synonymous change. Of the missense changes, five were predicted to be deleterious. Five changes are in exon 1, coding for the amino-terminal portion of HOXB13, with the remaining change in the DNA-binding homeobox domain, coded for in exon 2. Whether these non-recurrent missense changes contribute to prostate carcinogenesis is unknown. Much larger studies will be required to determine their possible statistical association with the risk of PCa.

One of the missense variants observed is the Nordic founder variant, G84E. Its presence in PCa cases of African descent has been previously reported by others including Witte et al. who demonstrated that the variant was on a haplotype of European origin, indicating that it was most likely the result of population admixture rather than an independent variant event [17].

The only recurrent change observed was a frameshift variant resulting in loss of the in-frame stop codon due to a deletion of one nucleotide, c.853delT at the cDNA level and resulting in p. Ter285KextX95 (aka X285K) at the protein level. The functional significance of this stop loss is unclear at present, although there are multiple examples where stop-loss variants confer functional consequences, typically via instability of either the mRNA transcript or resultant protein [18]. For the change described here, if translated, the stop-loss-containing mRNA would code for a HOXB13 protein that extended an additional 96 amino acids before reaching the next in-frame stop codon. Whether this C-terminal protein extension, immediately 3′ to the DNA-binding homeodomain, affects HOXB13 protein function, or leads to instability of the transcript and/or protein remains to be determined. Clinvar lists this variant as having uncertain significance (two entries), or likely benign (one entry), although no stability or functional studies of this variant have been reported.

The c.853delT variant was first reported by Akbari et al. in a study of 1843 PCa cases and 2225 controls [9]. One of 200 AA cases and one of 160 AA controls carried the variant. It was absent from EA and Asian cases and controls. In our experience sequencing *HOXB13* in germline DNA from over 5000 PCa cases of European descent, we have found only one example of X285K (Table 3)—this patient had high-grade disease (GS 8 at 66 yo) and admixed ancestry. Regarding the African ancestry-specific nature X285K, population MAF data from gnomAD show only a single occurrence of this variant out of 140,000 non-African-American individuals. We did find a different stop-loss mutation (p. X285SextX31, A to C c.854) in another EA patient with high-grade (GS 9) disease, at age 47.

Interestingly, putative founder variants have been seen in other ancestral populations, including Chinese and Japanese [19, 20]. In both instances, like G84E, the recurrent variants found in these populations convert a Glycine codon to a Glutamic acid codon, although at two different positions: G132E in Japanese and G135E in Chinese. All three of these G to E variants are located close to or

Table 5. Carrier rates of the HOXB13 mutation, c.853delT X285K, in AA populations with different ages at diagnosis.

Mutation	Age at diagnosis			
	PCa <50 year ($n = 170$)	PCa ≥ 50 year ($n = 878$)	OR (95% CI)	*P* value
Negative	165 (97.1%)	869 (99.0%)	Ref	Ref
c.853delT X285K	4 (2.4%)	4 (0.5%)	5.25 (1.00–28.52)	0.03

within conserved domains that all paralog group 13 HOX proteins share. These domains contain binding sites for the *MEIS* family of TALE homeobox cofactors, which are known to bind and modulate the transcriptional effects of HOXB13 [21, 22]. While these observations suggest disrupted HOXB13–MEIS interactions may underlie the pro-carcinogenic activity of G to E- mutated HOXB13, this remains largely unexplored [22, 23]. Furthermore, other than a more general dysregulation of HOXB13 function, whether these variant forms of HOXB13 share similar functional effects related to prostate carcinogenesis remains unknown.

HOXB13 G84E has been consistently shown to identify men at high risk for PCa in populations of men of European ancestry [7, 8]. Men carrying the G84E variant in *HOXB13* have a significantly increased risk of PCa and are more likely to have a family history of positive, early-onset disease. To date, no other variant in *HOXB13* has been found to have similar clinical implications. This study suggests that X285K variant carriers in the AA population have a higher risk of clinically significant PCa and an earlier age of onset. If confirmed, these results would provide a rationale for incorporating this variant when screening AA men for PCa risk.

The limitations of this study are several fold: although over 1000 patients were studied, the number of variants observed is low, thus due to the rarity of these changes, the relatively small numbers of carriers in this study resulted in low power to perform additional subgroup analysis in multiple GS groups (eg ≤ 6, =7 and ≥8), T stages, or other clinicopathologic variables. Further studies need to be conducted to assess and expand our study. It should also be noted that the interpretation of any results between cases and controls, using public datasets for the latter, should be done cautiously due to population heterogeneity and differing sequencing methods. In addition, we acknowledge the lack of generalisability of our observations to the general prostate cancer population overall due to the reliance on a hospital based, surgically treated study population. Other biases like those introduced by changes in screening and diagnostic practices, pathological grading over time, and referral patterns are possible, and could affect our results.

With respect to function, to make any causal inference beyond the associations from this study, mechanistic studies are necessary to understand how this variant might act to affect *HOXB13* activity and promote PCa in AA. Finally, even if confirmed, the low population frequency indicates that the X285K likely accounts for a very small proportion of PCa disparities.

In summary, we confirm the presence of a rare but recurrent germline variant in *HOXB13* in AA men with PCa, and provide initial data to suggest a potentially important association with risk of early-onset, clinically significant PCa. Markers such as these, if validated, are urgently needed to provide useful risk stratification information for PCa in the high-risk AA population.

DATA AVAILABILITY

The data of the study are available upon reasonable request from the corresponding author.

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AUTHOR CONTRIBUTIONS

WI conceived and designed the study. RN, MG, CS, DR, KC, PW, SC and LZ contributed materials and collected the data. RN and JW analysed the data. RN, WI and JX wrote the manuscript.

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COMPETING INTERESTS

KAC and WBI are coinventors on a patent (no. 9593380; Inst.) related to the discovery of HOXB13 as a prostate cancer susceptibility gene.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the Institutional Review Boards at Johns Hopkins University. The study was performed in accordance with the Declaration of Helsinki.

CONSENT TO PUBLISH

All the authors have reviewed and approved the current version of the manuscript.

ADDITIONAL INFORMATION

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