Varying parameters associated with prostate-specific antigen (PSA) level in prostate cancer cases and controls from three geographical regions.

Nishi Karunasinghe[1*], Tsion Zewdu Minas[2], Bo-Ying Bao[3], Arier Lee[4], Alice Wang [1], Shuotun Zhu[1], Jonathan Masters[5], Megan Goudie[6], Shu-Pin Huang[7,8], Frank J. Jenkins[9] and Lynnette R Ferguson[1,10]

1- Auckland Cancer Society Research Centre, Faculty of Medical and Health Sciences (FMHS), University of Auckland (UoA), New Zealand; 2- Laboratory of Human Carcinogenesis, National Cancer Institute, NIH, Bethesda, USA; 3- Department of Pharmacy, China Medical University, Taichung, Taiwan; 4- School of Population Health, UoA, New Zealand; 5- Urology Department, Whangarei Hospital, New Zealand; 6- Urology Department, Auckland City Hospital, New Zealand; 7- Department of Urology, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan; 8- Center for Cancer Research, Kaohsiung Medical University, Kaohsiung, Taiwan; 9- University of Pittsburgh Medical Center, Hillman Cancer Center, Pittsburgh, USA; 10- Emeritus Professor, FMHS, UoA, New Zealand, * - n.karunasinghe@auckland.ac.nz

Background

It is still being debated whether prostate-specific antigen (PSA)-based screening effectively reduces prostate cancer mortality in men. Some of the uncertainty could be related to existing deficiencies in the age-based PSA thresholds that are currently used in prostate cancer screening, without consideration of other factors that may alter PSA levels independently of the disease. Our previous work with a prostate cancer patient cohort from Auckland has shown that age-based PSA increase is restricted to those carrying the *aldo-keto reductase 1C3 (AKR1C3)* rs12529 G allele[1]. We have also recorded that NZ men carrying this allele and ever-tobacco smoking lifestyle show a delayed diagnosis of high-risk prostate cancer compared to US cohorts[2]. The current analyses is to understand the association of PSA with factors including this genotype in PSA outcomes in case and control cohorts from three geographical locations.

Methods

2781 men with prostate cancer and 1606 men without a cancer diagnosis, recruited for various studies in New Zealand (NZ), United States of America (US-EA - European Americans and US-AA - African Americans) and Taiwan (TW1-advanced, and TW2 - localised prostate cancer groups respectively) were considered in this analysis. Potential effects of demographic, lifestyle, clinical characteristics (for cases only), and the *aldo-keto reductase 1C3* (*AKR1C3*) rs12529 genetic polymorphisms on PSA level were considered in this evaluation. Prognostic stage criteria for cases were from D'Amico *et al* 1998[3] as recorded before[1&2].

Statistical analysis

- Analysis of continuous variables - Kruskal-Wallis One Way Analysis of Variance on Ranks test.
- Analysis of categorical variables - Chi Square test.
- Combined overall PSA data were right skewed, and were log transformed.
- Multiple linear regression analysis was carried out to test the association of PSA with ethnicity, BMI, *AKR1C3* rs12529 genotype, tobacco smoking status, alcohol consumption status, age at recruitment (for controls), age at diagnosis (for cases), disease prognostic stage (for cases), Gleason sum score (for cases) and the interaction effects.
- The Spearman Rank Order Correlation was used to analyse the correlation between age and log PSA for all cohorts with and without genetic stratification.
- Of the NZ patient cohort, only 17 (3.3%) are non-Caucasian. Therefore, non-Caucasian New Zealand patients were excluded from all analysis with ethnicity effect.

Results

Age and PSA (at diagnosis for cases and at recruitment for controls), BMI, proportion of men who are ever-smokers, Gleason sum score and prognostic stage groups (for cases) were significantly different between cohorts (Tables 1&2)

Table 1. Comparison of characteristics of prostate cancer cases from NZ, TW and US

	NZ (N=516)	TW1 (N=645)	TW2 (N=643)	US-AA (N=489)	US-EA (N=487)	P value
Demographic and lifestyle data						
Ethnicity	96.7% NZ European, 3.3% non-European*	100% Asian	100% Asian	100% US-African	100% US-European	
BMI (kg/m²) (median, inter quartile range, number)	27.0 (25.0, 30.0) [512]	24.2 (22.3, 26.2) [377]	24.7 (23.0, 26.4) [379]	27.7 (24.4, 31.2) [488]	27.5 (25.1, 30.3) [487]	<0.001
Ever-tobacco smoking (number and %)	287 (56)	NA	NA	353 (72)	299 (61)	< 0.00001
Ever-alcohol consumption (number and %)	366 (71)	NA	NA	418 (85)	437 (90)	< 0.00001
Clinical data						
Age (y) at diagnosis (median, inter quartile range, number)	66 (60, 71) [512]	73 (67, 78) [645]	66 (61,70) [643]	63 (57.3, 68) [488]	65 (60, 71) [490]	<0.001
PSA (ng/ml) at diagnosis (median, inter quartile range, number)	8.6 (5.8, 15.0) [468]	41.0 (15.7, 136) [622]	10.9 (7.0, 18.4) [622]	7.0 (5.2, 12.9) [484]	6.0 (4.6, 9.3) [481]	<0.001
Gleason sum score at diagnosis (median, inter quartile range, number)	7 (6, 7) [515]	7 (6, 8) [632]	7 (7, 7) [643]	7 (6, 7) [202]	6 (6,7) [232]	<0.001
Prognostic stage ≥IIB high-risk group (number and %)	262 (51)	553 (86)	184 (29)	133 (66)	126 (54)	

* Māori / Pacific/ Asian

Table 2. Characteristics of the controls study cohorts from NZ and US.

	NZ (N=572)	US-AA (N=486)	US-EA (N=548)	P value
BMI (kg/m²) (median, inter quartile range, number)	26 (24, 29) [547]	29 (26, 33) [486]	27.4 24.5, 31) [548]	<0.001
Ever-tobacco smoking (number and %)	195 (34)	302 (62)	324 (59)	<0.00001
Ever-alcohol consumption (number and %)	492 (86)	379 (78)	479 (84)	<0.0001
Age (y) at recruitment (median, inter quartile range, number)	54 (44, 64) [572]	64 (59, 69) [486]	66 (60,73) [548]	<0.001
PSA at recruitment (ng/ml) (median, inter quartile range, number)	0.9 (0.6, 1.9) [498]	0.4 0.2, 0.8) [412]	0.4 (0.2, 0.8) [476]	<0.001

NZ Māori/Pacific/Asian cases record similar *AKR1C3* rs12529 G allele frequencies (85-88%) to TW cases. All other cohorts recorded a G allele frequency between 38-45%.

Table 3. Comparison of the *AKR1C3* rs12529 genotype frequencies among cohorts.

	CC N (%)	CG N (%)	GG N (%)	G allele %	HW equilibrium statistics / P value
NZ European controls	181 (40)	202 (44)	71 (16)	38	1.36 P>0.05
NZ European cases	121 (33)	162 (44)	83 (23)	45	4.04 **P<0.05**
NZ Māori/Pacific & Asian cases	–	4 (31)	9 (69)	85	–
US-AA cases	58 (29)	105 (52)	39 (19)	45	0.48 P>0.05
US-EA cases	69 (30)	115 (49)	48 (21)	45	0 P>0.05
Taiwan cases-TW1	8 (1)	133 (22)	477 (77)	88	0.14 P>0.05
Taiwan cases-TW2	6 (1)	150 (23)	487 (76)	87	2.27 P>0.05

When data from all cases cohorts were considered together in multiple linear regression analysis, the factors significantly associated with PSA were ethnicity, prognostic stage, Gleason sum score, age at diagnosis, BMI and ever-smoking status (Table 4). Age at diagnosis interacting with ethnic group also had a significant association on PSA outcomes (P=0.007).

Table 4. Summary of multiple linear regression analysis for testing impacts on log PSA for cases cohorts considered together.

Parameter	Parameter Est. without lifestyle data	Pr > F	Parameter Est. with lifestyle data	Pr > F
Ethnicity (ref=European American)		<.0001		
African American	0.35		0.36	0.0002
NZ European	0.29		0.24	
Taiwanese TW1	1.69			
Taiwanese TW2	0.51			
Prognostic Stage (ref=<IIB)		<.0001		<.0001
>IIB	0.55		0.33	
Gleason sum score	0.18	<.0001	0.27	<.0001
Genotype (ref=CC)		0.6782		0.6527
CG	-0.02		-0.006	
GG	0.04		0.069	
Age at diagnosis	0.01	0.0004	0.01	0.003
BMI	-0.02	0.004	-0.01	0.136
Ever-smoker (ref=never smoker)	-	-	0.16	0.015
Alcohol consumer (ref= never alcohol consumer)	-	-	-0.07	0.3697
Model	R^2=0.396, Pr>F <0.0001		R^2=0.187, Pr>F <.0001	

When data from all cases cohorts were considered independently in multiple linear regression analysis, each cohort was presented with unique features significantly associated with PSA outcomes (Table 5).

Table 5. The association of log PSA with tested parameters in cases cohorts analysed independently.

	US-EA		US-AA		NZ-European		TW1		TW2	
Parameter	Estimate	Pr > F	Estimate	Pr > F	Estimate	Pr > F	Estimate	Pr > F	Estimate	Pr > F
Prognostic Stage >IIB high-risk group (ref=<IIB)	0.13	0.310	0.22	0.179	0.62	<.0001	1.79	<.0001	0.35	0.0003
Gleason sum score	0.26	0.001	0.62	<.0001	0.07	0.208	0.08	0.098	0.12	0.006
Genotype (ref=CC) for all except TW (ref=CG)		0.361		0.720		0.778				
CG	0.08		-0.047		-0.07		0.00	0.117	0.00	0.666
GG	0.024		0.105		-0.01		-2.35		0.44	
Age at diagnosis	0.01	0.491	0.02	0.073	0.02	0.0003	-0.02	0.894	0.01	0.186
BMI	-0.01	0.587	-0.04	0.012	0.01	0.259	-0.06	0.006	0.01	0.659
Ever-smoker (ref=never smoker)	0.16	0.697	0.13	0.697	0.22	0.018				
Alcohol consumer (ref= never alcohol consumer)	-0.07	0.595	0.21	0.325	-0.15	0.139				
Model	R^2=0.08, Pr>F <0.013		R^2=0.336, Pr>F <0.0001		R^2=0.206, Pr>F <0.0001		R^2=0.23, Pr>F <0.0001		R^2=0.111, Pr>F <0.0001	

When data from all controls cohorts were considered together in multiple linear regression analysis, the factors significantly associated with PSA were ethnicity (P<0.0001), age at recruitment (P<0.0001), BMI (P=0.0002) and ever-smoking status compared to never-smoking (P=0.036). When data from all controls cohorts were considered independently in multiple linear regression analysis, each cohort was presented with unique features significantly associated with PSA outcomes (Table 6).

Table 6. The association of log PSA with tested parameters in controls cohorts analysed independently.

	European American		African American		NZ European	
Parameter	Parameter Est.	Pr > F	Parameter Est.	Pr > F	Parameter Est.	Pr > F
Age	0.03	<.0001	0.05	<.0001	0.03	<.0001
BMI	-0.02	0.048	-0.03	0.008	-0.01	0.2039
Ever-smoker (ref=never- smoker)	-0.18	0.072	-0.25	0.034	0.04	0.6255
Ever-alcohol consumer (ref= never-alcohol consumer)	0.13	0.389	0.09	0.4812	-0.19	0.0785
Model	R^2=0.064, Pr>F <0.0001		R^2=0.13, Pr>F <0.0001		R^2= 0.282, Pr>F <0.0001	

Univariate analysis showed that NZ, US-AA and US-EA controls have an overall PSA correlation with age. This correlation remained significant despite stratification of NZ controls by the *AKR1C3 rs12529* genotypes. All cases except for the US-EA cases also showed a significant correlation between age and PSA with univariate analyses. However upon stratification, significant correlation was seen among cases carrying the GG genotype for NZ, TW1 and TW2 cases, CG genotype for NZ and US-AA cases and CC genotype for the US-AA cases.

Table 8. Spearman correlation statistics between age (age at diagnosis for cases and age at recruitment for controls) and log PSA stratified by ethnicity, case, control status and the *AKR1C3* rs12529 genotype.

		All	CC	CG	GG
NZ controls	r	0.556	0.517	0.519	0.616
	p	**2E-07**	**2E-07**	**2E-07**	**2.3E-09**
	n	498	181	202	71
NZ cases	r	0.303	0.129	0.287	0.426
	p	**6.7E-011**	0.160	**2.3E-04**	**7.4E-05**
	n	449	120	161	82
US-AA controls	r	0.344			
	p	**1.1E-12**			
	n	410			
US-AA cases	r	0.243	0.312	0.239	0.153
	p	**5.0E-04**	**0.017**	**0.014**	0.349
	n	202	58	105	39
US-EA controls	r	0.213			
	p	**2.9E-06**			
	n	475			
US-EA cases	r	0.0244	0.113	-0.063	0.110
	p	0.711	0.352	0.504	0.457
	n	232	69	115	48
TW1 cases	r	0.119	0.500	-0.00108	0.140
	p	**0.003**	0.182	0.990	**0.002**
	n	622	8	133	477
TW2 cases	r	0.113	0.0286	0.103	0.121
	p	**0.005**	1.000	0.217	**0.008**
	n	622	6	144	472

r= correlation coefficient; p = significance of probability; n= number of pairs tested

Discussion

The well-known PSA association with age (age at diagnosis for cases and age at recruitment for controls) was reproduced in pooled cohort analyses with multiple linear regression in both cases and controls. However, upon analyses of independent cases cohorts, this was reproduced only among NZ-European cases. Among controls, PSA was significantly associated with age in all tested cohorts with independent as well as pooled multiple regression analyses as well as in univariate analyses. This indicates that changes have taken place impacting general PSA increase with age upon cancer presentation in some cohorts. Association of PSA with BMI and tobacco smoking, even at the expense of age in US cohorts could be indicating a changing paradigm of parameters associated with PSA since this test was established for prostate cancer screening in 1994. Genetic impacts on age and PSA correlation recorded with univariate analysis may have value in establishing stratified PSA thresholds. Overall, our data suggests an insufficiency of universal age-based PSA thresholds for prostate cancer screening. This also suggests that PSA thresholds for prostate cancer screening need refreshing in different ethnicities, in different geographical locations, at different time points for its better utility. However, it is too early to know whether the current findings on variable factors affecting PSA outcomes in different cohorts are unique only to the current cohorts assessed or whether they can be generalised to these ethnicities from different geographical locations. Current findings require further validation with extended cohorts that will provide better statistical power for stratified analyses based on genotype, BMI and lifestyle factors. We have plans to carryout NZ-wide extended studies to assess the reproducibility of the current findings, and we welcome you to join us in our endeavour.

References

1. Karunasinghe N, Symes E, Gamage A *et al.* (2019) *PLoS ONE* 14:e0217373
2. Karunasinghe N, Ambs S, Wang A *et al.* (2018) *PLoS ONE* **13**, e0199122.
3. D'Amico et al (1998) *JAMA* 280:969-974

Acknowledgements

Stefan Ambs, from the Laboratory of Human Carcinogenesis, National Cancer Institute, NIH, Bethesda, USA is sincerely acknowledged for his support in organising the US prostate cancer cases and controls data for this analysis.