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DPP4 GENETIC VARIANTS INFLUENCE BASELINE PROSTATE-SPECIFIC ANTIGEN LEVELS: THE J-MICC STUDY

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ABSTRACT

Prostate specific antigen (PSA) testing plays a major role in prostate cancer screening; however, the low positive predictive value of PSA testing leads to many unnecessary biopsies. Genetic background is one of factors that could cause it. That's why an association between genetic background and PSA levels should be elucidated. This study aimed to investigate whether *DPP4* genetic variants are associated with baseline PSA levels. A cross-sectional study was performed on 2,074 Japanese men aged between 35 and 69 in the Shizuoka area from the Japan Multi-institutional Collaborative Cohort (J-MICC) Study. Three *DPP4* tagging single nucleotide polymorphisms (SNPs) were selected for genotyping: rs3788979 (A/G), rs7608798 (T/C), and rs2268889 (A/G). Higher mean serum PSA levels were significantly associated with an increase in the number of the rs7608798 C allele (p for trend = 0.02). A stratified analysis by age groups demonstrated that PSA levels had positive significant trends with the numbers of the minor alleles of rs3788979 or rs7608798 in the oldest group (men aged between 60 and 69) (p for trend=0.004 for rs3788979 and p for trend=0.001 for rs7608798). Haplotype analysis showed that the C-A (rs7608798-rs2268889) haplotype was significantly associated with increased PSA levels (p=0.006), compared with the most common haplotype, T-A. In summary, our study suggests that *DPP4* genetic variants influence baseline PSA levels, especially in men aged between 60 and 69.

Key Words: DPP-IV, Prostate cancer, Screening, Polymorphism, Cross-sectional study

INTRODUCTION

DPP4, located on chromosome 2q24.3, encodes dipeptidyl peptidase IV (DPP-IV), a cell-surface aminopeptidase.[1)] DPP-IV is involved not only in hyperglycemic action, but also in cancer biology processes, such as apoptosis, migration, invasion, metastasis, and sensitivity to chemotherapy.[2)] Previous studies reported that DPP-IV expression was markedly decreased or completely absent in tumors of many organs, including the prostate.[3,4)] This suggests that DPP-IV downregulation might be an important event in the progression of many cancers.[3)]

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The diagnosis of prostate cancer has been increasing steadily in almost all countries.[5] One of the factors that have caused this increase is the adoption of prostate-specific antigen (PSA), an abundant prostatic-secreted serine proteinase, for prostate cancer screening. PSA testing has allowed prostate cancer to be diagnosed at a curable point in more cases.[6] The European Randomized Study of Screening for Prostate Cancer reported that PSA-based screening has significantly reduced mortality from prostate cancer by 21%.[7]

However, a meta-analysis showed that the pooled positive predictive value for elevated PSA levels (greater than 4.0 ng/ml) was 25.1%, respectively.[8] The low positive predictive value of PSA testing leads to many unnecessary biopsies. One reason why PSA testing does not have sufficient positive predictive value is that PSA levels are affected by several factors other than prostate cancer. This increases the risk of generating false positive results.

Genetic background is one of factors that affect PSA levels. Several studies have reported associations between PSA levels and single nucleotide polymorphisms (SNPs) located in genomic regions with known genes: *JAZF1*, *MSMB*, *CTBP2*, *HNF1B*, *KLK3*, and *AR*.[9-13] To date, there are no epidemiological reports that indicate an association between *DPP4* genetic variants and PSA levels.

The aim of this study was to investigate whether *DPP4* genetic variants are associated with baseline PSA levels. The baseline PSA levels of young men were reported to be a stronger predictor of prostate cancer than family history, race, or suspicious digital rectal examination findings.[14] This cross-sectional study was conducted using baseline data from the Shizuoka area in the Japan Multi-institutional Collaborative Cohort (J-MICC) Study. The protocols of the J-MICC Study and Shizuoka Study were described in previous reports.[15,16]

METHODS

Study population

Subjects were obtained from a pool of 3,414 Japanese men aged between 35 and 69 who visited a health checkup center in Hamamatsu, Shizuoka, Japan from January 2006 to December 2007.[16] Subjects who met any of the following criteria were excluded: (i) having no anthropometric or genotyping data available (n=16); (ii) medical history of benign prostatic hypertrophy and prostatitis (n=41); (iii) taking medication that affects PSA levels (nonsteroidal anti-inflammatory drugs, statins, and 5-alpha-reductase inhibitors) (n=428); and (iv) unknown PSA levels (n=845) or 0 ng/ml (n=10). Subjects with a PSA level of 0 ng/ml were excluded because the data cannot be log-transformed. The final study group was 2,074 Japanese men, all of whom provided written informed consent before participation. The ethics committee of Nagoya University School of Medicine approved both the J-MICC Study and the Shizuoka Study (approval number 253 and 288, respectively).

Measurements

At the time of enrollment, self-administered questionnaires were collected from each subject. The questionnaires included medical and medication histories. Venous blood was drawn after an overnight fast and serum PSA levels were determined by chemiluminescence immunoassays (Abbott Laboratories, Abbott Park, IL, USA).

Glycosylated hemoglobin (HbA1c) analysis was performed using a standard high performance liquid chromatography method. HbA1c values based on the Japanese Diabetes Society (JDS) were converted to the National Glycohemoglobin Standardization Program (NGSP)-assigned HbA1c values using the following formula: HbA1c (NGSP) (%)=HbA1c (JDS) (%)+0.4%.[17] A

current medical history of diabetes mellitus was defined as HbA1c (NGSP) ≥6.1% or the use of hypoglycemic medication. HbA1c (NGSP) ≥6.1% was suggested by a previous study to identify diabetes mellitus patients.[18]

SNP selection & Genotyping

Using HapMap version 28[19] and Haploview version 4.2 (Broad Institute, Cambridge, MA, USA),[20] 17 SNPs with a minor allele frequency >10% in the Japanese subjects were identified within the linkage disequilibrium (LD) block. These SNPs included rs7608798, which was the only SNP that was shown to have a significant and compatible association with plasma total-cholesterol levels among the sequencing-derived *DPP4* SNPs in a previous study.[21] Subsequently, three *DPP4* haplotype tagging SNPs capturing 82.4% of the 17 SNPs were selected for genotyping: rs3788979 (A/G), rs7608798 (T/C), and rs2268889 (A/G). All the three SNPs are located in the intron regions of *DPP4*.

Each genomic DNA sample was extracted from the buffy coat fraction, preserved at –80°C, using a BioRobot® M48 (QIAGEN Group, Tokyo, Japan). The three SNPs were genotyped by PCR with confronting two-pair primers, as described in a previous study.[22] The primers were F1: 5'- GGA AGT TTT GAG ACA TGT AGT GAA G -3', R1: 5'- GTA GGG AAT GGT TTG CTT GGT -3', F2: 5'- CAA CAC TGC TGT ACT CAG G -3', and R2: 5'- AAT TGG CAA CAG ATG TGT CAA AG -3' for rs3788979, F1: 5'- GTT GGA ACA TGT CTG ATT GTG -3', R1: 5'- TGG TAT TGA CAA AAA AAA AAA AGT AAC ATC G -3', F2: 5'- TGT GCA GTT TTA AAA TGT GTG CAA T -3', and R2: 5'- GTG ATT TGT ATG GAA GTT GCA TTG -3' for rs7608798, and F1: 5'- GCA GAG CAT GAT CTG CAG TA -3', R1: 5'- AGA GCC TGG ACA ACT TAC T -3', F2: 5'- GGC TCA TAT GTC TAA CCT GG -3', and R2: 5'- AGC AGG ATT ATC ATC TAT GCT TTG C -3' for rs2268889.

Statistical analysis

The Hardy-Weinberg equilibrium was examined by the χ^2 test. To adjust the effect of age on PSA levels, all subjects were divided into three almost equal-sized groups by age groups (35–54, 55–59, and 60–69 years). Linear regression analysis was used to test for trends in PSA levels across the genotypes, and the number of minor alleles was included in the model. PSA levels between each genotype were also compared by linear regression analysis. The Bonferroni correction was applied for multiple testing. Covariates included age (continuous), BMI (continuous), and current medical history of diabetes mellitus.[23-26] PSA levels were normalized by taking the logarithm before the regression analyses.

Haplotypes were reconstructed on PHASE version 2.1,[27] and the strengths of LD (r^2) between the pairs of *DPP4* SNPs were measured using Haploview version 4.2.[20] Haplotype-based linear regression analysis under the dominant model was used to estimate haplotype effects. The most common haplotype was treated as the reference, and subjects with rare haplotypes (haplotype frequency <1%) were removed. A two-tailed p value of <0.05 was considered to be statistically significant. All analyses were conducted using Stata version 11.0 (Stata Corp., College Station, TX, USA).

RESULTS

DPP4 genetic variants

The genotype distribution of each SNP is shown in Table 1. The distributions of rs7608798 and rs2268889 followed the Hardy-Weinberg equilibrium (χ^2=0.03, p=0.86 for rs7608798; χ^2=0.05,

p=0.83 for rs2268889), while that of rs3788979 did not follow the Hardy-Weinberg equilibrium (χ^2=21.0, p<0.001).

Age-stratified analyses

Table 2 shows baseline PSA levels according to the *DPP4* SNPs. For all subjects, higher PSA levels correlated with an increased number of the minor allele of rs7608798 (p for trend=0.02). Stratified analysis by age groups (35–54, 55–59, and 60–69 years) demonstrated that none of

Table 1 Baseline characteristics of subjects by age groups.

		Age group, year		
	Total (n=2,074)	35–54 (n=718)	55–59 (n=678)	60–69 (n=678)
PSA (ng/ml), mean (SD)	1.3 (1.4)	1.1 (0.8)	1.4 (1.4)	1.7 (1.7)
BMI (kg/m^2), mean (SD)	23.4 (2.7)	23.6 (2.9)	23.4 (2.6)	23.1 (2.6)
Diabetes mellitus, n (%)	367 (17.7)	86 (12.0)	118 (17.4)	163 (24.0)
Genotype frequency, n (%)				
rs3788979				
AA	904 (43.6)	324 (45.1)	304 (44.8)	276 (40.7)
AG	857 (41.3)	294 (41.0)	259 (38.2)	304 (44.8)
GG	313 (15.1)	100 (13.9)	115 (17.0)	98 (14.5)
rs7608798				
TT	1,187 (57.3)	416 (57.9)	398 (58.7)	373 (55.0)
TC	766 (36.9)	259 (36.1)	236 (34.8)	271 (40.0)
CC	121 (5.8)	43 (6.0)	44 (6.5)	34 (5.0)
rs2268889				
AA	1,335 (64.4)	459 (63.9)	452 (66.7)	459 (63.9)
AG	656 (31.6)	233 (32.5)	194 (28.6)	229 (33.8)
GG	83 (4.0)	26 (3.6)	32 (4.7)	25 (3.7)

BMI, body mass index; PSA, prostate-specific antigen.

Table 2 Baseline PSA levels (ng/ml), mean (SD), according to *DPP4* SNPs by age groups.

			Age group, year					
	Total (n=2,074)		35–54 (n=718)		55–59 (n=678)		60–69 (n=678)	
Genotype	PSA	p[a,b]	PSA	p[a,b]	PSA	p[a,b]	PSA	p[a,b]
rs3788979								
AA	1.28 (1.13)	–	1.03 (0.67)	–	1.38 (1.33)	–	1.45 (1.27)	–
AG	1.38 (1.40)	0.23	1.05 (0.74)	1.00	1.38 (1.60)	1.00	1.69 (1.63)	0.06
GG	1.49 (1.75)	0.39	1.14 (1.06)	1.00	1.27 (1.18)	0.86	2.10 (2.56)	0.02
p for trend[a]	0.11		0.66		0.56		0.004	
rs7608798								
TT	1.29 (1.18)	–	1.02 (0.67)	–	1.37 (1.32)	–	1.49 (1.41)	–
TC	1.41 (1.50)	0.14	1.12 (0.91)	0.76	1.31 (1.53)	1.00	1.78 (1.81)	0.03
CC	1.60 (1.87)	0.13	0.95 (0.58)	1.00	1.59 (1.61)	0.67	2.43 (2.77)	0.02

Table 3 Baseline PSA levels (ng/ml) according to *DPP4* haplotypes in subjects aged 60–69 (n=675)[a].

Haplotype[b]							
rs7608798	–	rs2268889	Frequency (%)	Number copies	n (%)	PSA, mean (SD)	p[c,d]
T	–	A	75.0	2	371 (55.0)	1.49 (1.42)	[Reference]
C	–	G	20.4	1 or 2	251 (37.2)	1.77 (1.78)	0.08
C	–	A	4.7	1 or 2	61 (9.0)	2.28 (2.59)	0.006

PSA, prostate-specific antigen.
[a] Subjects with the T-G haplotype, in which frequency was less than 1%, were removed (n=3).
[b] Haplotypes were reconstruced on PHASE.
[c] Adjusted for body mass index (continuous) and medical history of diabetes mellitus.
[d] p values were calculated using the Bonferroni correction.

the *DPP4* SNPs had significant associations with PSA levels in the youngest and middle groups, while the PSA levels of subjects with the minor alleles of rs3788979 or rs7608798 were significantly higher than those with the major alleles in the oldest group (GG vs. AA for rs3788979, p=0.02; TC vs. TT and CC vs. TT for rs7608798, p=0.03 and 0.02, respectively). Moreover, PSA levels had positive significant trends with the numbers of the minor alleles of rs3788979 or rs7608798 in the group (p for trend=0.004 for rs3788979 and 0.001 for rs7608798). The trends were similar even after excluding subjects with a PSA level ≥10 ng/ml (p for trend=0.02 for rs3788979 and 0.003 for rs7608798).

Haplotype analyses

Haplotypes were reconstructed from rs7608798 and rs2268889 because this was the only pair of *DPP4* SNPs that was in tight LD (r^2=0.75) among all pairs of the three *DPP4* SNPs. rs3788979 was not tightly linked with the other *DPP4* SNPs (r^2=0.37 for rs7608798 and 0.43 for rs2268889).

Table 3 shows baseline PSA levels in men aged between 60 and 69 according to their haplotypes. The coding of the haplotypes refers to the allele at each locus, and each allele within the haplotype is ordered according to its genomic location (rs7608798-rs2268889). Subjects with the T-G haplotype were removed because its frequency was less than 1% (n=3). Haplotype-based linear regression analysis showed that the C-A haplotype was significantly associated with increased PSA levels (p=0.006), compared with the most common haplotype, T-A. On the other hand, the crude p value for the C-G haplotype was significant (p=0.04), but the Bonferroni corrected p value was not (p=0.08).

DISCUSSION

This study found that higher baseline PSA levels were significantly associated with the minor alleles of two *DPP4* SNPs (rs3788979 and rs7608798) in men aged between 60 and 69. Moreover, the C-A haplotype reconstructed from rs7608798 and rs2268889 was significantly associated with increased PSA levels in the same age group.

As this study was a cross-sectional study, it is unclear whether differences in PSA levels according to the *DPP4* SNPs reflect susceptibility to prostate cancer. On the other hand, the relation of DPP-IV to cancer biology is extremely complex and DPP-IV might influence virtually all stage of cancer development and growth.[2]

Therefore, the results of this study might help to elucidate the effect of DPP-IV on prostate carcinogenesis. The observation that there were no associations between the *DPP4* SNPs and

PSA levels in men aged less than 60 might suggest that DPP-IV is not involved in aggressive, life-threatening prostate cancer in younger men, i.e., early onset prostate cancer.[28] Further follow-up studies, including cohort studies, are necessary to ascertain whether *DPP4* genetic variants are preventative or risk factors of prostate cancer and whether they are associated with prostate cancer-specific mortality.

Ethnic origin is identified as an established risk factor for prostate cancer.[5] The American Cancer Society reported that, in the United States from 2002 to 2006, Asian-Americans/Pacific Islanders had the lowest incidence of prostate cancer among racial and ethnic groups: one third that of African-Americans and half that of whites.[29] The HapMap project showed that the major allele of all the three *DPP4* SNPs in Japanese and Han Chinese was the opposite of that in Europeans and Sub-Saharan Africans.[19] Therefore, these differences in the allelic frequencies of the *DPP4* SNPs might partially explain the lower risk of prostate cancer in Asians than in African-Americans and whites.

This study had several limitations. First, it is unknown how the *DPP4* SNPs, as well as the SNPs tightly linked to them, affect the function of *DPP4*. Second, the biological mechanisms how DPP-IV influences PSA levels have not been elucidated. Third, it is possible that the polymorphisms in another gene strongly linked to the *DPP4* polymorphisms might affect PSA levels. Within 1M base pairs, *TANK*, *PSMD14*, *TBR1*, *SLC4A10*, *GCG*, *FAP*, *IFIH1*, *GCA*, and *KCNH7* were reported to exist, but there were no reports that showed the association between PSA levels and them. Fourth, the effects of more influential genetic traits on PSA levels were not removed. Fifth, the effects of medical history and medication on PSA levels could not be completely removed, because self-administered questionnaires, not medical records, were used to identify the subjects with them. Finally, subjects who had undiagnosed prostate cancer at their enrollment, as well as those who underwent digital rectal examination or prostate needle biopsy or who ejaculated semen before PSA measurements, were not excluded. This might slightly weaken the statistical power to detect the associations between the *DPP4* SNPs and PSA levels.

In summary, our study suggests that *DPP4* genetic variants influence baseline PSA levels. It is expected that further investigations of *DPP4* genetic variants will lead to the development of a superior screening method for prostate cancer.

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