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LETTER TO THE EDITOR

Prostate Cancer

Prostate cancer antigen 3 and genetic risk score as markers for the detection of prostate cancer in the Chinese population

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Dear Editor,

We report here the performance of prostate cancer antigen 3 (*PCA3*) and genetic risk score (GRS) in predicting prostate cancer (PCa) from the prostate biopsy. To the best of our knowledge, this is the first report of simultaneously evaluating these two biomarkers in the same study.

The incidence of PCa has increased in China over the last two decades, likely due to a combination of factors such as increase in life expectancy, better detection method of PCa, and life style changes.[1] PCa is typically diagnosed from the prostate biopsy in patients with elevated prostate specific antigen (PSA) levels. However, for moderately elevated PSA levels, its specificity for PCa is relatively low because other prostate disorders may also lead to elevated PSA levels.[2] Therefore, additional biomarkers are needed to complement PSA to better identify patients that may benefit from prostate biopsy.

Two recently proposed biomarkers of PCa are promising. The first is a urine biomarker (*PCA3*), a noncoding RNA that was first reported in 1991 to have higher expression in prostate tumors than normal or benign prostate tissue.[3] Since then several studies found that it can also be detected in the urine after digital rectal exam (DRE).[4,5] A urine *PCA3* test for considering a repeat biopsy in men 50 years of age or older who have had one or more previous negative prostate biopsies by Hologic Gen-Probe was approved by the European Medicines Agency and the United States Food and Drug Administration in 2012. The second is GRS derived from multiple PCa risk-associated single nucleotide polymorphisms (SNPs) identified from genome-wide association studies (GWAS).[6,7] Although each of these two biomarkers has been evaluated individually, the combined effect of these two biomarkers in predicting PCa from the prostate biopsy have not been reported.

We assessed the joint effect of these two biomarkers in a biopsy cohort of the Chinese population. Subjects in this study were patients scheduled for needle prostate biopsy at Huashan Hospital, Shanghai, China during 2012 and 2013. Blood samples and post-DRE urine were collected for each patient before biopsy. The study was approved by the Institutional Ethics Review Board at Huashan Hospital and written informed consent was completed for each patient.

Because the Hologic *PCA3* kit is not currently available in China, urine *PCA3* RNA level was measured using an in-house qPCR method and its level was normalized by urine levels of *PSA* mRNA. *PCA3* level was calculated using $\Delta Ct = Ct_{PCA3} - Ct_{PSA}$.[8] Twenty-nine PCa risk-associated SNPs discovered from GWAS and confirmed in the Chinese population ($P < 0.05$) were genotyped using MassARRAY iPLEX (Sequenom, Inc., CA, USA).[9] A GRS was calculated for each subject based on their genotypes at these 29 SNPs and weighted by odd ratios of these SNPs derived from an external study.[10] Association of PCa diagnosis with *PCA3*, GRS, and other clinical variables were tested using both univariate and multivariate analyses. Total PSA, *PCA3*, free to total PSA ratio, and GRS were log-transformed prior to statistical tests.

The key demographic and clinical variables for the 99 patients in the cohort, as well as their association with PCa risk, are presented in **Table 1**. Based on univariate analysis, higher *PCA3* ($P = 0.0002$), higher total PSA ($P = 0.0002$), higher GRS ($P = 0.0008$), lower free to total PSA ratio ($P = 0.007$), and smaller prostate volume ($P = 0.0003$) were each associated with increased risk for PCa. The performance for discriminating PCa from non-PCa, measured by area under the curve (AUC), was 0.73 for total PSA, 0.77 for *PCA3*, and 0.70 for GRS.

We also examined in detail the added value of *PCA3* and GRS to total PSA in discriminating prostate biopsy outcomes (**Table 2**). Compared to Model 1 with total PSA alone where AUC was 0.73, the AUC increased to 0.81 for Model 2 with total PSA and GRS ($P = 0.05$), and to 0.84 for Model 3 with total PSA and *PCA3* ($P = 0.01$). The AUC for Model 4 with all three variables (total PSA, GRS and *PCA3*) was further increased to 0.86, although the improvement was not statistically significant over Model 2 ($P = 0.08$) or Model 3 ($P = 0.34$).

When all six variables were considered together in a multivariate analysis, only three variables were independently associated with PCa risk from a multivariate analysis; total PSA ($P = 0.001$), prostate volume ($P = 0.0001$), and *PCA3* ($P = 0.027$). The AUC of the best multivariate model for discriminating PCa from non-PCa was excellent at 0.94.

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Table 1: Key demographic and clinical variables in subjects

Variables	All	Biopsy outcomes		P^a	AUC
		PCa	Non-PCa		
Number (%) of subjects	99 (100)	42 (42.42)	57 (57.58)		
Univariate analysis					
Age (n=93)					
Mean (s.d.), year	72.11 (8.36)	73.68 (7.65)	70.94 (8.74)	0.12	0.57
Total PSA (n=94)					
Median (Q1–Q3), ng ml^{-1}	11.45 (8.65–20.48)	18.11 (10.62–53.25)	10.54 (7.33–14.82)	0.0002	0.73
Mean (s.d.), ng ml^{-1}	57.39 (257.33)	121.88 (393.33)	11.66 (6.46)		
Prostate volume (n=89)					
Median (Q1–Q3), ml	48.00 (36.00–65.00)	39.00 (32.00–51.00)	59.50 (41.00–74.50)	0.0003	0.76
Mean (s.d.), ml	54.57 (25.00)	42.51 (16.99)	62.39 (26.34)		
F/T ratio (n=61)					
Median (Q1–Q3)	0.15 (0.10–0.21)	0.10 (0.08–0.16)	0.18 (0.13–0.23)	0.007	0.73
Mean (s.d.)	0.16 (0.08)	0.12 (0.07)	0.19 (0.08)		
PCA3 (n=90)					
Median (Q1–Q3)	104.67 (2.02–354.43)	242.43 (111.59–558.16)	16.65 (0.42–135.45)	0.0002	0.77
Mean (s.d.)	592.23 (1642.70)	815.67 (1490.70)	428.95 (1741.41)		
GRS (n=94)					
Median (Q1–Q3)	0.99 (0.56–1.38)	1.15 (0.85–1.77)	0.78 (0.46–1.25)	0.0008	0.70
Mean (s.d.)	1.13 (0.75)	1.42 (0.84)	0.92 (0.61)		
Multivariable analysis					
Total PSA				0.001	0.94
Prostate volume				0.0001	
PCA3				0.027	

^{a}Based on logistic regression analysis. AUC: area under the curve; F/T ratio: free to total PSA ratio; GRS: genetic risk score; PCa: prostate cancer; PCA3: prostate cancer antigen 3; PSA: prostate specific antigen; Q1: first quartile; Q3: third quartile; s.d.: standard deviation
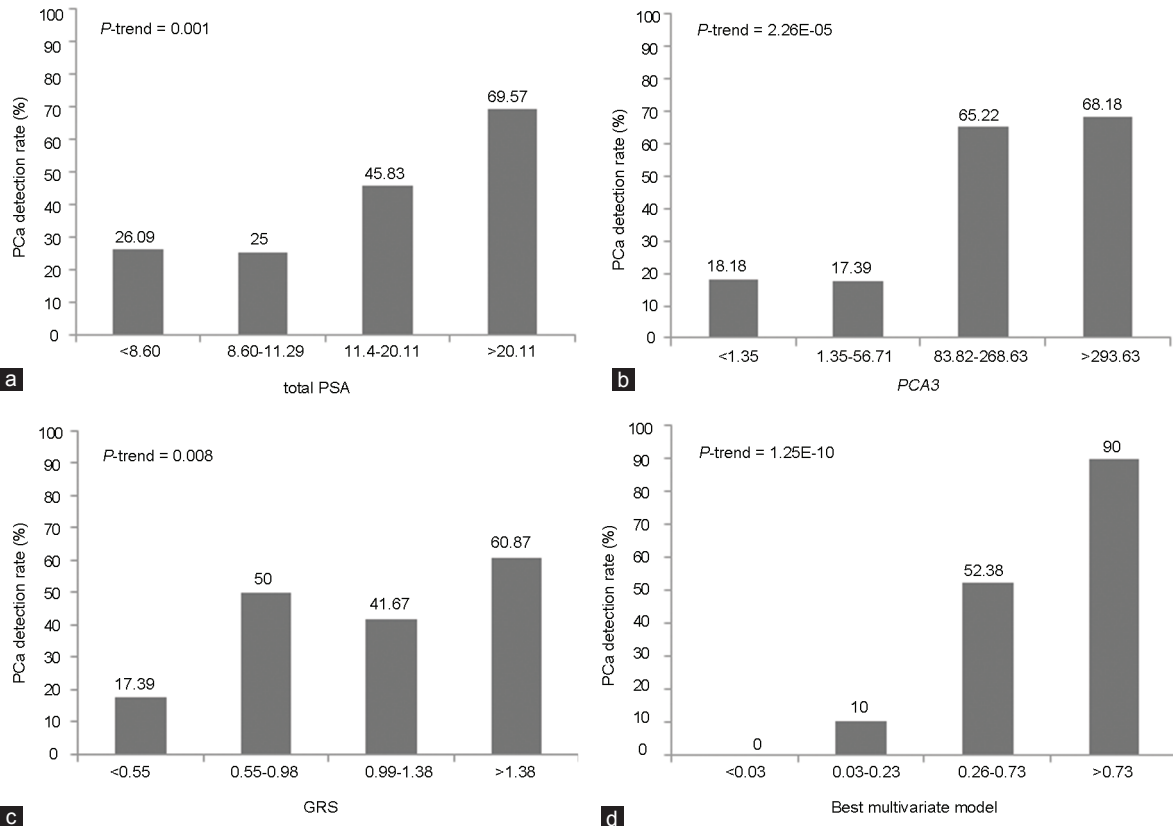

Figure 1: Prostate cancer detection rates among patients with (**a**) total prostate specific antigen, (**b**) prostate cancer antigen 3, (**c**) genetic risk score and (**d**) best multivariate model.

Table 2: AUC comparison between models

Model	Variables	AUC	P^a	P^b	P^c
1	Total PSA	0.73	-	-	-
2	Total PSA, GRS	0.81	0.05	-	-
3	Total PSA, *PCA3*	0.84	0.01	-	-
4	Total PSA, *PCA3*, GRS	0.86	0.01	0.08	0.34

[a]Compare with Model 1; [b]Compare with Model 2; [c]Compare with Model 3. AUC: area under the curve; GRS: genetic risk score; PCA3: prostate cancer antigen 3; PSA: prostate specific antigen

To assess the predictive performance of these variables using a more clinically meaningful measurement, we estimated the PCa detection rate for patients at each quartile based on total PSA, *PCA3*, GRS, and the best multivariate model (**Figure 1**). PCa detection rate from biopsy increased with increasing quartiles for total PSA ($P\text{-}_{trend} = 0.001$), *PCA3* ($P\text{-}_{trend} = 2.26\text{E}{-}05$), GRS ($P\text{-}_{trend} = 0.008$), and the best model ($P\text{-}_{trend} = 1.25\text{E}{-}10$). The best multivariate model had the highest discriminative performance; the PCa detection rate was 0% for patients at lowest quartile and 90% for patients at highest quartile.

In conclusion, results from this study suggest that (1) either *PCA3* or GRS provides added value to total PSA in predicting PCa, (2) combining both *PCA3* and GRS may further improve the performance of total PSA in discriminating biopsy outcome, and (3) model with total PSA, prostate volume, and *PCA3* has an excellent predictive performance of PCa. While these findings are promising, caution should be taken due to the small sample size. Large studies of Chinese and other populations are needed to confirm these findings.

AUTHOR CONTRIBUTIONS

HMW, JX participated in the conception and design of study. RN and YSW collected the samples. HMW performed the laboratory experiment. HTC performed all the statistical analyses. HMW drafted the manuscript. PW, FL, DKJ, and JX assisted in the revision of the manuscript. JX and DRL supervised the study. All authors read and approved the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

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