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Levels of Beta-Microseminoprotein (MSP) in Blood and Risk of Prostate Cancer in Multiple Populations

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Notes

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Abstract

Background: A common genetic variant (rs10993994) in the 5' region of the gene encoding β-microseminoprotein (MSP) is associated with circulating levels of MSP and prostate cancer risk. Whether MSP levels are predictive of prostate cancer risk has not been evaluated.

Methods: We investigated the prospective relationship between circulating plasma levels of MSP and prostate cancer risk in a nested case-control study of 1,503 cases and 1,503 controls among African American, Latino, Japanese, Native Hawaiian and White men from the Multiethnic Cohort study. We also examined the ability of MSP to serve as a biomarker for discriminating prostate cancer cases from controls. All statistical tests are two-sided.

Results: In all racial and ethnic groups, men with lower MSP levels were at greater risk of developing prostate cancer (odds ratio [OR] = 1.02 per one unit decrease in MSP, $p<0.001$ in the PSA-adjusted analysis). Compared to men in the highest decile of MSP, the multivariable PSA-adjusted odds ratio was 3.64(95% confidence interval [CI], 2.41-5.49) for men in the lowest decile. The positive association with lower MSP levels was observed consistently across racial/ethnic populations, by disease stage and Gleason score, for men with both high and low levels of PSA and across all genotype classes of rs10993994. However, we did not detect strong evidence of MSP levels in improving prostate cancer prediction beyond that of PSA.

Conclusions: Regardless of race/ethnicity or rs10993994 genotype, men with low blood levels of MSP have increased risk of prostate cancer.

Introduction

Although prostate cancer is the most common cancer among men in the U.S., risk factors for the disease remain largely unknown. More than 40 common low-risk genetic variants have been discovered through genome-wide association studies (GWAS) of prostate cancer (1-6). One such variant is a single nucleotide polymorphism (rs10993994) in the 5' region of the *microseminoprotein-β (MSMB)* gene which encodes for this protein (MSP) (2,6). The risk allele (T nucleotide) for prostate cancer has been shown to be strongly associated with lower circulating MSP levels in multiple populations, with the variant accounting for as much as 50% of the variation in MSP levels in blood or in seminal fluid (7,8).

MSP is one of the most highly secreted proteins from the prostate, and circulating levels have been shown to be positively correlated (r~0·2), with both levels of total and free PSA (8). In contrast to PSA, where risk of prostate cancer increases with higher PSA levels, MSP levels measured in serum, urine and prostate tissue have been shown to be statistically significantly lower in men with prostate cancer, and even lower in men with aggressive disease (9,10). The reproducible association of rs10993994 with prostate cancer risk and circulating MSP levels implicates MSP in the etiology of prostate cancer (11). However, a prospective study has yet to examine MSP levels as a risk factor for incident prostate cancer and as a potentially clinically informative marker for early detection.

To determine the prospective relationship between circulating pre-diagnostic levels of MSP and prostate cancer risk we measured MSP and PSA in a nested multiethnic case-control study of prostate cancer among men in the Multiethnic Cohort (MEC).

Methods

Study Population

The MEC consists of more than 215,000 men and women in California and Hawaii aged 45-75 at recruitment, and comprises mainly five self-reported racial/ethnic populations: African Americans, Japanese, Latinos, Native Hawaiians, and Whites (12). Between 1993 and 1996, adults enrolled in the study by completing a 26-page mailed questionnaire asking detailed information about demographic factors, personal behaviors, and prior medical conditions. Potential participants were identified through driver's license files from Departments of Motor Vehicles, voter registration lists, and Health Care Financing Administration data files. Between 1995 and 2006, blood specimens were collected prospectively from ~67,000 participants for genetic and biomarker analyses. Information regarding previous PSA testing was collected at the time of blood draw as well as from a second questionnaire completed between 1999 and 2000. Informed consent was provided by all study participants and the Institutional Review Boards at the University of Southern California and University of Hawaii approved of the study protocol.

Incident prostate cancer, as well as stage and Gleason score was identified by linkage of the cohort to the Surveillance, Epidemiology, and End Results cancer registries covering Hawaii and California. Invasive prostate cancer was classified according to the International Classification of Disease for Oncology, Third Edition code C619 (prostate). The nested case-control study of prostate cancer in the MEC includes 1,503 men diagnosed with incident prostate cancer after blood collection and 1,503 male controls without prostate cancer at the time the cases and controls were selected (June 2010). Controls were matched to cases in a 1:1 ratio based on race/ethnicity, location, birth year, year of blood collection, hours of fasting and time of collection (1,005 cases were matched on birth year within 5 years, collection date within 0.5 years, hours of fasting time within 2 hours and collection time within 3 hours).

Laboratory Assays

Measurements of MSP and PSA in blinded samples of EDTA anti-coagulated blood plasma were performed in Dr. Lilja's laboratory at Lund University (Malmö, Sweden). In brief, the MSP immunoassay was conducted using the AutoDelfia 1235 automatic immunoassay system (Perkin-Elmer Life Sciences, Turku, Finland). Production and purification of the polyclonal rabbit anti-MSP antibody, and protocols for the biotinylation and Europium labeling of the anti-MSP antibody have been previously described (13). To measure free and total PSA, we used the dual-label DELFIA Prostatus® total/free PSA-Assay (Perkin-Elmer, Turku, Finland) (14), which is calibrated against the WHO 96/670 (PSA-WHO) and WHO 68/668 (free PSA-WHO) standards. Using two identical AutoDelfia 1235 automated instruments to measure MSP, each sample was run in duplicate with coefficients of variation (CV) being ≤9.0% at 22.8 ng/mL and ≤6.6% at 55 ng/mL of MSP. For the measurements of free PSA, CVs were 7.3% at 0.14 ng/mL, 3.3% at 0.63 ng/mL, and 5.1% at 2.6 ng/mL; CVs for measuring total PSA were 10.6% at 0.47 ng/mL, 7.4% at 2.6 ng/mL, and 8.4% at 14.2 ng/mL. For 37 blinded duplicates, the coefficients of variation were 3.6%, 5.2%, and 3.3% for MSP, total PSA, and free PSA respectively. The detectable ranges are: 0.10-250 ng/mL for total PSA, 0.04-250 ng/mL for free PSA, and 0.2-90 ng/mL for MSP; values above the detectable limits were assigned the highest detectable values. Of the 3,006 samples, 2,999 were analyzed for the MSP and PSA measures.

Statistical Analysis

We excluded men who were determined not to be one of the five racial/ethnic populations (n=22), men missing information about body mass index (BMI) (n=3) and men with levels below the limits of detection for either total PSA, free PSA or MSP (n=12), resulting in 1,480 cases and 1,481 controls for analysis. Analysis of variance was used to test for differences in demographic variables and biomarker levels by case-control status, racial/ethnic group and rs10993994 genotype, adjusted for matching factors [age, area (Hawaii, Los Angeles), hours of fasting, year of collection and time of collection] and body mass index (BMI,

kg/m^2). MSP and PSA levels were log transformed to better meet the model assumptions and the results are presented as geometric means. The relationship between levels of free or total PSA and MSP levels was summarized using Spearman's rank correlation.

Unconditional logistic regression models of prostate cancer were used to examine its association with MSP levels, adjusted for the matching factors (listed above), BMI, total PSA and free PSA. Total and free PSA were included in the models as linear and squared terms to better capture their associations with prostate cancer risk. MSP was parameterized as indicator variables reflecting quartile and decile membership. Laboratory batch was not adjusted for as it did not influence the results. We assessed heterogeneity in the association of MSP levels and risk by a Wald test of the interaction terms between each covariate and MSP as well as stratified analysis.

We also examined the association of MSP and the risk of prostate cancer by disease stage (localized, n=1,116; regional/advanced, n=169) and Gleason score (≥7, n=640; <7, n=688). In analyses stratified by stage and Gleason score odds ratios were estimated comparing cases in each subgroup to all controls. Case-only analyses were performed to test for differences by disease subgroup. for undetected disease among men with shorter follow-up time. We also conducted analysis stratified by self-reported PSA testing prior to blood collection.

We also evaluated the predictive ability of MSP to discriminate prostate cancer cases and controls in all subjects and in analyses stratified by case characteristics, PSA and the time between blood draw and diagnosis. In these analyses, area under the curve statistics (AUCs) for ROC curves from logistic regression models were estimated before and after accounting for age, BMI, total and free PSA (linear and squared terms) and MSP. All statistical tests are two-sided.The statistical analyses were conducted using SAS software version 9.2 (SAS Institute Inc., Cary, NC, USA).

Results

The mean age of cases and controls at blood draw (baseline) was 72.1 and 70.6 years, respectively (Table 1). MSP levels were positively associated with age in cases but not in controls and were inversely associated with BMI and weight in both cases and controls (Supplemental Table 1). For cases, the mean number of years between blood draw and cancer diagnosis ranged from 3.0 in White cases to 3.8 in Native Hawaiian cases (subject range: <1 year to 12 years, 2 months).

The levels of both total and free PSA measured at the time of blood draw were statistically significantly higher ($p<0.001$) in men who were subsequently diagnosed with prostate cancer than in men who did not develop prostate cancer (Table 1, Supplemental Figures 1a-c). In contrast, mean plasma levels of MSP at baseline were statistically significantly lower in cases than controls ($p=0.003$; Table 1, Supplemental Figure 1d). We found statistically significant, yet modest, correlations between PSA measures and MSP at baseline (correlation coefficients of 0.23-0.26, $p<0.001$, in cases and controls). Although there were no statistically significant differences in the levels of total or free PSA across racial/ethnic populations, among controls, African American men had the lowest mean MSP levels at baseline (p-value for differences by race/ethnicity <0.001) (Table 1).

Given the lower levels of MSP in prostate cancer cases compared with controls, and the positive correlation of MSP and PSA, the difference in mean MSP levels between cases and controls was statistically significantly greater following adjustment for PSA ($p<0.001$, Table 1). In analyses adjusted for total and free PSA, lower MSP levels were strongly associated with greater prostate cancer risk (OR=1.02 per unit decrease in MSP, $p<0.001$ adjusted for PSA; and OR=1.01, $p=0.006$ unadjusted for PSA) (Figure 1). Compared to men in the highest quartile of MSP, the PSA-adjusted odds ratio was 1.58 (95% CI, 1.23-2.03) for men in the third quartile, 1.77 (95% CI, 1.37-2.27) for men in the second quartile and 2.08 (95% CI, 1.61-2.68) for men in the lowest quartile (Table 2). The PSA-adjusted odds ratio was 3.64 (95% confidence interval [CI], 2.41-5.49) for men in the lowest decile, when compared to men in the highest decile of

MSP (Figure 1). We observed evidence of heterogeneity in the MSP-risk association by racial/ethnic group (p=0.04) with statistically significant differences noted between Whites and African Americans (p for interaction=0.008), Whites and Latinos (p for interaction=0.02), and African Americans and Native Hawaiians (p for interaction=0.02). We did not observe evidence of heterogeneity of the association by age, disease stage or Gleason score, PSA levels, the number of years between providing a blood sample and a subsequent prostate cancer diagnosis (Supplemental Table 2) or prior PSA testing (Supplemental Table 3).

Among the 1,221 cases and 1,230 controls with rs10993994 genotype information available from previous studies in the MEC (8,15), we confirmed the strong association between the prostate cancer risk variant and MSP levels in each population (Supplemental Table 4), with the genotype accounting for 46 to 58% of the variation in circulating MSP levels in controls. We detected evidence of heterogeneity of the association between MSP and prostate cancer risk by rs10993994 genotype (p=0.03 for interaction). Stratified analysis shows that the association is strongest for men with the CC genotype (Table 3). An association was observed for the lowest compared to the highest quartile of MSP levels for each genotype group; the trend was statistically significant both for men with the TT genotype, which is associated with higher risk of prostate cancer, as well as for men with the low-risk CC genotype, indicating that the association of MSP on risk is independent of genotype.

Despite the highly statistically significant association of MSP and prostate cancer risk, MSP levels did not importantly enhance discrimination of prostate cancer cases from controls above that of PSA. Among all cases and controls the AUC changed from 0.839 to 0.843 when including MSP in models that included total and free PSA. Similar small increases in the AUCs were noted when MSP was added in models that included free and total PSA for each ethnic group, by disease stage and Gleason score, and for men at increased prostate cancer risk based on their total PSA level at baseline (Supplemental Table 5).

Discussion

In this prospective study, we provide evidence that lower circulating levels of MSP are associated with increased risk of prostate cancer. An association with the biomarker levels was observed in all racial/ethnic populations, in all rs10993994 genotypes and irrespective of levels of total or free PSA. However, we found little evidence that circulating MSP levels improve prostate cancer prediction beyond that of total and free PSA.

Together with PSA, MSP is one of the most abundantly expressed proteins from the prostate (16,17) and one of the most abundant secreted proteins in human seminal fluid (18). Serum MSP levels have been shown to be lower in men with aggressive prostate cancer (9) as well as in men with a prostate cancer recurrence after radical prostatectomy (19). Urinary MSP levels have also been shown to be lower in men with prostate cancer than in men with no history of prostate cancer (10). In the prostate, MSP expression is lower in malignant tissue than in benign prostate tissue, with levels found to be even lower in men with more aggressive tumors (10,20,21). It is not clear whether the lower expression in malignant tissue and the lower levels observed in the blood and in the urine are a reflection of the disease or whether the change is an etiologic event that is important in the pathogenesis of the cancer. All previous studies to examine MSP as a marker of risk have been cross-sectional with urinary or blood measurements taken among cases already diagnosed with prostate cancer. Here we show that a lower level of MSP measured in blood drawn five years before diagnosis is associated with a statistically significantly increased risk of subsequent diagnosis of prostate cancer. The inverse association that we detected was not influenced by the duration of time between blood draw and a diagnosis, which suggests that the lower levels in cases is unlikely to be the result of undiagnosed disease. However one limitation of our study is the length of time of follow-up. Given the long latency period for prostate cancer, longer follow-up of the cohort will be required to better assess the impact of potentially undiagnosed disease on our findings.

The biological function of MSP in the prostate is not known. It is also unclear why MSP levels decrease (in contrast to PSA) during tumor progression. MSP has been implicated to have a role in regulating cellular growth in the prostate through apoptosis as well as to function as a suppressor of tumor growth (22). Silencing of MSP expression in prostate tumor tissue has been associated with promotor methylation of the gene mediated by a Polycomb group member protein, enhancer of zeste homolog-2 (EZH2) (23). MSP has the capacity to form very high affinity complexes with cysteine-rich secretory protein-3 (CRISP3) in seminal fluid (24), a protein implicated through structural similarity of the C-terminal domain to possibly function as a potassium-channel inhibitor (25), however the functional significance or biological role of MSP alone or bound to CRISP3 is not currently known.

Consistent with numerous prior reports (26,27), the level of total PSA in blood was a remarkably strong predictor of prostate cancer risk in our study. The increase in the effect size associated with lower MSP levels following adjustment for PSA is consistent with negative confounding due to a modest positive correlation between these markers and the very strong association of PSA and prostate cancer risk. Both accurate measurement of PSA and modeling of its relationship with prostate cancer are important, as residual, and unaccounted for, negative confounding would conceal the true strength of the association of MSP with prostate cancer risk.

GWAS have been criticized because the variants identified have modest associations, account for only a small fraction of disease heritability and have yet to translate into mechanisms that are successful, beyond those currently available, to effectively identify, prevent or treat disease. The counter to this criticism is that the risk loci are providing biological insights into genes and pathways that are unambiguously important in disease etiology. While previous studies have focused on MSP as a biomarker of potential utility in screening (9,10,19), the identification of a common risk variant at the MSP locus from GWAS (2,6), and subsequent studies showing that this variant is involved in the regulation of the protein (7,8), has highlighted

the protein as directly involved in the pathogenesis of the disease. Our investigation of MSP as a biomarker and risk factor for the disease was based on the risk association with the genetic locus, and demonstrate the value of GWAS in revealing and strengthening biological links with disease, which in the case of prostate cancer is important given how little is known about factors that influence individual risk.

Despite the highly statistically significant association of MSP with prostate cancer risk, we found little evidence that circulating MSP levels improve prostate cancer prediction beyond that of total and free PSA. Findings from our prospective analysis are consistent with the cross-sectional study of Nam et al. (9) which also found no improvement by considering MSP in prostate cancer prediction among men with PSA >4 or an abnormal DRE. However in contrast to this previous report (9) we observed no improvement in distinguishing aggressive versus non-aggressive disease when MSP was combined with total PSA and free PSA. This difference may be due to the association of PSA being artificially inflated in our study due to verification bias, which is another potential limitation of our study. Prostate cancer is more likely to be detected among men with elevated PSA levels as this is an indication for biopsy in the U.S. Indeed, the AUC for PSA and prostate cancer (0.822) was much higher than typically reported (26,27). Accordingly, we suggest additional studies to determine the value of MSP in predicting outcome of biopsy following an elevated PSA level as well as studies to address the specificity of MSP for lethal disease.

In summary, our data provide strong evidence in support of an association between the MSP protein measured in blood and prostate cancer risk across multiple racial/ethnic populations. However, this study does not contribute definitive evidence as to whether MSP levels can importantly enhance prostate cancer prediction beyond that of total and free PSA

Figure Legend

Figure 1. The Association of Blood MSP Levels and Prostate Cancer Risk.

Shown is the association of MSP levels in the blood with risk of prostate cancer diagnosis. The red circles are PSA-adjusted odds ratios (and bars, 95% CIs) for each decile of MSP compared with the highest decile (black circle, reference). The blue circles are PSA-unadjusted odds ratios (and bars, 95% CIs) for each decile of MSP compared with the highest decile (black circle, reference). The red and blue lines show the linear association from each model starting from the highest decile of MSP. The p-values are for the linear association of MSP with prostate cancer risk.

References

1. Eeles RA, Kote-Jarai Z, Al Olama AA*, et al.* Identification of seven new prostate cancer susceptibility loci through a genome-wide association study. *Nat Genet* 2009;41(10):1116-1121.

2. Eeles RA, Kote-Jarai Z, Giles GG*, et al.* Multiple newly identified loci associated with prostate cancer susceptibility. *Nat Genet* 2008;40(3):316-321.

3. Gudmundsson J, Sulem P, Gudbjartsson DF*, et al.* Genome-wide association and replication studies identify four variants associated with prostate cancer susceptibility. *Nat Genet* 2009;41(10):1122-1126.

4. Gudmundsson J, Sulem P, Rafnar T*, et al.* Common sequence variants on 2p15 and Xp11.22 confer susceptibility to prostate cancer. *Nat Genet* 2008;40(3):281-283.

5. Kote-Jarai Z, Olama AA, Giles GG*, et al.* Seven prostate cancer susceptibility loci identified by a multi-stage genome-wide association study. *Nat Genet* 2011;43(8):785-791.

6. Thomas G, Jacobs KB, Yeager M*, et al.* Multiple loci identified in a genome-wide association study of prostate cancer. *Nat Genet* 2008;40(3):310-315.

7. Xu X, Valtonen-Andre C, Savblom C*, et al.* Polymorphisms at the Microseminoprotein-beta locus associated with physiologic variation in beta-microseminoprotein and prostate-specific antigen levels. *Cancer Epidemiol Biomarkers Prev* 2010;19(8):2035-2042.

8. Waters KM, Stram DO, Le Marchand L*, et al.* A common prostate cancer risk variant 5' of microseminoprotein-beta (MSMB) is a strong predictor of circulating beta-microseminoprotein (MSP) levels in multiple populations. *Cancer Epidemiol Biomarkers Prev* 2010;19(10):2639-2646.

9. Nam RK, Reeves JR, Toi A*, et al.* A novel serum marker, total prostate secretory protein of 94 amino acids, improves prostate cancer detection and helps identify high grade cancers at diagnosis. *J Urol* 2006;175(4):1291-1297.

10. Whitaker HC, Kote-Jarai Z, Ross-Adams H*, et al.* The rs10993994 risk allele for prostate cancer results in clinically relevant changes in microseminoprotein-beta expression in tissue and urine. *PLoS One* 2010;5(10):e13363.

11. Whitaker HC, Warren AY, Eeles R*, et al.* The potential value of microseminoprotein-beta as a prostate cancer biomarker and therapeutic target. *Prostate* 2010;70(3):333-340.

12. Kolonel LN, Henderson BE, Hankin JH*, et al.* A multiethnic cohort in Hawaii and Los Angeles: baseline characteristics. *Am J Epidemiol* 2000;151(4):346-357.

13. Vaisanen V, Peltola MT, Lilja H*, et al.* Intact free prostate-specific antigen and free and total human glandular kallikrein 2. Elimination of assay interference by enzymatic digestion of antibodies to F(ab')2 fragments. *Anal Chem* 2006;78(22):7809-7815.

14. Mitrunen K, Pettersson K, Piironen T*, et al.* Dual-label one-step immunoassay for simultaneous measurement of free and total prostate-specific antigen concentrations and ratios in serum. *Clin Chem* 1995;41(8 Pt 1):1115-1120.

15. Haiman CA, Chen GK, Blot WJ*, et al.* Characterizing genetic risk at known prostate cancer susceptibility loci in African Americans. *PLoS Genet* 2011;7(5):e1001387.

16. Ulvsback M, Lindstrom C, Weiber H*, et al.* Molecular cloning of a small prostate protein, known as beta-microsemenoprotein, PSP94 or beta-inhibin, and demonstration of transcripts in non-genital tissues. *Biochem Biophys Res Commun* 1989;164(3):1310-1315.

17. Lundwall A, Lilja H. Molecular cloning of human prostate specific antigen cDNA. *FEBS Lett* 1987;214(2):317-322.

18. Lilja H, Abrahamsson PA. Three predominant proteins secreted by the human prostate gland. *Prostate* 1988;12(1):29-38.

19. Reeves JR, Dulude H, Panchal C*, et al.* Prognostic value of prostate secretory protein of 94 amino acids and its binding protein after radical prostatectomy. *Clin Cancer Res* 2006;12(20 Pt 1):6018-6022.

20. Bjartell AS, Al-Ahmadie H, Serio AM*, et al.* Association of cysteine-rich secretory protein 3 and beta-microseminoprotein with outcome after radical prostatectomy. *Clin Cancer Res* 2007;13(14):4130-4138.

21. Abrahamsson PA, Lilja H, Falkmer S*, et al.* Immunohistochemical distribution of the three predominant secretory proteins in the parenchyma of hyperplastic and neoplastic prostate glands. *Prostate* 1988;12(1):39-46.

22. Shukeir N, Arakelian A, Kadhim S*, et al.* Prostate secretory protein PSP-94 decreases tumor growth and hypercalcemia of malignancy in a syngenic in vivo model of prostate cancer. *Cancer Res* 2003;63(9):2072-2078.

23. Beke L, Nuytten M, Van Eynde A*, et al.* The gene encoding the prostatic tumor suppressor PSP94 is a target for repression by the Polycomb group protein EZH2. *Oncogene* 2007;26(31):4590-4595.

24. Udby L, Lundwall A, Johnsen AH*, et al.* beta-Microseminoprotein binds CRISP-3 in human seminal plasma. *Biochem Biophys Res Commun* 2005;333(2):555-561.

25. Guo M, Teng M, Niu L*, et al.* Crystal structure of the cysteine-rich secretory protein stecrisp reveals that the cysteine-rich domain has a K+ channel inhibitor-like fold. *J Biol Chem* 2005;280(13):12405-12412.

26. Lilja H, Cronin AM, Dahlin A*, et al.* Prediction of significant prostate cancer diagnosed 20 to 30 years later with a single measure of prostate-specific antigen at or before age 50. *Cancer* 2011;117(6):1210-1219.

27. Vickers AJ, Cronin AM, Bjork T*, et al.* Prostate specific antigen concentration at age 60 and death or metastasis from prostate cancer: case-control study. *BMJ* 2010;341:c4521.

Table 1: Characteristics of prostate cancer cases and controls by race/ethnicity.

Characteristic	White	African American	Latino	Japanese	Native Hawaiian	All Groups	P-value[†]	P-value[‡]
n, case/control	288/287	349/345	258/263	499/499	86/87	1,480/1,481		
Age (yr)*	70.8±7.9/69.7±8.0	72.9±7.0/70.4±6.5	71.3±6.7/ 70.0±6.5	73.1±7.7/ 72.2±7.6	69.0±7.2/66.4±6.3	72.1±7.5/70.6±7.3	<0.001	
Body mass index (kg/m^2)*	26.6±3.9/26.8±4.2	27.4±4.2/27.7±4.1	27.0±3.5/27.2±3.8	25.3±3.4/25.2±3.5	29.3±5.3/27.6±5.8	26.6±4.0/26.6±4.1	0.98	
MSP (ng/mL)[§]	22.7±1.0/24.9±1.1	18.4±0.8/17.5±0.8	21.3±1.0/22.1±1.0	18.4±0.6/20.7±0.7	16.4±1.2/23.2±1.7	19.7±0.4/21.1±0.4	0.003	<0.001
MSP (ng/mL)[<]	21.1±0.9/26.0±1.1	17.2±0.7/18.9±0.8	20.2±0.9/23.9±1.1	17.4±0.6/21.7±0.7	15.2±1.1/24.5±1.8	18.5±0.4/22.4±0.4	<0.001	<0.001
Total PSA (ng/mL)[¶]	4.2±0.2/1.4±0.08	4.5±0.3/1.4±0.08	4.2±0.3/1.2±0.08	3.9±0.2/1.3±0.06	4.7±0.5/1.2±0.1	4.2±0.1/1.3±0.03	<0.001	0.37
Free PSA (ng/mL)[¶]	0.9±0.04/0.4±0.02	0.9±0.05/0.4±0.02	0.8±0.04/0.4±0.02	0.8±0.03/0.4±0.02	1.0±0.09/0.4±0.04	0.9±0.02/0.4±0.01	<0.001	0.84
Percent Free PSA[¶]	0.2±0.01/0.3±0.01	0.2±0.01/0.3±0.01	0.2±0.01/0.3±0.01	0.2±0.01/0.3±0.01	0.2±0.01/0.3±0.02	0.2±0.002/0.3±0.003	<0.001	0.001

*Means ± standard deviation at time of blood draw. [†]F-test of case-control differences from covariance analysis. [‡]F-test of racial/ethnic differences among controls from covariance analysis. [§]Geometric means (±SE) adjusted by covariance analysis for the matching factors (age, area, hours of fasting, year of collection, time of collection) and body mass index (kg/m^2). [<]Geometric means (± standard error) adjusted by covariance analysis for the matching factors (age, area, hours of fasting, year of collection, time of collection), body mass index (kg/m^2), total PSA (log transformed), free PSA (log transformed), and, race/ethnicity (5 groups) in the pooled analysis. [¶]Geometric means (±SE) adjusted by covariance analysis for the matching factors (age, area, hours of fasting, year of collection, time of collection), body mass index (kg/m^2), MSP and race/ethnicity (5 groups) in the pooled analysis.

Table 2: The association of MSP levels (quartiles) with prostate cancer risk by race/ethnicity.

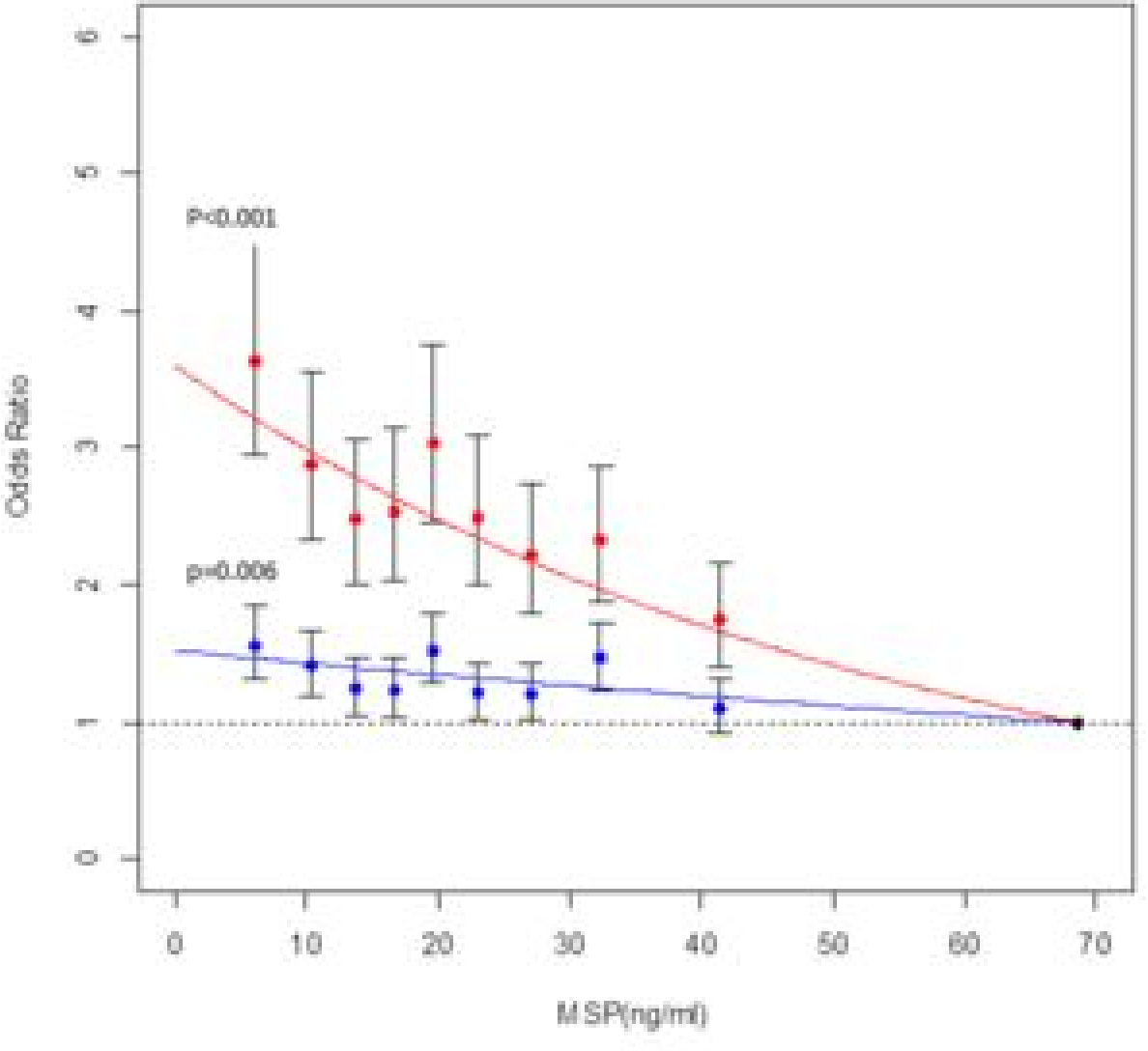
	Q4* (highest)	Q3	Q2	Q1 (lowest)	P-value‡
White					
Odds ratio (95% CI)†	1.0 (ref.)	4.74(2.48-9.06)	5.13(2.66-9.91)	4.31(2.16-8.58)	<0.001
African American					
Odds ratio (95% CI)†	1.0 (ref.)	1.23(0.76-1.98)	1.49(0.92-2.44)	1.20(0.73-1.98)	0.05
Latino					
Odds ratio (95% CI)†	1.0 (ref.)	1.42(0.75-2.68)	1.75(0.94-3.26)	1.81(0.95-3.43)	0.11
Japanese					
Odds ratio (95% CI)†	1.0 (ref.)	1.50(0.96-2.35)	1.48(0.94-2.32)	2.20(1.42-3.40)	<0.001
Native Hawaiian					
Odds ratio (95% CI)†	1.0 (ref.)	2.88(0.7-11.78)	2.64(0.57-12.23)	6.94(1.77-27.18)	0.01
All Groups					
Odds ratio (95% CI)§	1.0 (ref.)	1.58(1.23-2.03)	1.77(1.37-2.27)	2.08(1.61-2.68)	<0.001
P_{Het}<					0.04
All Groups					
Odds ratio (95% CI)¶	1.0 (ref.)	1.09(0.88-1.34)	1.17(0.95-1.44)	1.27(1.03-1.57)	0.006

*Quartiles are based on the distribution in each control population and for all controls in the pooled analysis. †Odds ratios were adjusted for the matching factors (age, area, hours of fasting, year of collection, time of collection), body mass index (kg/m^2), total PSA (linear and squared terms) and free PSA (linear and squared terms). ‡P-value for the linear association of MSP modeled as a continuous variable. §Odds ratios were adjusted for the matching factors (age, area, hours of fasting, year of collection, time of collection), body mass index (kg/m^2), total PSA (linear and squared terms), free PSA (linear and squared terms) and race/ethnicity (5 groups). <P-value for heterogeneity across racial/ethnic groups. ¶Odds ratios were adjusted for the matching factors (age, area, hours of fasting, year of collection, time of collection), body mass index (kg/m^2) and race/ethnicity (5 groups).

Table 3. The association of MSP levels (quartiles) and prostate cancer risk by rs10993994 genotype.

	Q4*	Q3	Q2	Q1	P-value‡	P-value§
rs10993994 Genotype						
TT genotype						
MSP (ng/mL), cases/controls<	21.5/22.1	11.4/11.9	7.8/7.7	4.5/5.1		
n (cases/controls)†	71/70	92/72	79/72	77/72		
Odds ratio (95% CI)	1.00	1.45(0.83-2.53)	1.77(1.00-3.15)	2.04(1.10-3.80)	0.04	
CT genotype						
MSP (ng/mL), cases/controls<	39.8/38.6	22.6/22.6	17.2/17.0	12.0/13.0		
n (cases/controls)†	180/146	166/146	120/146	122/147		
Odds ratio (95% CI)	1.00	1.59(1.08-2.32)	1.42(0.94-2.16)	1.75(1.14-2.69)	0.16	
CC genotype						
MSP (ng/mL), cases/controls<	64.4/65.2	36.3/37.6	28.5/28.6	20.3/19.8		
n (cases/controls)†	64/89	92/90	82/89	76/91		
Odds ratio (95% CI)	1.00	2.82(1.56-5.08)	3.14(1.71-5.77)	3.02(1.56-5.85)	<0.001	0.03

*****Quartiles are defined separately for each genotype class. †Odds ratios were adjusted for the matching factors (age, area, hours of fasting, year of collection, time of collection), body mass index (kg/m^2), total PSA (linear and squared terms), free PSA (linear and squared terms) and race/ethnicity (5 groups). ‡P-value for the linear association of MSP modeled as a continuous variable. §P-value for test of heterogeneity in the linear association of MSP and prostate cancer risk by genotype. <Geometric means adjusted by covariance analysis for the matching factors (age, area, hours of fasting, year of collection, time of collection), body mass index (kg/m^2), total PSA (log transformed), free PSA (log transformed) and race/ethnicity (5 groups).


P<0.001
p=0.006
Odds Ratio
0
1
2
3
4
5
6
0
10
20
30
40
50
60
70
MSP(ng/ml)