Original Article
Prostate cancer

pISSN: 2287-4208 / eISSN: 2287-4690
World J Mens Health 2023 Oct 41(4): 960-968
https://doi.org/10.5534/wjmh.220190

The World Journal of MEN's HEALTH

Germline DNA-Repair Genes and HOXB13 Mutations in Korean Men with Metastatic Prostate Cancer: Data from a Large Korean Cohort

Sang Hun Song[1,2,*], Hak-Min Kim[3,*], Yu Jin Jung[4], Ha Rim Kook[2,5], Sungwon Jeon[6,7], Jong Bhak[6,7,8,9], Jin Hyuck Kim[1,2], Hakmin Lee[1,2], Jong Jin Oh[1,2], Sangchul Lee[1,2], Sung Kyu Hong[1,2], Seok-Soo Byun[1,3,4]

[1]Department of Urology, Seoul National University Bundang Hospital, Seongnam, [2]Department of Urology, Seoul National University College of Medicine, [3]Procagen, [4]Department of Medical Device Development, Seoul National University College of Medicine, Seoul, [5]Seoul N Urology Clinic, Seongnam, [6]Korean Genomics Center, Ulsan National Institute of Science and Technology, [7]Clinomics Inc., [8]Department of Biomedical Engineering, Ulsan National Institute of Science and Technology College of Information-Bio Convergence Engineering, Ulsan, [9]Personal Genomics Institute, Genome Research Foundation, Osong, Korea

Purpose: Germline mutations in DNA damage repair (DDR) genes such as *BRCA2* have been associated with prostate cancer (PC) risk but has not been thoroughly evaluated for metastatic prostate cancer (mPC) in Asian men. This study attempts to evaluate frequency of DDR mutations in the largest cohort of Koreans.

Materials and Methods: We recruited 340 patients with mPC unselected for family history of cancer and compared to 495 controls. Whole genome sequencing was applied to assess germline pathogenic/likely pathogenic variants (PV/LPVs) in 26 DDR genes and *HOXB13*, including 7 genes (*ATM*, *BRCA1/2*, *CHEK2*, *BRIP1*, *PALB2*, and *NBN*) associated with hereditary PC. Comparisons to published Caucasian and Japanese cohorts were performed.

Results: Total of 28 PV/LPVs were identified in 30 (8.8%) patients; mutations were found in 13 genes, including *BRCA2* (15 men [4.41%]), *ATM* (2 men [0.59%]), *NBN* (2 men [0.59%], and *BRIP1* (2 men [0.59%]). Only one patient had *HOXB13* mutation (0.29%). A lower rate of overall germline variant frequency was observed in Korean mPC compared to Caucasians (8.8% *vs*. 11.8%), but individual variants notably differed from Caucasian and geographically similar Japanese cohorts. PV/LPVs in DDR genes tended to increase gradually with higher Gleason scores (GS 7, 7.1%; GS 8, 7.5%; GS 9–10, 9.9%).

Conclusions: *BRCA2* was the most frequently mutated gene common to different cohorts supporting its importance, but differences in variant distribution in Korean mPC underscore the need for ethnic-specific genetic models. Future ethnic-specific analyses are warranted to verify our findings.

Keywords: Asians; DNA damage; Genetics; Prostatic neoplasms

Received: Sep 15, 2022 **Revised:** Dec 30, 2022 **Accepted:** Jan 16, 2023 **Published online** Mar 27, 2023
Correspondence to: Seok-Soo Byun https://orcid.org/0000-0001-7979-9035
Department of Urology, Seoul National University Bundang Hospital, 82 Gumi-ro 173beon-gil, Bundang-gu, Seongnam 13620, Korea.
Tel: +82-31-787-7343, **Fax:** +82-31-787-4057, **E-mail:** seoksoo.byeon@gmail.com
*These authors contributed equally to this work as co-first authors.

INTRODUCTION

With considerable advances in identifying risk variants in the genomic landscape of prostate cancer (PC), germline mutation in nominal genes have garnered interest for prediction of cancer prognosis and treatment response. As such, recent guidelines instruct implementation of germline screening in men with high familial risk or early onset of PC based on results from large genome-wide association studies (GWAS) [1]. Currently, genetic factors contribute to approximately 5% to 15% of all PC cases, especially in carriers of rare pathogenic mutations (RPMs) in DNA damage repair (DDR) genes such as *BRCA1*, *BRCA2*, *ATM*, and *CHEK2* [2]. Such patients with either germline or somatic pathogenic variants (PVs) show differential response to treatment and have aggressive forms of the disease. With the approval of poly ADP-ribose polymerase (PARP) inhibitors such as Olaparib in castration-resistant PC with BRCA mutation either germline and/or somatic, identification of such DDR mutations may provide a chance of early therapy.

Amidst evidence that support genetic testing to identify high-risk patients and guide eligibility for active surveillance as well as provide individualized methods of treatment based on genomic imprints, reports on germline mutation of risk variants for PC in Asian population are limited. Multi-ethnic based GWAS summary statistics are conducted primarily in Caucasian and European men, and large proportions of PC-associated single nucleotide polymorphisms (SNPs) lack significance when compared to East Asian cohorts [3]. Men of Asian ancestry were found to have differential levels of SNP-based composite genetic risk scores compared to African and European men when using the same risk variants [1], implying the need for ethnic-specific analysis to achieve robust performance suitable for clinical implementation. Metastatic PC (mPC) in Asians deserve further evaluation, not only due to the increase in incidence but also due to Asian men harboring more adverse phenotypes [4]. The objective of this study was to identify the frequency of germline mutation in DDR genes & highly penetrant *HOXB13* in patients with mPC in Korean populations.

MATERIALS AND METHODS

1. Study population

Clinical variables including age at diagnosis and metastasis, initial PSA, and Gleason score (GS) were included. Histopathologic analysis was based on sextant transrectal ultrasound or MRI-fusion targeted biopsy as well as transurethral prostatectomy specimen, and radical prostatectomy (RP)-proven pathologic staging and grade were used when available. Total 340 patients treated or undergoing treatment for any mPC were included, defined as metastasis outside the prostate to lymph nodes, bone, and other organs. All mPC patients were recruited from a single tertiary hospital as part of a prospective biobank collected in all PC patients collected at initial presentation or at identification of metastatic status (*de novo*, progression, or recurrence). Allele frequency for healthy controls were obtained from 2 multicenter databases, Korean Variome Center (KoVariome) and Ulsan 10K Genomes Project (U10K). Total 495 healthy male controls were used (n=145 from KoVariome and n=350 from U10K), defined as male over 40 with no history of any cancer.

2. Ethics statement

Patient recruit and clinical data collection was conducted at from a single tertiary medical center (Seoul National University Bundang Hospital) as a prospective biobank approved by the Institutional Review Board (B-167/355-302). Informed consent was obtained for all subjects diagnosed for PC between 2008 and 2021.

3. Sequencing, variant calling & annotation

Selection of DDR genes were based on 20 genes from the pivotal study by Prichard et al [2], including *ATM*, *ATR*, *BAP1*, *BARD1*, *BRCA1*, *BRCA2*, *BRIP1*, *CHEK2*, *FAM175A*, *GEN*, *MLH1*, *MRE11A*, *MSH2*, *MSH6*, *NBN*, *PALB2*, *PMS2*, *RAD61C*, *RAD51D*, and *XRCC2*. *HOXB13* with 6 additional PC-associated DDR genes proven in previous literature (*BLM*, *CDK12*, *CHEK1*, *FANCA*, *RAD51B*, *RAD54L*) were further included. *HOXB13*, a well-known tumor suppressor gene implicated in PC pathogenesis, was included as per recommendation for its inclusion in recent guidelines for germline testing. Whole genome sequencing was performed using DNBSEQ-T7 sequencing platform (MGI-Tech, Shenzhen, China), with 150 bp

(base pair) read length and average 38× depth of coverage. Samples were obtained from both saliva (n=11) and blood (n=329) and DNA extracted per manufacturer instructions. Reads were aligned to human reference genome GRCh38 (hg38) using the Burrows-Wheeler Aligner (BWA-MEM) [5], after quality filtration of raw FASTQ sequencing with FastQC and adapter sequence trimming with Cutadapt [6] (Supplement Fig. 1). Fastp [7] quality filter were applied prior to alignment, and duplicate reads were removed with Picard v2.21.8 [8]. GATK4 was used for Base Quality Score Recalibration and variant calling [9]. Annotations of single nucleotide variant (SNV) and indel (insertion-deletion mutations) were identified using SnpEff [10], and structural variants (SVs) and copy number variations (CNVs) using Lumpy [11] and CNVnator [12] frameworks, respectively. PVs and likely PVs (LPVs) for SNV and indels were further annotated using the ClinVar database (ver. 20211120) [13]. Slightly low mapping rates when DNA was extracted *via* saliva (Supplement Fig. 2), but no significant differences were observed in overall analysis.

4. Statistical analysis

Association and odds ratio (OR) for case-controls and inter-cohort comparisons were conducted with Fisher's exact and logistic regression analysis, respectively. Cochran-Armitage test was used for trend. All tests were two-sided with p-value less than 0.05 considered significant unless quoted otherwise. Statistical tests were performed using the R package (ver. 4.0.5).

RESULTS

1. Clinical characteristics

Median age at diagnosis and metastasis for patients with mPC were 68 and 71 years old, respectively (Supplement Table 1). Median PSA was 37.6±507.1 ng/mL, with 8.4% GS 7 (3+4 or 4+3), 28.2% GS 8, and 63.4% GS 9 or higher. Of all patients, 127 (37.4%) were de novo mPC with no previous treatment, whereas 212 (62.4%) were recurrent mPC (progressed from localized disease) with overt metastases detected at imaging (32.1%) or suspected for biochemical recurrence (BCR) after initial treatment with potential micro-metastases (30.3%). Family history was collected in 285 participants, of whom only 15 (5.3%) had a positive family history. Nodal metastasis was identified in 48 (14.1%) patients who underwent RP.

2. Germline variant mutation in DDR genes and *HOXB13*

Results from the ClinVar annotation statistics for 26 DDR genes and *HOXB13* are found in Table 1. Total 1,442 single nucleotide variants and 376 indels were identified, with the vast majority of variants corresponding to benign (BV), likely-benign (LBV) or unknown significance (VUS). Six SNVs and 11 indels were classified as PV/LPV, constituting 0.42% and 3.2% of all SNVs and indels, respectively. Six stop-gain and frameshift variants not identified from ClinVar were putatively considered as PV/LPVs and included in downstream analysis, and categorization conducted through InterVar [14] based on the American College of Medical Genetics and Genomics (ACMG) guideline [15] has been further noted in Table 2. Of all patients, 30

Table 1. Variant significance classification from ClinVar annotation

Variant significance	SNV	%	Indel	%
Pathogenic	3	0.21	11	2.93
Pathogenic/likely pathogenic	1	0.07	1	0.27
Likely pathogenic	2	0.14	0	0.00
Benign	1,045	72.47	252	67.02
Benign/likely benign	52	3.61	30	7.98
Conflicting interpretations of pathogenicity	85	5.89	13	3.46
Likely benign	131	9.08	51	13.56
Uncertain significance	123	8.53	18	4.79
Total	1,442	100	376	100

SNV: single nucleotide variant.

Table 2. PV/LPVs (n=28) in Korean mPC (n=30)

Gene	Allele change	Amino acid change	Consequence	ClinVar	No.
ATM	c.5288_5289insGA	p.Tyr1763fs	FS	PV, LPV	1
ATM	c.9022C>T	p.Arg3008Cys	MS	PV, LPV	1
BRCA1	c.2216_2217delAA	p.Lys739fs	FS	PV	1
BRCA2	c.632-1G>T	-	SpV	LPV	1
BRCA2	c.658_659delGT	p.Val220fs	FS	PV	1
BRCA2	c.1399A>T	p.Lys467[a]	NS	PV	2
BRCA2	c.2798_2799delCA	p.Thr933fs	FS	PV	2
BRCA2	c.3744_3747delTGAG	p.Ser1248fs	FS	PV	1
BRCA2	c.5073dupA	p.Trp1692fs	FS	PV	1
BRCA2	c.5148T>G	p.Tyr1716[a]	NS	PV[a]	1
BRCA2	c.5576_5579delTTAA	p.Ile1859fs	FS	PV	1
BRCA2	c.5795_5799delATAAC	p.His1932fs	FS	PV	1
BRCA2	c.6262delA	p.Thr2088fs	FS	PV	1
BRCA2	c.7480C>T	p.Arg2494[a]	NS	PV	1
BRCA2	c.8488-1G>A	-	SpV	PV	1
BRCA2	c.9253delA	p.Thr3085fs	FS	PV	1
BRIP1	c.1378_1379delGA	p.Asp460fs	FS	PV	1
BRIP1	c.1203_1204delTG	p.Ala402fs	FS	LPV[a]	1
FAM175A	del exon 4-7	-	Large del	-	1
GEN1	c.606_618delAATACTTCTTGGC	p.Ile203fs	FS	VUS[a]	1
HOXB13	c.395G>A	p.Gly132Glu	MS	VUS	1
MSH6	c.3916_3920dupGCTAA	p.Asn1307fs	FS	PV	1
NBN	c.1523dupT	p.Ser509fs	FS	LPV[a]	1
NBN	c.585-2A>G	-	SpV	LPV	1
PMS2	del exon 13-14	-	Large del	-	1
RAD51C	del exon 6-9+3'UTR	-	Large del	-	1
RAD51D	c.212C>A	p.Ser71[a]	NS	LPV[a]	1
RAD54L	c.1650_1660dupGAAGCGAGCCA	p.Lys554fs	FS	VUS[a]	1

FS: frameshift, LPV: likely pathogenic variant, mPC: metastatic prostate cancer, MS: missense, NS: nonsense: PV: pathogenic variant, SpV: splice variant, VUS: variant of uncertain significance.
[a]These variants do not exist in the ClinVar database but have been annotated *via* InterVar.

(8.8%) harbored total 28 PV/LPVs in 13 out of 20 genes (Table 2). Twenty-five variations were SNV and indels, and 3 variations were large deletions corresponding to CNVs and SVs. *BRCA2* variants were most commonly observed (4.41%), followed by *ATM*, *BRIP1*, *NBN* (each 0.59%), and *BRCA1*, *FAM175A*, *GEN1*, *MSH6*, *PMS2*, *RAD51C*, and *RAD51D* (each 0.29%) (Table 3, Fig. 1).

CNV and SV analysis indicated 3 large putatively pathogenic deletions in *PMS2*, *FAM175A*, and *RAD51C* (Table 2, Supplement Fig. 3-5). In the patient with *PMS2* mutation, copy number deletion was found in exons 13 and 14 with about 3 kbp deletion in a homozygous pattern (2 copy loss). *FAM175A* and *RAD51C* showed deletions in exons 6–9 to 3' untranslated region (UTR) and exons 4–7, respectively, with both heterozygous deletion profiles (single copy loss). Small 30 to 40 bp deletions in the intron region of *BRCA2* were identified, located at the 13th and 23rd introns in 3 and 11 patient samples, respectively.

Sub-analysis for variant frequency and clinicopathologic correlation showed a positive trend for increasing GS, with germline mutations in 7% and 10% of GS ≤7 and ≥9 patients, respectively (Fig. 2). Although statistical significance for trend was not achieved (p=0.47), the results confirm previous findings [16] that the frequency of PV/LPV increase with GS, suggesting that the degree of aggressiveness may be higher in men with pathogenic germline mutations.

The World Journal of MEN's HEALTH

Table 3. Germline variant frequency comparison between Korean mPC (n=340), Korean healthy controls (n=495), and Caucasian mPC (n=692)

Gene	Korean mPC (n=340)		Korean healthy control (n=495)		Caucasian mPC[a] (n=692)		Korean mPC *vs.* Korean healthy control	
	No. of mut	% of men	No. of mut	% of men	No. of mut	% of men	p-value	OR (95% CI)
BRCA2	15	4.41	2	0.40	37	5.35	<0.001	11.37 (2.6–50.1)
ATM	2	0.59	2	0.40	11	1.59	0.7	1.46 (0.2–10.4)
BRIP1	2	0.59	0	0.00	1	0.14	-	-
NBN	2	0.59	0	0.00	2	0.29	-	-
BRCA1	1	0.29	1	0.20	6	0.87	0.79	1.46 (0.1–23.4)
FAM175A	1	0.29	0	0.00	1	0.14	-	-
GEN1	1	0.29	0	0.00	2	0.29	-	-
MSH6	1	0.29	0	0.00	1	0.14	-	-
PMS2	1	0.29	0	0.00	2	0.29	-	-
RAD51C	1	0.29	0	0.00	1	0.14	-	-
RAD51D	1	0.29	0	0.00	3	0.43	-	-
ATR	0	0.00	1	0.20	2	0.29	-	-
BAP1	0	0.00	0	0.00	0	0.00	-	-
BARD1	0	0.00	0	0.00	0	0.00	-	-
CHEK2	0	0.00	1	0.20	10	1.45	-	-
MLH1	0	0.00	1	0.20	0	0.00	-	-
MRE11A	0	0.00	0	0.00	1	0.14	-	-
MSH2	0	0.00	1	0.20	1	0.14	-	-
PALB2	0	0.00	0	0.00	3	0.43	-	-
XRCC2	0	0.00	0	0.00	0	0.00	-	-
Sum	28[b]	8.2	9	1.8	84	11.8	-	-

mPC: metastatic prostate cancer.
[a]Data for the Caucasian mPC cohort was retrieved from Pritchard et al (N Engl J Med 2016;375:443-53) [2].
[b]Excluding *HOXB13* and *RAD54L*.

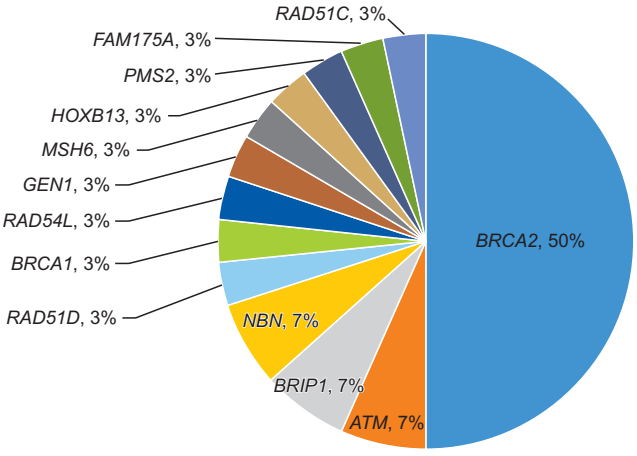

Fig. 1. Distribution of pathogenic/likely pathogenic variants (PV/LPVs) in Korean metastatic prostate cancer (mPC).

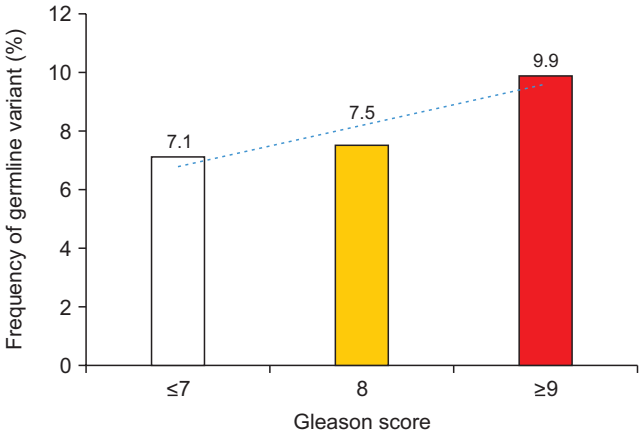

Fig. 2. Association between Gleason score and variant frequency in Korean metastatic prostate cancer (mPC) cohort.

3. Comparison analysis to different cohorts

Analysis of Korean healthy males (n=495) revealed 9 (1.8%) mutation variants in *BRCA2*, *ATM*, *BRCA1*, *ATR*, *CHEK2*, *MLH1*, and *MSH2* in decreasing order (Table 3). *BRCA2* mutation was significantly more likely to be present in Korean mPC compared to healthy counterparts (OR 11.37, p<0.001). *ATM* and *BRCA1*, considered PV for PC, was not significantly

associated for increased risk for developing mPC (p=0.7 and 0.79, respectively). Further comparison to Caucasian mPC as reported by Pritchard et al [2] showed that while *BRCA2* is the most frequently in both ethnicities (4.41% *vs.* 5.35% in Koreans and Caucasians, respectively), mutations in *ATM* and *CHEK2* which displayed 2nd and 3rd highest frequency in Caucasian mPC were not found or had very low frequency in Koreans (0.59% & 0.0% *vs.* 1.59% & 1.45% in Koreans and Caucasians, respectively) (Table 3). Overall variant frequency was also ultimately lower in Koreans compared to Caucasians with no overlap of PV/LPV germline variants, underlying an ethnic difference in genetic predisposition for mPC.

To further assess the germline variant profile of DNA repair genes within the East-Asian ethnicity, Korean mPC was compared to the germline carrier frequency reported in a large Japanese cohort [17] (Supplement Table 2). Eight genes including *BRCA1/2* and *ATM* were compared, with *BRCA2* commonly found to be most frequent in both studies. However, *BRCA2* frequency in the Korean mPC cohort was approximately 4-times higher than that of the Japanese cohort, with gene-based association test showing the most significant p-value in the *BRCA2* gene with overall statistical significance. Only 1 patient had *HOXB13* mutation (Gly132Glu), which was 2nd most frequent in Japanese.

DISCUSSION

Despite modifications in recent guidelines to support the use of genetic testing for assessment of germline mutations in men with high familial risk, evaluation amongst different racial and ethnic cohorts are lacking, with established literature suggesting varying genetic profiles depending on ancestry. Unlike previous studies in germline PC, we selected only men with proven metastatic cancer status to assess the linkage of germline DDR mutation with progression to mPC in the largest volume of Koreans to our knowledge. Approximately 9% of all mPC harbored at least one deleterious variant in DDR genes, with *BRCA2* mutation most predominant. Three novel CNVs in *PMS2*, *RAD51C*, and *FAM175A* were identified, with higher GS patients showing a gradual increase in PV/LPV variant frequency. Comparison with healthy controls as well as Caucasian and Japanese cohorts indicate a distinct mutation profile in Korean mPC, further supporting the need for ethnic-specific appraisal of germline susceptibility in PC.

Germline mutations in DDR genes have especially been associated with increased risk for PC-mortality and early age at diagnosis [18] as well as GS reclassification during active surveillance [19], notably in *BRCA1/2* and *ATM* carriers. Markedly high proportion of germline *BRCA2* mutation in this study affirms previous literature suggesting localized *BRCA2*-mutant tumors harbor increased frequency of CNV than those without mutations [20]. Half of mutation carriers had variants in *BRCA2*, attesting to the hypothesis that *BRCA2* aberrations may accelerate mutation as which occurs during hormone therapy, leading to aggressive, metastatic forms of the disease [21]. While *BRCA1* and *BRCA2* are both RPMs of importance as suggested in early literature, *BRCA2* seems to play a more pivotal role in East Asian mPC. Study of germline PVs in more than 7000 Japanese patients [17] lay further support, as PC patients were 5.6 times more likely to harbor *BRCA2* mutation (p<0.001) but failed to show statistical significance for *BRCA1* (p=0.06). However, PV carriers had increased risk of aggressive (GS ≥8) tumors, which supports the upward trend for frequency of germline PVs from 7% in GS ≤7 to nearly 10% in GS ≥9 revealed from this study (Fig. 2).

Surprisingly, no overlap of individual variants was observed when compared to the Caucasian cohort [2], with considerable difference in overall distribution of RPMs. Overall germline variant carriers in Korean mPC were over 2-fold higher than in Japanese PC cohort (6.76% *vs.* 2.88%), but this can be attributed to the relatively low percentage of mPC (M1) patients included in the latter study (8.0%, n=297) [17]. Further comparison to 139 mPC from a UK biobank with all European ancestry found only 1 shared variant in *BRCA2* (p.Thr3085fs) out of 22 PV identified [22]. Mutation in *ATM* and *CHEK2*, found in relatively high frequency in Caucasian mPC, did not show significant overlap in Korean mPC nor increased mPC risk compared to Korean healthy controls (OR 1.46, p=0.79), with *CHEK2* mutation found only in a healthy male. Lack of RPM distribution overlap aside from *BRCA2* suggest that while germline mutation in DDR substantially increases risk for mPC, different genomic factors may drive carcinogenesis depending on ethnicity. A recent meta-analysis of germline RPMs further suggest that amidst

The World Journal of MEN's HEALTH

the myriad of gene panels recommended in current guidelines, only *BRCA2*, *ATM*, *NBN*, *CHEK2*, and *PALB2* show significant association with PC progression to lethal or metastatic disease [23]. *HOXB13*, while associated with high PC risk in European and Caucasian cohorts, was found in only 1 Korean patient with mPC despite being the 2nd most frequently observed in Japanese PC [17]. Gly132Glu variant, classified as VUS in ClinVar, seems to be specific in East Asian and Korean men [24] and should be considered for ethnic-specific panels along with Gly135Glu. Difference in germline PV profiles even when compared to Japanese men of same East Asian ancestry provides evidence for a more tailored approach when assessing genetic risk, especially during the construction of risk scores from cancer-associated SNPs [25], as population-based scores tend to outperform generalized, multiethnic models [26].

While family history was collected in 285 participants (83.4%), only 5% had a positive history of PC. This suggests that while familial PC history may contribute to PC development and must be adequately screened for cascade testing, it may not influence oncologic severity and likelihood for metastasis. Association analysis conducted in a Japanese cohort with 473 men of *BRCA1/2* family history further support this hypothesis [27], as familial *BRCA* mutations failed to show any correlation to GS ≥8 nor metastasis at diagnosis. Study of germline variants showed similar results, with family history failing to achieve statistical significance despite a relatively large sample size of combined 20,000 case and controls [17]. Only 9% in an earlier Korean study had 1st to 2nd degree family history of PC [24].

Of the 6 variants not reported in ClinVar, 4 were categorized as putative PV/LPV and 2 as VUS *via* InterVar (Table 3). Frameshift mutations considered VUS were absent from controls and potentially pathogenic based on the ACMG guidelines, but were found in *GEN1* and *RAD54L* genes with little known mechanism of disease, and hence categorized as VUS. Interestingly, 30 to 40 bp mutations in the introns of *BRCA2* were also identified in a relatively large portion of patients. The significance of such deletions in the 13th and 23rd introns are unknown, despite being found in 11 and 3 samples, respectively. Previous research has suggested that variants in the splicing site of intron 13 altered the maturation of mRNA and may play a role in breast and ovarian cancer [28], but no definite associations in PC can be made. However, intronic variants newly discovered from GWAS studies rather than conventional exome-based analyses have been linked to cellular signaling and differentiation that ultimately result in PC progression [29], and as such, these novel mutations may play a role in metastatic conversion. Also, the discovered intronic alterations may potentially correspond to transcription disruption and downstream loss-of-function in *BRCA2* [28], though validation in large cohorts is required to assess functionality of such rare intronic variants. Findings from our research further support the need to study CNVs *via* whole-genome rather than exome-wide studies to evaluate whether these variants actually affect genomic pathogenesis in PC.

Our study is not without limitations. First, despite a relatively large cohort reported in Asian PC, the number of included participants in this study are modest to comprehensively represent populational burden and result in statistical significance. Also, this was a single institutional study, allowing the collection of well-conditioned data at the inevitable risk of selection bias. Lastly, comparison to a previous Korean study of germline PV shows identical mutations in only 2 out of 6 PVs (c.658_659delGT in *BRCA2* and c.395G>A in *HOXB13*), most likely due to the lack of variant coverage in the sample population. However, as only 297 patients mPC patients were included in a previous analysis of East Asian PC and 30 men in the earlier Korean cohort, our study best represents germline mutation of mPC in East Asia. Also, novel CNVs were detected from this study, whose role in mPC pathogenesis need to be further discussed in future research. Patients with germline mutations should be carefully monitored and accurately stratified for early intervention rather than active surveillance [30].

CONCLUSIONS

We successfully identified 26 DDR and *HOXB13*-related deleterious variants in 30 Korean men with aggressive mPC. Germline PV/LPV profiles show comparable frequency of overall carriers (8.8%) but distinctly different distribution when contrasted to previous studies in European and even geographically nearby Japanese cohorts, with *BRCA2* playing a dominant role. These results illustrate further evidence for population-based, ethnic-specific analyses for genetic testing

as well as highlighting the potential differences that exist even in common East Asian ancestry. These findings may further emphasize the importance of genomic background in Korean PC. Future combinatory efforts must be made in larger, multiethnic trials to identify PVs of variable importance depending on ancestry.

Conflict of Interest

Hak-Min Kim, Yu Jin Jung are employees, and Seok-Soo Byun is the chief executive officer of Procagen. Sang Hun Song, Hak-Min Kim, Hakmin Lee, Jong Jin Oh, Sangchul Lee and Seok-Soo Byun have an equity interest in Procagen. All other authors report no conflicts or other disclosure.

Funding

This research was supported by the Seoul National University Bundang Hospital (FundRef ID: 10.13039/100016275) Research Fund (no. 13-2017-0006, 18-2018-0025, 18-2018-0003, 14-2020-0030, 13-2021-0010, 13-2022-0009) and the Korean Urological Oncology Society (KUOS 22-02).

Acknowledgements

We appreciate the support from the SNUBH Research foundation and KUOS.

Author Contribution

Conceptualization: HMK, SSB. Data curation: YJJ, HRK, SJ. Formal analysis: HMK, SJ, JB. Funding acquisition: SHS, SSB. Investigation: SHS, YJJ, HRK, JHK, HL, SSB. Methodology: HMK. Project administration: SSB. Resources: SHS, YJJ, SJ, JB, HL, JJO, SL, SKH, SSB. Software: HMK, YJJ, JB. Supervision: HL, JJO, SL, SKH. Validation: HMK. Visualization: SHS, HMK. Writing – original draft: SHS, HMK. Writing – review & editing: all authors.

Supplementary Materials

Supplementary materials can be found *via* https://doi.org/10.5534/wjmh.220190.

Data Sharing Statement

All data generated or analysed during this study are included in this published article and its supplementary information files.

REFERENCES

1. Conti DV, Darst BF, Moss LC, Saunders EJ, Sheng X, Chou A, et al. Trans-ancestry genome-wide association meta-analysis of prostate cancer identifies new susceptibility loci and informs genetic risk prediction. Nat Genet 2021;53:65-75. Erratum in: Nat Genet 2021;53:413.
2. Pritchard CC, Mateo J, Walsh MF, De Sarkar N, Abida W, Beltran H, et al. Inherited DNA-repair gene mutations in men with metastatic prostate cancer. N Engl J Med 2016;375:443-53.
3. Carlaw KR, Woo HH. Evaluation of the changing landscape of prostate cancer diagnosis and management from 2005 to 2016. Prostate Int 2017;5:130-4.
4. Kimura T, Egawa S. Epidemiology of prostate cancer in Asian countries. Int J Urol 2018;25:524-31.
5. Li H. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv 2013. doi: https://doi.org/10.48550/arXiv.1303.3997 [Epub].
6. Martin M. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet J 2011;17:10-2.
7. Chen S, Zhou Y, Chen Y, Gu J. fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics 2018;34:i884-90.
8. Broad Institute. Picard: a set of command line tools (in Java) for manipulating high-throughput sequencing (HTS) data and formats such as SAM/BAM/CRAM and VCF [Internet]. Cambridge (MA): Broad Institute; c2020 [cited 2022 Aug 1]. Available from: https://broadinstitute.github.io/picard/index.html.
9. DePristo MA, Banks E, Poplin R, Garimella KV, Maguire JR, Hartl C, et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nat Genet 2011;43:491-8.
10. Cingolani P, Platts A, Wang le L, Coon M, Nguyen T, Wang L, et al. A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w1118; iso-2; iso-3. Fly (Austin) 2012;6:80-92.
11. Layer RM, Chiang C, Quinlan AR, Hall IM. LUMPY: a probabilistic framework for structural variant discovery. Genome Biol 2014;15:R84.
12. Abyzov A, Urban AE, Snyder M, Gerstein M. CNVnator: an approach to discover, genotype, and characterize typical and atypical CNVs from family and population genome sequencing. Genome Res 2011;21:974-84.
13. Landrum MJ, Lee JM, Benson M, Brown GR, Chao C, Chitipiralla S, et al. ClinVar: improving access to variant interpretations and supporting evidence. Nucleic Acids Res

2018;46:D1062-7.
14. Li Q, Wang K. InterVar: clinical interpretation of genetic variants by the 2015 ACMG-AMP guidelines. Am J Hum Genet 2017;100:267-80.
15. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al.; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17:405-24.
16. Helfand BT, Loeb S, Kan D, Catalona WJ. Number of prostate cancer risk alleles may identify possibly 'insignificant' disease. BJU Int 2010;106:1602-6.
17. Momozawa Y, Iwasaki Y, Hirata M, Liu X, Kamatani Y, Takahashi A, et al. Germline pathogenic variants in 7636 Japanese patients with prostate cancer and 12 366 controls. J Natl Cancer Inst 2020;112:369-76.
18. Na R, Zheng SL, Han M, Yu H, Jiang D, Shah S, et al. Germline mutations in ATM and BRCA1/2 distinguish risk for lethal and indolent prostate cancer and are associated with early age at death. Eur Urol 2017;71:740-7.
19. Carter HB, Helfand B, Mamawala M, Wu Y, Landis P, Yu H, et al. Germline mutations in ATM and BRCA1/2 are associated with grade reclassification in men on active surveillance for prostate cancer. Eur Urol 2019;75:743-9.
20. Castro E, Jugurnauth-Little S, Karlsson Q, Al-Shahrour F, Piñeiro-Yañez E, Van de Poll F, et al. High burden of copy number alterations and c-MYC amplification in prostate cancer from BRCA2 germline mutation carriers. Ann Oncol 2015;26:2293-300.
21. Taylor RA, Fraser M, Rebello RJ, Boutros PC, Murphy DG, Bristow RG, et al. The influence of BRCA2 mutation on localized prostate cancer. Nat Rev Urol 2019;16:281-90.
22. Mijuskovic M, Saunders EJ, Leongamornlert DA, Wakerell S, Whitmore I, Dadaev T, et al. Rare germline variants in DNA repair genes and the angiogenesis pathway predispose prostate cancer patients to develop metastatic disease. Br J Cancer 2018;119:96-104. Erratum in: Br J Cancer 2019;120:867.
23. Shi Z, Lu L, Resurreccion WK, Yang W, Wei J, Wang Q, et al. Association of germline rare pathogenic mutations in guideline-recommended genes with prostate cancer progression: a meta-analysis. Prostate 2022;82:107-19.
24. So MK, Ahn HK, Huh J, Kim KH. Germline pathogenic variants in unselected Korean men with prostate cancer. Investig Clin Urol 2022;63:294-300.
25. Song SH, Kim E, Woo E, Kwon E, Yoon S, Kim JK, et al. Prediction of clinically significant prostate cancer using polygenic risk models in Asians. Investig Clin Urol 2022;63:42-52.
26. Na R, Ye D, Qi J, Liu F, Lin X, Helfand BT, et al. Race-specific genetic risk score is more accurate than nonrace-specific genetic risk score for predicting prostate cancer and high-grade diseases. Asian J Androl 2016;18:525-9.
27. Ishiyama Y, Shimbo M, Iizuka J, Deshpande G, Tanabe K, Hattori K. Association between prostate cancer characteristics and BRCA1/2-associated family cancer history in a Japanese cohort. PLoS One 2020;15:e0244149.
28. Pensabene M, Spagnoletti I, Capuano I, Condello C, Pepe S, Contegiacomo A, et al. Two mutations of BRCA2 gene at exon and splicing site in a woman who underwent oncogenetic counseling. Ann Oncol 2009;20:874-8.
29. Jiang Y, Meyers TJ, Emeka AA, Cooley LF, Cooper PR, Lancki N, et al. Genetic factors associated with prostate cancer conversion from active surveillance to treatment. HGG Adv 2022;3:100070.
30. Song SH, Kim JK, Lee H, Lee S, Hong SK, Byun SS. A single-center long-term experience of active surveillance for prostate cancer: 15 years of follow-up. Investig Clin Urol 2021;62:32-8.