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Genome-wide association study of serum prostate-specific antigen levels based on 1000 Genomes imputed data in Japanese: the Japan Multi-Institutional Collaborative Cohort Study

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ABSTRACT

Prostate cancer is emerging as a significant global public health burden. The incidence and prevalence of prostate cancer has increased in Japan, as westernized lifestyles become more popular. Recent advances in genetic epidemiology, including genome-wide association studies (GWASs), have identified considerable numbers of human genetic factors associated with diseases. Several GWASs have reported significant loci associated with serum prostate-specific antigen (PSA) levels. One GWAS, which was based on classic GWAS microarray measurements, has been reported for Japanese so far. In the present study,

we conducted a GWAS of serum PSA using 1000Genomes imputed GWAS data (n =1,216) from the Japan Multi-Institutional Collaborative Cohort (J-MICC) Study, to detect candidate novel genetic loci that influence serum PSA levels in Japanese. The association of SNPs/genetic variants with serum PSA as a continuous variable was tested using the linear Wald test. SNP rs10000006 in *SGMS2* (sphingomyelin synthase 2) on chromosome 4 had genome-wide significance (P <5×10^{-8}), and eight variants on three chromosomes (chromosomes 12, 14, 15) had genome-wide suggestive levels of significance (P <1×10^{-6}). With an independent data set from the J-MICC Shizuoka Study (n = 2,447), the association of the *SGMS2* SNP with blood PSA levels was not replicated. Although our GWAS failed to detect novel loci associated with serum PSA levels in the Japanese cohort, it confirmed the significant effects of previously reported genetic loci on PSA levels in Japanese. Importantly, our results confirmed the significance of *KLK3* SNPs also in Japanese, implying that consideration of individual genetic information in prostate cancer diagnosis may be possible in the future.

Keywords: PSA, GWAS, genetic polymorphisms, J-MICC Study

Abbriations:
PSA: prostate-specific antigen
GWAS: genome-wide association study
SNP: single nucleotide polymorphism
J-MICC Study: Japan Multi-Institutional Collaborative Cohort Study

INTRODUCTION

Prostate cancer is an emerging global public health burden. The incidence and prevalence of prostate cancer has increased in Japan, as westernized lifestyles become more popular.[1] The highly sensitive prostate-specific antigen (PSA) test for prostate cancer is one of the standard blood exams used in actual clinical settings, including outpatient clinic or health checks in Japan.[2]

The PSA test is an established routine clinical test not only for the early detection of prostate cancer, but also as a marker of the clinical progression of prostate cancer.[3,4] In preventive medicine and medical checkups, relatively high false positive rates or relatively low specificity can be an annoying issue.[5] For some cancer detection markers, genetic predispositions to higher than normal serum levels of these markers have been reported. Among them, high CA 19–9 levels caused by *Lewis* and *secretor* gene polymorphisms are considered promising,[6] which suggests the establishment of personalized diagnostic criteria based on individual genetic information may be possible in the future.

Recent advances in genetic epidemiology, including genome-wide association studies (GWASs), have identified considerable numbers of human genetic factors associated with diseases, including cardiovascular and metabolic diseases, and cancers.[7-10] Several GWASs have reported significant loci associated with serum PSA levels.[11] Until now, only one GWAS, which was based on classic GWAS microarray measurements of SNPs (single nucleotide polymorphisms), has been reported for Japanese.[12] Recent GWASs in Chinese revealed a novel locus at 1q32.1 associated with serum PSA levels.[13] Among the SNPs in *KLK3*, one was established as a genetic factor that influenced blood PSA levels across races and ethnicities.[14,15]

The Japan Multi-Institutional Collaborative Cohort (J-MICC) Study is a large population-based genome cohort study in which about 100,000 participants from 15 study areas of Japan are being followed up for 20 years for cancer incidence.[16] The purpose is to find effective ways of cancer prevention based on genetic information. In the present study, we conducted a GWAS using 1000Genomes imputed data to detect novel genetic loci that influence serum PSA levels in a

Japanese cohort, for the possible establishment of personalized PSA testing in the near future.

METHODS

Study subjects

The J-MICC Study is a large-scale cohort study that is being conducted at 13 independent research institutes. The main objective is to detect gene–environment interactions mainly for cancer prevention.[16] The baseline survey was started in 2005 and was completed in March 2014 with about 100,000 individuals throughout Japan. In the finding phase of the J-MICC Study (J-MICC GWAS data ver. 20190729), the GWAS genotyping results (described below) and serum PSA measurement data of 1216 men from six independent study sites (Okazaki, Shizuoka-Hamamatsu, Kyoto, Kagoshima, Tokushima, and Shizuoka-Sakuragaoka) were used. All the participation assented to taking part in this project. Individuals with a history of prostate cancer were excluded.

In the replication phase, one data set of 5006 participants from the J-MICC Shizuoka area was used.[17] Briefly, 5006 health check examinees who resided in the Shizuoka Prefecture completed a self-administered questionnaire and provided blood samples. Of these, samples and data from 2447 of the male study participants with serum PSA data and no history of prostate cancer, and participants who were not included in the data set used in the finding phase, were used for the replication analyses.

The characteristics of the study subjects are described in Table 1. Subjects with present illness or past history of prostate diseases (coded as C61 [prostate cancer] and N40 [benign prostate hyperplasia] according to the International Classification of Disease, version 10) were excluded, except for participants from the Okazaki area for whom only past history of cancer data were available and considered. Written informed consent was obtained from all participants. The study

Table 1 Study characteristics

Variable	Discovery	Replication
Male (n, %)	1,216 (100.0%)	2,447 (100.0%)
Age (mean ± sd)	57.2 ± 7.8	52.5 ± 8.7
PSA (ng/ml)	1.30 ± 1.15	1.35 ± 1.34
Site (n, %)		
Okazaki	428 (35.2%)	-
Shizuoka (Hamamatsu)	314 (25.8%)	2,447 (100.0%)
Kyoto	122 (10.0%)	-
Kagoshima	249 (20.5%)	-
Tokushima	29 (2.4%)	-
Shizuoka (Sakuragaoka)	74 (6.1%)	-
SGMS2 rs10000006 SNP (n, %)		
T/T	1,095 (90.1%)	2,058 (84.1%)
T/C	117 (9.6%)	364 (15.9%)
C/C	4 (0.3%)	25 (1.0%)

PSA: prostate specific antigen
SGMS2: *sphingomyelin synthase 2*

protocol was approved by the Ethics Committees of the Nagoya University Graduate School of Medicine and each participating Institute. All the research procedures were conducted according to the Ethical Guidelines for Human Genome and Genetic Sequencing Research and the Ethical Guidelines for Medical and Health Research Involving Human Subjects in Japan.

Measurement of PSA in serum samples

PSA levels in the serum samples were measured using a chemiluminescent method. The clinical reference ranges were set at <4.0 ng/mL.

Genotyping and quality control

For the participants in the discovery phase (Stage I), DNA samples were extracted automatically from the buffy coat using the BioRobot M48 Workstation (QIAGEN group, Tokyo, Japan). Genotyping of the samples in the discovery phase was performed using the Illumina HumanOmniExpressExome v1.2 platform (Illumina, San Diego, California) at the RIKEN Center for the Integrated Medical Sciences (Yokohama, Japan). Identity-by-descent was detected using PLINK 1.9 software (https://www.cog-genomics.org/plink2). In the sample quality check, subjects with identity-by-descent proportions >0.1875 and outliers detected by principal component analysis[18] of the 1000Genomes reference panel (phase 3)[19] whose ancestries were estimated to be outside the Japanese population were excluded from the analysis. SNPs with genotype call rates <0.98, Hardy-Weinberg equilibrium exact test P values <1×10⁻⁶, or minor allele frequencies (MAFs) <0.01 were excluded. After quality control filtering, 14,091 individuals and 575,802 SNPs remained for further analyses.

The samples in the replication phase were genotyped for SNP rs10000006 in *SGMS2* by PCR-CTPP (PCR with confronting two-pair primers).[20] The primers used were F1: 5′-GGTG-GAAGGCAAAAGGCAC-3′, F2: 5′-CCAACTGAATTAACTGTATTAGTCTGTTTTC-3′, R1: 5′-ATTGTTTATGAGGTTTGGCAGTGT-3′, and R2: 5′-CGTCTGCTGCCATGTAAAACA-3′. The SNPs are underlined. The thermal cycler conditions were: denaturing at 95°C for 10 min, followed by 30 cycles of 95°C for 1 min, 61°C for 1 min, and 72°C for 1 min, then final extension at 72°C for 5 min, A representative gel for the genotyping is shown in Figure 1.
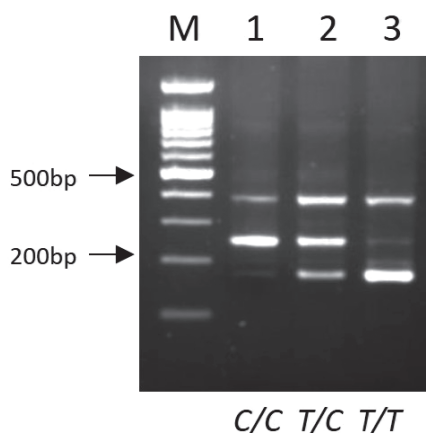

Fig. 1 Genotyping of the *SGMS2* rs10000006
Lane M = 100-bp marker; lane 1 = *C/C* genotype (249- and 381-bp bands); lane 2 = *T/C* genotype (170-, 249- and 381-bp bands); lane 3 = *T/T* genotype (170- and 381-bp bands).
SGMS2: *sphingomyelin synthase 2*

Genotype imputation

Genotype imputation was conducted using SHAPEIT v2 (https://mathgen.stats.ox.ac.uk/genetics_software/shapeit/shapeit.html#home) and Minimac3 (http://genome.sph.umich.edu/wiki/Minimac3) software based on the 1000 Genomes Project cosmopolitan reference panel (phase 3).[21] After the genotype imputation, variants with MAFs <0.05 and R^2 <0.3 were excluded, resulting in 6,288,024 variants for the 1216 subjects from the six study areas for the final analyses.

Replicability of reported PSA-related GWAS loci

We also examined the replicability of previously reported PSA-related GWAS loci[11,14] with the loci detected with our J-MICC Study samples. Among the 40 SNPs reported, we selected the SNPs detected by the unconditional GWAS,[11] from which SNPs that failed to pass the quality control filtering were excluded. In total, 23 genetic loci on 15 chromosomes were examined.

Statistical analysis

We examined the associations of the SNPs with serum PSA levels using EPACTS software (http://genome.sph.umich.edu/wiki/EPACTS). The association of SNPs with serum PSA as a continuous variable was tested using the linear Wald test, where the number of minor alleles was defined as the independent variable. To adjust for the covariates, age and the first five principal components were considered. Variants with MAFs ≥0.05 were taken into account in the main analysis, whereas this criterion was not adopted when examining the replicability of previously reported SNPs. Manhattan and Q-Q plots were drawn using the 'qqman' function in R (https://cran.r-project.org/web/packages/qqman/index.html). Genome-wide significance levels were defined as $P < 5 \times 10^{-8}$ and genome-wide suggestive levels of significance were defined as $P < 1 \times 10^{-6}$ in all the analyses. In the replication phase or in the verification of replicability of the reported loci, the significance threshold was set at $P < 0.05$, which is considered nominally significant.

RESULTS

The characteristics of the participants included in the discovery and replication data sets are provided in Table 1. Significant SNPs/genetic variants associated with serum PSA are listed in Table 2. SNP rs10000006 in *SGMS2* (sphingomyelin synthase 2) on chromosome 4 had genome-wide significance, and eight variants on three chromosomes (chromosomes 12, 14, 15) had genome-wide suggestive levels of significance. Manhattan and Q-Q plots of the results are shown in Figures 2 and 3. The genomic inflation factor of lambda was close to 1 (lambda = 0.99874; range 0.99–1.01), suggesting that the population structure was well adjusted.

We examined the replicability of the GWAS significant SNP rs10000006 in *SGMS2*, with the independent data set for the Shizuoka Study. The association between SNP rs10000006 in *SGMS2* and blood PSA levels was not replicated in the independent samples by linear regression analysis (Table S1 and Figure 4).

We also examined the associations of previously reported PSA-related GWAS loci[11] with the PSA levels in the present study. Among the 25 genetic variants (on 23 genetic loci) examined, six SNPs on six independent genetic loci were nominally significant ($P < 0.05$). The results of these analyses are described in Table 3. The direction of effect was inverse in 3 of the 6 variants and the same in the other 3 variants, compared with the effects reported previously.[11]

Table 2 Genetic variants associated with PSA levels with the suggestive level ($P < 1 \times 10^{-6}$) in the current J-MICC GWAS

rs#	Geno/Imp	Cytoband	Position	Gene	Function	Major Allele	Minor Allele	MAF	r^2	n	β	S.E.	P
rs10000006	Imputed	4q25	108826383	*SGMS2*	intronic	*T*	*C*	0.0514	0.543	1216	0.594	0.102	7.06×10^{-9}
rs59071933	Imputed	14q22.3	56941657	(intergenic)	intergenic	*C*	*A*	0.0596	0.878	1216	0.484	0.095	4.15×10^{-7}
rs72768427	Imputed	15q25.3	87866346	(intergenic)	intergenic	*A*	*G*	0.0609	0.888	1216	0.473	0.094	4.96×10^{-7}
rs72768428	Imputed	15q25.3	87866662	(intergenic)	intergenic	*G*	*A*	0.0609	0.891	1216	0.473	0.094	4.96×10^{-7}
rs72768429	Imputed	15q25.3	87866712	(intergenic)	intergenic	*G*	*A*	0.0609	0.891	1216	0.473	0.094	4.96×10^{-7}
rs72724291	Imputed	14q22.3	56946429	(intergenic)	intergenic	*G*	*T*	0.0604	0.897	1216	0.471	0.095	7.37×10^{-7}
rs141052148	Imputed	12q14.3	67140107	(intergenic)	intergenic	*A*	*T*	0.0526	0.880	1216	0.504	0.102	8.23×10^{-7}
rs78582243	Imputed	12q14.3	67164098	(intergenic)	intergenic	*G*	*C*	0.0518	0.880	1216	0.505	0.103	9.77×10^{-7}
rs60050830	Imputed	15q25.3	87864957	(intergenic)	intergenic	*ATG*	*A*	0.0604	0.892	1216	0.462	0.094	9.77×10^{-7}

Genetic variants on genetic loci that fulfilled the suggestive level ($P < 1 \times 10^{-6}$) are shown.
PSA: prostate specific antigen
S.E.: standard error

Table 3 Associations of previously reported PSA-related GWAS loci with the PSA levels in the present study

rs#	Genotyped/ Imputed	Cytoband	Position	Gene	Function	Minor allele	Major allele	MAF	n	β	S.E.	P
rs6662386	Genotyped	1p22.3	88190037	(intergenic)	intergenic	T	C	0.17804	1216	-0.0847	0.0604	0.161
rs4951018	Imputed	1q32.1	205636334	SLC45A3	intronic	C	A	0.43586	1216	0.0285	0.046	0.540
rs2556375	Imputed	2p16.1	60759747	BCL11A	intronic	T	G	0.1764	1216	-0.0395	0.0601	0.511
rs397735760	Imputed	4q31.22	146874227	(intergenic)	intergenic	AT	A	0.12212	1216	0.0003	0.0697	0.997
rs10023685	Imputed	4q32.1	157534249	(intergenic)	intergenic	A	C	0.37294	1216	-0.0137	0.047	0.769
rs10023685	Imputed	4q32.1	157534249	(intergenic)	intergenic	G	C	0.37294	1216	-0.0137	0.047	0.769
rs37004	Imputed	5p15.33	1356684	(intergenic)	intergenic	T	C	0.075247	1216	-0.0643	0.083	0.441
rs6920449	Imputed	6p21.1	43710348	(intergenic)	intergenic	C	T	0.34622	1216	-0.0376	0.048	0.437
rs10486567	Genotyped	7p15.2	27976563	JAZF1	intronic	A	G	0.10321	1216	-0.0304	0.075	0.686
rs13272392	Imputed	8p21.2	23528511	(intergenic)	intergenic	A	T	0.37541	1216	-0.1563	0.048	0.001
rs10505477	Genotyped	8q24.21	128047443	RP11-382A18.1	ncRNA_intronic	G	A	0.31579	1216	-0.1044	0.049	0.033
rs6478343	Imputed	9q33.1	120732749	(intergenic)	intergenic	C	T	0.023438	1216	0.0126	0.153	0.934
rs59482735	Imputed	9q33.2	123643426	(intergenic)	intronic	TAA	T	0.28372	1216	3.5×10^{-5}	0.051	0.999
rs10993994	Genotyped	10q11.23	51549496	TIMM23B	intronic	C	T	0.44367	1216	-0.0858	0.046	0.061
rs10886902	Genotyped	10q26.12	123049264	(intergenic)	intergenic	C	T	0.21546	1216	0.1252	0.056	0.026
rs4378355	Imputed	11p13	34783417	(intergenic)	intergenic	C	G	0.37911	1216	-0.0393	0.048	0.411
rs12285347	Genotyped	11q22.2	102396607	MMP7	intronic	C	T	0.060855	1216	0.0540	0.094	0.567
rs11067228	Genotyped	12q24.21	115094260	(intergenic)	intergenic	G	A	0.36184	1216	-0.0644	0.048	0.177
rs202346	Imputed	13q14.3	51087443	DLEU1	ncRNA_intronic	A	C	0.15831	1216	-0.0249	0.063	0.693
rs9921192	Imputed	16p13.3	4349111	(intergenic)	intergenic	A	T	0.33923	1216	-0.0501	0.047	0.288
rs9921192	Imputed	16p13.3	4349111	(intergenic)	intergenic	C	T	0.33923	1216	-0.0501	0.047	0.288
rs11263761	Imputed	17q12	36097775	HNF1B	intronic	A	G	0.34539	1216	0.1300	0.047	0.006
rs11084596	Genotyped	19q12	32104979	(intergenic)	intergenic	C	T	0.49753	1216	-0.0893	0.045	0.046
rs17632542	Imputed	19q13.33	51361757	KLK3	exonic	C	T	0.00082	1216	-0.8366	0.801	0.297
rs2735839	Imputed	19q13.33	51364573	KLK3	intergenic	A	G	0.40337	1216	0.1859	0.046	6.23×10^{-5}

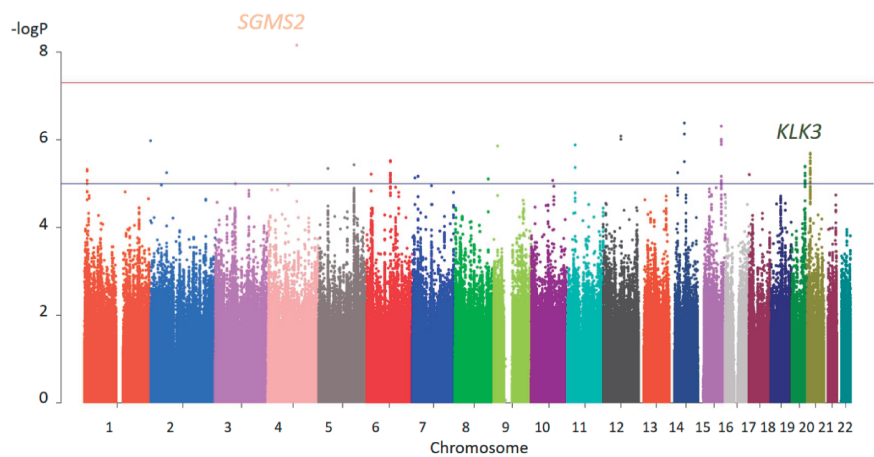PSA: prostate specific antigen
S.E.: standard error

Fig. 2 Manhattan plot for the PSA GWAS of the J-MICC Study

PSA: prostate specific antigen

GWAS: genome-wide association study

The red line indicates the genome-wide significant level ($P < 5 \times 10^{-8}$) and the blue line indicates the suggestive level ($P < 1 \times 10^{-6}$).
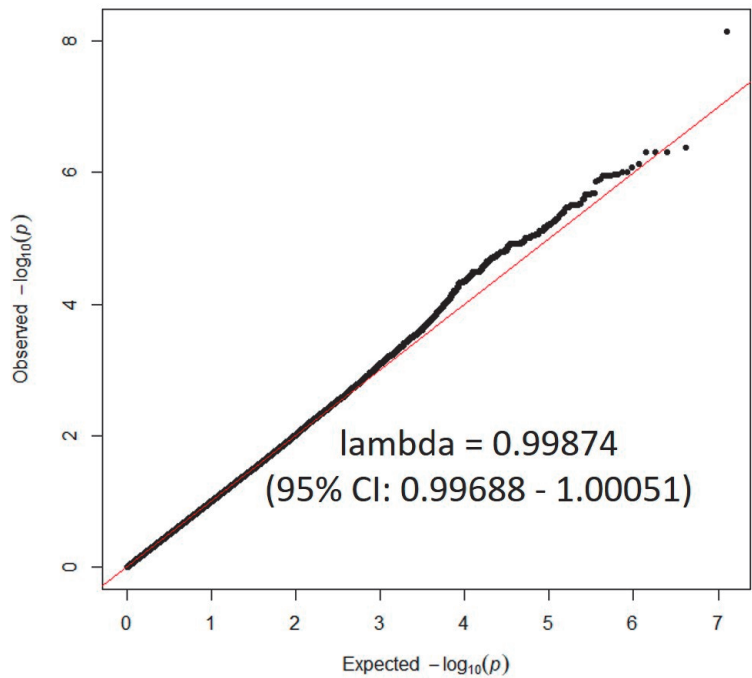

Fig. 3 Q-Q plot for the PSA GWAS of the J-MICC Study.

PSA: prostate specific antigen;

GWAS: genome-wide association study

Table S1 Replicability of the association of the SNP rs10000006 in *SGMS2*
with serum PSA levels in the independent data set of the Shizuoka Study

Variable	β	S.E.	*P*	95%CI
SGMS2 rs10000006 *C* allele*	-0.037	0.052	0.477	(-0.139, 0.065)

Estimation of the replicability was conducted based on linear regression with a genetic additive model.

PSA: prostate specific antigen

S.E.: standard error

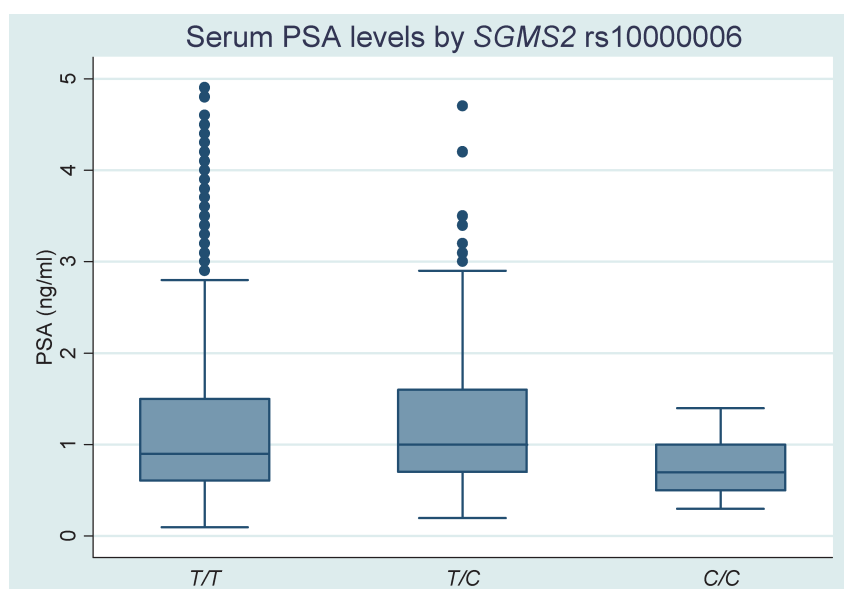*SGMS2*: *sphingomyelin synthase 2*

*Adjusted for age

Fig. 4 Examination of the replicability of the association of the *SGMS2* rs10000006 SNP
with serum PSA levels in the independent data set of the Shizuoka Study

SGMS2: *sphingomyelin synthase 2*

*The box plots indicate the medians and the inter-quartile ranges (IQR). The upper (lower) limits of the whisker plots represent the most extreme values within 1.5 IQR from the nearer quartiles (i.e., 75 percentiles or 25 percentiles).

DISCUSSION

Although the present study is the first GWAS of serum PSA levels based on 1000Genomes imputed data, only one genetic locus in *SGMS2* reached the GWAS significance level ($P < 5 \times 10^{-8}$), and this was not replicated in our independent data set of the Shizuoka Study. *SGMS2* encodes a member of the sphingomyelin synthase family, which plays roles in sphingomyelin biosynthesis in the Golgi lumen and in the formation of the plasma membrane.[22] Even after considering the scarce evidence of a link between *SGMS2* and carcinogenesis, such as its role in the promotion of an aggressive breast cancer phenotype by disruption of the homeostasis of ceramide and sphingomyelin,[23] the contribution of this *SGMS2* SNP to the regulation of serum/plasma PSA

levels seems implausible based on the present PSA-related GWAS results. We concluded that no novel genetic locus associated with PSA levels was found, presumably because of the relatively small sample size in our GWAS data set of male participants with known serum PSA levels.

The present GWAS replicated some of the previously reported genetic loci, including the *KLK3* locus, which confirmed the importance of these loci in regulating human blood PSA levels also in Japanese. Our investigation of the replicability of previously reported PSA loci based on multi-ethnic imputed GWAS from a European cohort[11] found that only six of the 25 genetic loci had nominal significance in our Japanese PSA-related GWAS. Among them, only three of the six loci effected the serum PSA levels in the same direction, suggesting different genetic factors affected PSA levels across ethnicities. Of the reported SNPs examined, the nonsynonymous SNP at position 51361757 on chromosome 19 in the GRCh37hg19 reference sequence, which is within the coding region of *KLK3*,[11] was not detected in our data set probably because it had a low MAF (0.00082). This finding suggests there may be different allele distributions among races and ethnicities. The reported association of SNP rs2735839 in *KLK3* with serum PSA[14] was replicated in our data set with a significant *P*-value of 6.232×10^{-5}, which supports the important role of *KLK3* SNPs in modulating PSA levels across races and ethnicities. *KLK3* encodes PSA, so its role in modulating PSA levels by genetic variations in this locus is considered biologically plausible[14]. With regard to the *KLK3* rs2735839 SNP, recent evidence demonstrated that those with the *A* allele of rs2735839 indicated significantly lower blood PSA levels,[14,15] whereas they were shown to be susceptible to more clinically aggressive prostate cancer.[24] Although the detailed mechanisms remain unclarified, there might be some possibility that subjects with the *G* allele of rs2735839 might be more likely to be diagnosed as having prostate cancer in earlier stage compared to those without.[24]

Determinants of blood tumor markers, such as genetic factors or health conditions other than the tumor itself, have been reported.[6,25] For PSA, some genetic factors, such as SNP rs2735839 in *KLK3*, have been shown to modulate blood PSA by recent studies including ours.[14] Blood PSA levels are known to be affected by some other conventional factors.[25] Therefore, interpreting blood PSA laboratory test results in actual clinical settings should be done with care, taking all these factors into consideration.

Although the present GWAS failed to detect novel loci associated with serum PSA levels in Japanese, it confirmed that previously reported genetic loci also significantly influenced PSA levels in Japanese. In particular, our results confirmed the significant effects of *KLK3* SNPs also in Japanese, which suggests the consideration of individual genetic information in prostate cancer diagnosis may be possible in the future. Further investigations with sufficiently large populations are warranted.

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CONFLICTS OF INTEREST DISCLOSURE

We have no financial relationship to disclose.

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