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Variants in *HOXB13*, G132E and F127C, Are Associated With Prostate Cancer Risk in Japanese Men

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Abstract. *Background/Aim: Several studies have reported on the relationship between HOXB13 variants and an increased prostate cancer (PC) risk. To our knowledge there are not many studies on HOXB13 mutations in Japanese patients with prostate cancer, and there many issues remain uninvestigated. We herein clarified the association between HOXB13 genetic variants and PC risk in a Japanese population. Patients and Methods: PC patients were diagnosed at the Gunma University Hospital and affiliated hospitals from 1994 to 2016. Sanger sequencing was performed on the coding regions of the HOXB13 gene in 152 familial PC (FPC) patients. Genotyping was performed on single nucleotide variants (SNVs) found in Sanger sequencing in 230 FPC patients from 152 pedigrees and 197 sporadic PC (SPC) patients and 144 controls. Allelic frequency and clinical data for each variant were studied in cases and controls. Results: G132E and F127C were identified in FPC patients. The frequencies of G132E and F127C were significantly higher compared to the control group (p=0.039). In three families, seven PC patients shared the G132E variant, within second-to-third-degree relatives. It was not possible to clarify to pathogenicity of each SNV alone. Conclusion: We found two significant variants of the HOXB13 gene, G132E, F127C by analyzing and comparing gene samples from PC and non-PC patients. Furthermore, the HOXB13 G132E variant was found significantly increased in the FPC group.*

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In 2020, 191,930 new cases of prostate cancer (PC) and 33,330 deaths were anticipated in the US, where PC is the most frequent non-dermatological cancer in men. PC is the second-leading cause of cancer-related death in men in the US, exceeded only by lung cancer (1). The incidence of PC varies greatly among populations: the PC-specific death rate of African-American men is twice that of non-Hispanic white men in the US. Asian men have a very low incidence of PC, although both the incidence and mortality have gradually increased, attributable to both environmental factors (particularly a Western diet) and genetic factors (2, 3). In Japan, the incidence of PC has recently increased; according to the National Cancer Center (2017), PC is the most common cancer in Japan and the sixth-leading cause of cancer death in Japanese men (4).

PC exhibits familial clustering. The first-degree relatives of an affected individual are two-to-three-fold more likely to develop PC than others. Independent segregation analyses revealed that both an early age of PC onset and the existence of multiple affected family members were strong predictions of risk (5-9). Of all PC cases, 5-10% are believed to be primarily caused by high-risk (inherited) genetic factors or PC susceptibility genes. The results of several large case-control and cohort studies on various populations suggest that a family history is a major PC risk factor (6, 10). Hereditary PC is distinguished by early age at onset and autosomal-dominant inheritance (11).

Genome-wide association studies have identified more than 170 common genetic variants [including single nucleotide variants (SNVs)] associated with PC (12). Several rare variants increase the risk of morbidity. A study conducted in the US found that *HOXB13* G84E was pathogenic (13). A study conducted in China found that G84E was absent, but G135E was present (14). In a study conducted in Japan, Hayano *et al*. identified *BRCA2*, *HOXB13*, and *TRRAP* genes as susceptibility genes (15).

We collected clinical and genomic data on PC patients living in the suburbs of the Gunma Prefecture, Japan. We performed targeted *HOXB13* sequencing and genotyping and explored the clinical characteristics of PC patients.

Patients and Methods

Study subjects. We defined PC patients with a family history of PC as familial PC (FPC) patients and those without such history as sporadic PC (SPC) patients. We included 571 men: 427 PC patients (230 FPC patients from152 pedigrees and 197 SPC patients) and 144 controls. All PC patients were diagnosed at the Gunma University Hospital and affiliated hospitals between 1994-2016. We screened *HOXB13* sequence variants in germline DNA samples of one affected proband from each of 152 pedigrees affected by FPC. Controls were recruited from the outpatient clinics of Gunma University Hospital; the most common diagnosis was benign prostate hypertrophy (BPH). Controls were excluded if they evidenced an abnormal prostate-specific antigen level (>4.0 ng/ml), an abnormal digital rectal examination, or a previous PC diagnosis.

Study design. In the first experiment,152 FPC probands underwent Sanger sequencing of the *HOXB13* coding regions. We then genotyped about the SNVs found in the first sequencing in 230 FPC patients, 197 SPC patients, and 144 controls. The frequencies and clinical features of all variants were studied in 152 FPC probands, 197 SPC patients, and 144 controls.

Sequence analysis and genotyping. Genomic DNA was isolated from whole blood cells using a GENOMIX kit (Talent srl, Trieste, Italy). Sanger sequencing of *HOXB13* coding regions employed gene-specific primers. PCR was performed in a reaction volume of 10 μl containing 10 ng genomic DNA, 2.0 pmol of each primer, 10×EX Taq buffer, 2.5 mmol/l deoxynucleoside triphosphates, and 0.25 U TaKaRa Ex Taq DNA polymerase (Hot Start Version) (Takara Bio Inc., Shiga, Japan). PCR amplification employed a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA). The conditions were 94˚C for 1 min, followed by 35 cycles of 30 s at 94˚C, 30 s at 60˚C, and 30 s at 72˚C, followed by elongation at 72˚C for 5 min. The PCR products were mixed with Big Dye Terminator ver. 3.1 Matrix Standard Kit reagents (Thermo Fisher Scientific, Waltham, MA, USA) and formamide, and analyzed on an ABI PRISM 310 Genetic Analyzer (Thermo Fisher Scientific). Data were analyzed using SEQUENCHER (Gene Codes) software. SNP markers were genotyped via real-time PCR using the Taq Man SNP genotyping assay (ID C_2160574_30, Applied Biosystems). Each reaction mixture (5 μl) contained 10 ng genomic DNA, 2.5 μl Premix Ex Taq, and 0.25 μl Allelic Discrimination Assay Mix (Applied Biosystems). PCR amplification proceeded in an C1000 thermal cycler (a CFX96 real-time PCR detection system; Bio-Rad Laboratories Inc., Hercules, CA, USA). The cycling conditions were 95˚C for 30 s, followed by 40 cycles of 5 s at 95˚C and 30 s at 60˚C. Genotypes were automatically called and manually verified.

Allele frequency database. Allele frequency data from more than 60,000 individuals stored in the genome Aggregation Database (gnomAD) served as references (https://gnomad.broadinstitute.org). To gather Japanese data, we accessed the database of the Japanese Multi Omix Reference Panel (jMORP) (16) and the Human Genetic Variation Database (HGVD, ver. 2.3) (17). jMORP was constructed using whole-genome sequencing data from more than 8000 healthy individuals. HGVD collected exome variant data on more than 1000 Japanese individuals.

Table I. *Patient characteristics*.

	PC patients	FPC	SPC
No. of patients	427	152*	197
Mean age at diagnosis (SD), yrs	69.1 (7.4)	67.5 (7.8)	69.3 (7.0)
Family history of PC			
Yes (%)	230 (53.9)		
No (%)	197 (46.1)		
Gleason score			
Indolent (<8) (%)	239 (56.0)	98 (64.5)	74 (37.6)
Aggressive (≥8) (%)	186 (43.6)	53 (34.9)	122 (61.9)
Unknown	2 (0.5)	1 (0.7)	1 (0.5)
Clinical stage			
Localized (%)	305 (71.4)	118 (77.6)	118 (59.9)
Metastatic (%)	116 (27.2)	33 (21.7)	76 (38.6)
Unknown (%)	6 (1.4)	1 (0.7)	3 (1.5)

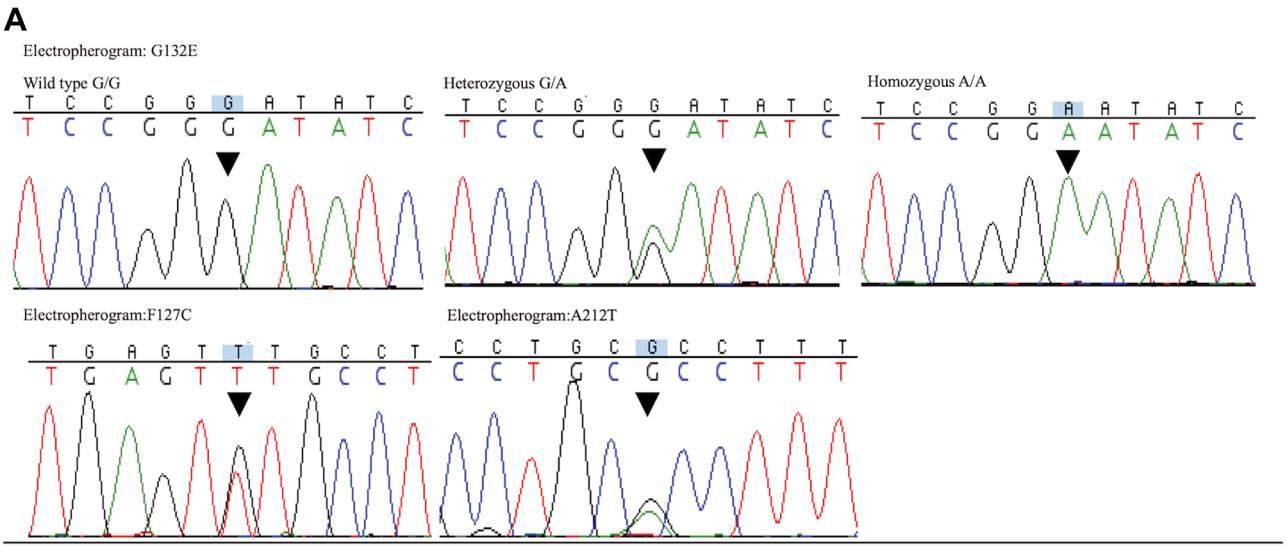
PC: Prostate cancer; FPC: familial prostate cancer; SPC: sporadic prostate cancer; S.D.: standard deviation. *Number of probands.

Statistical analysis. Case-control association analysis was performed using the Fisher's exact test. The two-sided Fisher's exact test was used for all comparisons except age at diagnosis. Welch's *t*-test was used to compare ages at diagnosis. A p-value <0.05 was considered significant. To eliminate selection bias in the statistical analysis of genotyping, excluding duplication within the same family, 152 probands were used in genotyping analysis. However, clinical data was evaluated for all PC patients. All analyses were performed with the aid of SPSS ver. 25 (IBM, Chicago, IL, USA).

Ethics statement. All patients provided written informed consent. The study protocol was approved by the Institutional Review Board of Gunma University Hospital (approval no. 5.2013.12.2, HS2017-263) and adhered to the standards of the International Conference on Harmonization/Good Clinical Practice and the 1964 Helsinki declaration and later amendments, or comparable ethical standards.

Results

The mean age at PC diagnosis was 69.1 years (standard deviation 7.4 years) in all PC patients. Table I shows the clinical characteristics of all PC patients and controls in this study. We sequenced the entire *HOXB13* coding regions of 152 FPC patients; analyzed the data using SEQUENCHER; and detected five SNVs, of which two (rs8556 and rs9900627) were synonymous substitutions (no amino acid change) and three non-synonymous. G132E refers to a change in the second position of codon 132 (GGA to GAA; glycine to glutamic acid; p. Gly132Glu). F127C refers to a change in the second position of codon 127 (TTT to TGT; phenylalanine to cysteine; p. Phe127Cys). A212T(rs145059285) refers to a change in the first position of codon 212 (GCC to ACC; alanine to threonine; p. Ala212Thr). We genotyped the three non-synonymous variants (G132E, F127C, and A212T) in 427 PC patients and 144 controls. We found that 20 PC patients



B

Genomic location GRCh37	rs ID	cDNA change	Genotype	Protein change	Clinical significance	Minor allele frequency			
						gnomAD v2.1.1	jMORP	HGVD	This study
17:46805561	rs1286034091	NM_006361.6:c.395G>A	Het/Hom	p.G132E	Uncertain significance	EA:0.00005437, AA:0,E:0	0.0004	0.0004	0.013
17:46805576	N/A	NM_006361.6:c.380T>G	Het	p.F127C	Not reported	Not reported	Not reported	Not reported	0.003
17:46804373	rs145059285	NM_006361.6:c.634G>A	Het	p.A212T	conflicting interpretaions of pathogenicity	EA:0.0004,AA:0, E 0	0.0081	0.0074	0.007
17:46805590	rs8556	NM_006361.6:c.366C>T	Het/Hom	p.(=)	Benign	EA:0.038,AA:0.271,E:0.157	0.0414	0.0372	0.204
17:46805443	rs9900627	NM_006361.6:c.513T>C	Het/Hom	p.(=)	Benign	EA:.0.185,AA:0.109,E:0.089	0.1665	0.1713	0.167

Figure 1. *Germline variants detected in this study. (A) Electropherograms of the result of sanger sequencing. Variants are indicated by an arrow. (B) Variant status detected in this study. The clinical significance of each variant is taken from clinVar. PC: Prostate cancer; gnomAD; Genome Aggregation Database; jMORP: Japanese Multi Omix Reference Panel; HGVD: Human Genetic Variation Database; EA: East Asian; AA: African American; E: European.*

and 1 control carried variants: G132E in 11 FPC patients; F127C in 4 FPC patients; and A212T in 3 FPC patients, 2 SPC patients, and 1 control. We compared the minor allelic frequencies of all five SNVs in 349 unrelated PC patients (152 FPC probands and 197 SPC patients) and 144 controls using the available databases (Figure 1). Non-synonymous variants (G132E, F127C, and A212T) were rare. G132E was found only in East Asia and was more common in our FPC patients than in the general populations. We identified only one homozygous G132E PC patient; homozygosity has been not reported in the public databases. F127C was not found in any database, including dbSNP of the National Center for Biotechnology Information. The A212T frequency did not differ between our PC patients and general populations. We drew family trees to place the various carriers (Figure 2). In three families, seven PC patients shared G132E with second-to-third-degree relatives. In pedigrees 2 and 9, this was the dominant mode of inheritance. In pedigree 66, three PC patients with F127C were observed in one family. In pedigrees 52 and 59, both families had two FPC patients, but they did not share A212T. Table II lists the genotypes of all variants along with significant differences between cases (152 FPC probands and 197 SPC patients) and 144 controls with and without variants. Patients were significantly more likely to host either of the two *HOXB13* variants than controls (p=0.039). FPC patients were significantly more likely to carry G132E than SPC patients (p=0.001) (Table III). Table IV lists the clinical characteristics of PC patients with and without the two variants. The age of onset was significantly lower among G132E carriers. None of SPC patients carried G132E and F127C variant. No other clinical findings differed significantly between the patient groups.

Discussion

The homeobox genes encode a highly conserved family of transcription factors that play essential role in embryonic development and tissue homeostasis. Humans have four HOX gene clusters on different chromosomes (18). HOX genes also play increasingly important roles in terms of genetic predispositions toward many types of cancer. A large-scale twin study revealed that hereditary factors contributed significantly to colorectal, breast, and PC development (19). *HOXB13* (located on chromosome 17q21.2) encodes a

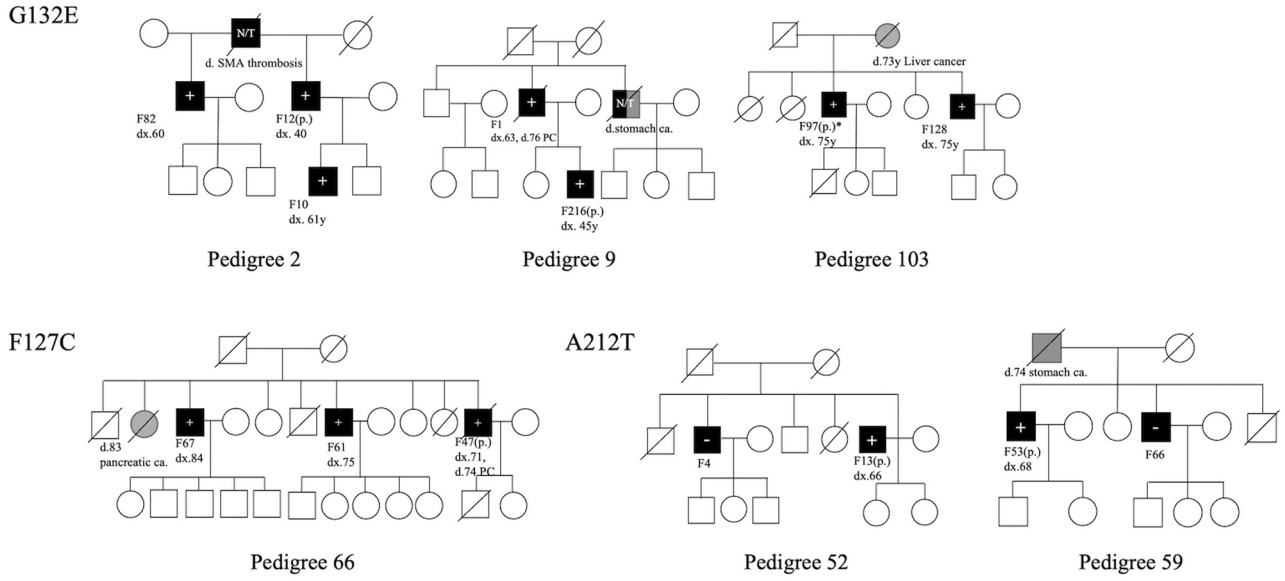


Figure 2. *Family trees of patients with each variant carrier. Pedigrees of patients with the HOXB13 G132E (pedigree 2,9,103), F127C (pedigree 66) and A212T (pedigree 52, 59). Black rectangles represent patients with PC. Patient's sample number in this study, age at diagnosis (dx.), age at death (d.) and cause of death described in the margin. Described as (p.) for proband. Patient of variant carriers are represented by (+) symbol, and non-carriers are represented by (–) symbol. N/T: Not typed. *Homozygous carrier.*

homeobox B transcription factor playing important roles in prostate development and tumorigenesis. Many studies have reported significant associations between *HOXB13* and various cancers (20-24). Remarkably, inherited *HOXB13* variants have not been widely assessed in terms of PC risk.

We focused on *HOXB13* variants in PC patients living in the suburbs of the Gunma Prefecture, Japan. We identified five SNVs and three missense variants (G132E, F127C, and A212T). Ewing *et al*. (13) reported that *HOXB13* G84E was associated with a significantly increased risk of hereditary PC patients. The Genome Aggregation Database (25) indicates that G84E has been found in African, Ashkenazi Jewish, and Latino populations; but G132E and G135E only in East Asians. However, a similar analysis of a Chinese cohort reported G135E but not G132E (14).

Momozawa *et al*. sequenced eight genes associated with hereditary PC in 7636 unselected Japanese patients with PC and 12,366 male controls; they categorized the variants according to the ACMG/AMP guidelines, G132E was classified as a pathogenic variant in PC patients (26). In that study, the proportion of G132E carriers was 0.73% in the prostate cancer group and 0.1% in the control group. In our study, the proportion of G132E carriers was 2.6% in the PC group and no carriers in control. Higher results of in our study may be biased by the limited number of cases in the suburbs of Gunma. However, as examined by Momozawa *et al*., it seems that the proportion of G132E rate was significantly higher in the PC group compared to the control group.

Table II. *Genotype in each variant and statistical differences between cases and controls with or without variants of prostate cancer (PC) patients.*

	No. controls	No. cases*	*p*-Value
G132E/F127C			
GG and TT	144	339	0.039
GA/AA or TG/GG	0	10	
G132E			
GG	144	341	0.11
GA/AA	0	8	
F127C			
TT	144	347	1.0
TG/GG	0	2	

FPC: Familial prostate cancer; SPC: sporadic prostate cancer. *Cases were 152 FPC probands and 197 SPC patients.

Hayano *et al*. (15) performed exome sequencing (followed by Sanger sequencing) when seeking putative, causative germline variants in 140 Japanese PC patients from 66 families. They found BRCA2, *HOXB13*, or TRRAP variants in seven large PC families (three or four patients per family; one variant per family). They also identified two or more variants per family in another 59 small PC families (two patients per family). They concluded that *HOXB13* variants F127C and G132E were deleterious and were significantly more common in PC patients than controls. However, one limitation of that study was the absence of healthy controls.

Table III. *Genotype in each variant and statistical differences between prostate cancer (PC) patients with or without family history.*

Genotype	No.FPC	No.SPC	OR (95%CI)	*p*-Value
G132E				
GG	144	197	1.00 (referent)	0.001
GA/AA	8	0	0 (0-0.44)	
F127C				
TT	150	197	1.00 (referent)	0.189
TG/GG	2	0	0 (0-4.10)	

FPC: Familial prostate cancer; SPC: sporadic prostate cancer; OR: odds ratio; 95%CI: 95% confidence interval.

Table IV. *The clinical characteristics of prostate cancer (PC) patients with and without two variants.*

	G132E (%)	F127C (%)	*p*-Value
No. of patients	11	4	
Carrier frequency (%)	2.6	0.9	
Mean age at diagnosis (SD), yrs	62.7 (11.0)	75.3 (5.3)	0.004*
Family history of PC			
Yes (%)	11 (100)	4 (100)	0.001**
No (%)	0 (0)	0 (0)	
Gleason score			
Indolent (<8) (%)	5 (45.5)	3 (75)	
Aggressive (≥8) (%)	6 (54.5)	1 (25)	
Unknown	0 (0)	0 (0)	
Clinical stage			
Localized (%)	7 (63.6)	2 (50)	
Metastatic (%)	4 (36.4)	2 (50)	
Unknown (%)	0 (0)	0 (0)	

*Statistically significant differences in mean age at diagnosis between with and without G132E variant. **Statistically significant differences between G132E variant carrier with family history of PC and non-carrier without family history of PC.

We compared PC samples from both FPC and SPC patients, as well as BPH samples from healthy controls. In addition to F127C and G132E, we found an A212T variant, which was less common than the other variants and thus may not be associated with PC. Generally, hereditary tumors develop at a young age and are highly malignant. The age of onset was significantly lower among G132E carriers, but no other clinical findings differed significantly between the patient groups.

Our study had several limitations. First, we included a relatively small number of participants whose clinical characteristics varied. Our results differ from those of Momozawa *et al*., who enrolled many more patients. We considered the results published by Momozawa *et al*. are very useful because they were obtained from many patient samples. However, our study was also meaningful because it was a conclusion drawn from the detailed follow-up data. Second, our work was retrospective in nature, and the FPC and SPC groups differed significantly in terms of prognostic indicators such as the Gleason score. Third, the analysis restricted to a limited regional area in Japan with concerns as to generalizability of the findings, and the overall limited implications for the clinical practice represent limitations to this contribution. There are some reports comparing prostate cancer tissue removed after diagnosis with non-cancer tissue by immunohistological examination. Jae HC *et al*. reported that the expression level of sirtuins (SIRT) 2 in the SIRT family affects the incidence of cancer (27). In this study, we could not investigate the pathological examination using the excised specimen, the immunohistological examination, and the association with the presence or absence of the mutation of *HOXB13*. I would like to make it an issue for future study.

In conclusion, we found two significant variants of the *HOXB13*, G132E and F127C, by comparing PC and non-PC patients as in the previous report. G132E is a very rare variant found only in East Asia. It might be a unique Japanese variant that is also different from the G135E seen in the Chinese. Compared to a healthy Japanese population, G132E was significantly more common in the FPC group. It may be associated with Japanese PC. G132E and F127C were shared in a hereditary PC pedigree. Two of them met Carter's definition of hereditary prostate cancer (3 PC patients in a nuclear family). Only one family shared one variant, it cannot be concluded that it is a deleterious variant of hereditary PC in this study. Although it is a small study, it is valuable accumulation of prostate cancer families in the Gunma suburbs. If follow-up surveys are possible, we would like to plan further evaluation of the family line.

Conflicts of Interest

Kazuhiro Suzuki has potential financial conflicts of interest as below. Consultancies: Takeda Pharmaceutical, Astellas Pharma, Daiichi-Sankyo, AstraZeneca, Sanofi, Janssen, Bayer; Grants received: Takeda Pharmaceutical, Astellas Pharma, Daiichi-Sankyo, Ono Pharmaceutical. All other Authors have declared that no conflicts of interest exist.

Authors' Contributions

Concept and design: SK, HM, KS, YM; Acquisition of data: SK, HM, NO, MA, YM; Analysis of interpretation of data: YS, SA, SK, HM, HK, YM; Drafting of the manuscripts: SK, YM; Critical revision of the manuscript for important intellectual content: KS, YM; Statistical analysis: KS, YM; Obtaining funding: none; Administrative, technical, or material support: KS; Supervision: KS.

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References

1 American Cancer Society: Cancer Facts and Figures 2020. Atlanta, GA, USA, American Cancer Society, 2020. Available at: https://www.cancer.org/content/dam/cancer-org/research/cancer-facts-and-statistics/annual-cancer-facts-and-figures/2020/cancer-facts-and-figures-2020.pdf [Last accessed on June 30, 2022]

2 Ito K: Prostate cancer in Asian men. Nat Rev Urol *11(4)*: 197-212, 2014. PMID: 24595118. DOI: 10.1038/nrurol.2014.42

3 Stanford JL, Stephenson RA, Coyle LM, Cerhan J, Correa R, Eley JW, Gilliland F, Hankey B, Kolonel LN, Kosary C, Ross R, Severson R and West D: Prostate Cancer Trends 1973-1995, SEER Program, National Cancer Institute, NIH Pub. No. 99-4543, Bethesda, MD, USA, 1999.

4 Cancer Registry and Statistics. Cancer Information Service, National Cancer Center, Ministry of Health, Labour and Welfare, National Cancer Registry, Japan. Available at: https://www.ncc.go.jp/en/cis/divisions/stat/index.html [Last accessed on June 30, 2022]

5 Steinberg GD, Carter BS, Beaty TH, Childs B and Walsh PC: Family history and the risk of prostate cancer. Prostate *17(4)*: 337-347, 1990. PMID: 2251225. DOI: 10.1002/pros.2990170409

6 Carter BS, Beaty TH, Steinberg GD, Childs B and Walsh PC: Mendelian inheritance of familial prostate cancer. Proc Natl Acad Sci USA *89(8)*: 3367-3371, 1992. PMID: 1565627. DOI: 10.1073/pnas.89.8.3367

7 Ghadirian P, Howe GR, Hislop TG and Maisonneuve P: Family history of prostate cancer: a multi-center case-control study in Canada. Int J Cancer *70(6)*: 679-681, 1997. PMID: 9096649. DOI: 10.1002/(sici)1097-0215(19970317)70:6<679::aid-ijc9>3.0.co;2-s

8 Stanford JL and Ostrander EA: Familial prostate cancer. Epidemiol Rev *23(1)*: 19-23, 2001. PMID: 11588848. DOI: 10.1093/oxfordjournals.epirev.a000789

9 Matikaine MP, Pukkala E, Schleutker J, Tammela TL, Koivisto P, Sankila R and Kallioniemi OP: Relatives of prostate cancer patients have an increased risk of prostate and stomach cancers: a population-based, cancer registry study in Finland. Cancer Causes Control *12(3)*: 223-230, 2001. PMID: 11405327. DOI: 10.1023/a:1011283123610

10 Grönberg H, Damber L and Damber JE: Familial prostate cancer in Sweden. A nationwide register cohort study. Cancer *77(1)*: 138-143, 1996. PMID: 8630920. DOI: 10.1002/(SICI)1097-0142(19960101)77:1<138::AID-CNCR23>3.0.CO;2-5

11 Carter BS, Bova GS, Beaty TH, Steinberg GD, Childs B, Isaacs WB and Walsh PC: Hereditary prostate cancer: epidemiologic and clinical features. J Urol *150(3)*: 797-802, 1993. PMID: 8345587. DOI: 10.1016/s0022-5347(17)35617-3

12 Takata R, Takahashi A, Fujita M, Momozawa Y, Saunders EJ, Yamada H, Maejima K, Nakano K, Nishida Y, Hishida A, Matsuo K, Wakai K, Yamaji T, Sawada N, Iwasaki M, Tsugane S, Sasaki M, Shimizu A, Tanno K, Minegishi N, Suzuki K, Matsuda K, Kubo M, Inazawa J, Egawa S, Haiman CA, Ogawa O, Obara W, Kamatani Y, Akamatsu S and Nakagawa H: 12 new susceptibility loci for prostate cancer identified by genome-wide association study in Japanese population. Nat Commun *10(1)*: 4422, 2019. PMID: 31562322. DOI: 10.1038/s41467-019-12267-6

13 Ewing CM, Ray AM, Lange EM, Zuhlke KA, Robbins CM, Tembe WD, Wiley KE, Isaacs SD, Johng D, Wang Y, Bizon C, Yan G, Gielzak M, Partin AW, Shanmugam V, Izatt T, Sinari S, Craig DW, Zheng SL, Walsh PC, Montie JE, Xu J, Carpten JD, Isaacs WB and Cooney KA: Germline mutations in HOXB13 and prostate-cancer risk. N Engl J Med *366(2)*: 141-149, 2012. PMID: 22236224. DOI: 10.1056/NEJMoa1110000

14 Lin X, Qu L, Chen Z, Xu C, Ye D, Shao Q, Wang X, Qi J, Chen Z, Zhou F, Wang M, Wang Z, He D, Wu D, Gao X, Yuan J, Wang G, Xu Y, Wang G, Dong P, Jiao Y, Yang J, Ou-Yang J, Jiang H, Zhu Y, Ren S, Zhang Z, Yin C, Wu Q, Zheng Y, Turner AR, Tao S, Na R, Ding Q, Lu D, Shi R, Sun J, Liu F, Zheng SL, Mo Z, Sun Y and Xu J: A novel germline mutation in HOXB13 is associated with prostate cancer risk in Chinese men. Prostate *73(2)*: 169-175, 2013. PMID: 22718278. DOI: 10.1002/pros.22552

15 Hayano T, Matsui H, Nakaoka H, Ohtake N, Hosomichi K, Suzuki K and Inoue I: Germline variants of prostate cancer in Japanese families. PLoS One *11(10)*: e0164233, 2016. PMID: 27701467. DOI: 10.1371/journal.pone.0164233

16 Tadaka S, Saigusa D, Motoike IN, Inoue J, Aoki Y, Shirota M, Koshiba S, Yamamoto M and Kinoshita K: jMorp: Japanese multi omics reference panel. Nucleic Acids Res *46(D1)*: D551-D557, 2018. PMID: 29069501. DOI: 10.1093/nar/gkx978

17 Higasa K, Miyake N, Yoshimura J, Okamura K, Niihori T, Saitsu H, Doi K, Shimizu M, Nakabayashi K, Aoki Y, Tsurusaki Y, Morishita S, Kawaguchi T, Migita O, Nakayama K, Nakashima M, Mitsui J, Narahara M, Hayashi K, Funayama R, Yamaguchi D, Ishiura H, Ko WY, Hata K, Nagashima T, Yamada R, Matsubara Y, Umezawa A, Tsuji S, Matsumoto N and Matsuda F: Human genetic variation database, a reference database of genetic variations in the Japanese population. J Hum Genet *61(6)*: 547-553, 2016. PMID: 26911352. DOI: 10.1038/jhg.2016.12

18 Shah N and Sukumar S: The Hox genes and their roles in oncogenesis. Nat Rev Cancer *10(5)*: 361-371, 2010. PMID: 20357775. DOI: 10.1038/nrc2826

19 Lichtenstein P, Holm NV, Verkasalo PK, Iliadou A, Kaprio J, Koskenvuo M, Pukkala E, Skytthe A and Hemminki K: Environmental and heritable factors in the causation of cancer – analyses of cohorts of twins from Sweden, Denmark, and Finland. N Engl J Med *343(2)*: 78-85, 2000. PMID: 10891514. DOI: 10.1056/NEJM200007133430201

20 Edwards S, Campbell C, Flohr P, Shipley J, Giddings I, Te-Poele R, Dodson A, Foster C, Clark J, Jhavar S, Kovacs G and Cooper CS: Expression analysis onto microarrays of randomly selected cDNA clones highlights HOXB13 as a marker of human prostate cancer. Br J Cancer *92(2)*: 376-381, 2005. PMID: 15583692. DOI: 10.1038/sj.bjc.6602261

21 Wang Z, Dahiya S, Provencher H, Muir B, Carney E, Coser K, Shioda T, Ma XJ and Sgroi DC: The prognostic biomarkers HOXB13, IL17BR, and CHDH are regulated by estrogen in breast cancer. Clin Cancer Res *13(21)*: 6327-6334, 2007. PMID: 17975144. DOI: 10.1158/1078-0432.CCR-07-0310

22 Miao J, Wang Z, Provencher H, Muir B, Dahiya S, Carney E, Leong CO, Sgroi DC and Orsulic S: HOXB13 promotes ovarian cancer progression. Proc Natl Acad Sci USA *104(43)*: 17093-17098, 2007. PMID: 17942676. DOI: 10.1073/pnas.0707938104

23 Jung C, Kim RS, Zhang H, Lee SJ, Sheng H, Loehrer PJ, Gardner TA, Jeng MH and Kao C: HOXB13 is downregulated in colorectal cancer to confer TCF4-mediated transactivation. Br J Cancer *92(12)*: 2233-2239, 2005. PMID: 15928669. DOI: 10.1038/sj.bjc.6602631

24 Zhang E, Han L, Yin D, He X, Hong L, Si X, Qiu M, Xu T, De W, Xu L, Shu Y and Chen J: H3K27 acetylation activated-long non-coding RNA CCAT1 affects cell proliferation and migration by regulating SPRY4 and HOXB13 expression in esophageal squamous cell carcinoma. Nucleic Acids Res *45(6)*: 3086-3101, 2017. PMID: 27956498. DOI: 10.1093/nar/gkw1247

25 Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, O'Donnell-Luria AH, Ware JS, Hill AJ, Cummings BB, Tukiainen T, Birnbaum DP, Kosmicki JA, Duncan LE, Estrada K, Zhao F, Zou J, Pierce-Hoffman E, Berghout J, Cooper DN, Deflaux N, DePristo M, Do R, Flannick J, Fromer M, Gauthier L, Goldstein J, Gupta N, Howrigan D, Kiezun A, Kurki MI, Moonshine AL, Natarajan P, Orozco L, Peloso GM, Poplin R, Rivas MA, Ruano-Rubio V, Rose SA, Ruderfer DM, Shakir K, Stenson PD, Stevens C, Thomas BP, Tiao G, Tusie-Luna MT, Weisburd B, Won HH, Yu D, Altshuler DM, Ardissino D, Boehnke M, Danesh J, Donnelly S, Elosua R, Florez JC, Gabriel SB, Getz G, Glatt SJ, Hultman CM, Kathiresan S, Laakso M, McCarroll S, McCarthy MI, McGovern D, McPherson R, Neale BM, Palotie A, Purcell SM, Saleheen D, Scharf JM, Sklar P, Sullivan PF, Tuomilehto J, Tsuang MT, Watkins HC, Wilson JG, Daly MJ, MacArthur DG and Exome Aggregation Consortium: Analysis of protein-coding genetic variation in 60,706 humans. Nature *536(7616)*: 285-291, 2016. PMID: 27535533. DOI: 10.1038/nature19057

26 Momozawa Y, Iwasaki Y, Hirata M, Liu X, Kamatani Y, Takahashi A, Sugano K, Yoshida T, Murakami Y, Matsuda K, Nakagawa H, Spurdle AB and Kubo M: Germline pathogenic variants in 7636 Japanese patients with prostate cancer and 12 366 controls. J Natl Cancer Inst *112(4)*: 369-376, 2020. PMID: 31214711. DOI: 10.1093/jnci/djz124

27 Choi JH, Choi SM, Lee SW, Jeh SU, Hyun JS, Lee MH, Lee C, Kam SC, Kim DC, Lee JS and Hwa JS: Expression patterns of ERα, ERβ, AR, SIRT1, SIRT2, and SIRT3 in prostate cancer tissue and normal prostate tissue. Anticancer Res *41(3)*: 1377-1386, 2021. PMID: 33788729. DOI: 10.21873/anticanres.14895

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