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Original Paper

Prostate Disease Risk Factors among a New Zealand Cohort

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Key Words
Prostate disease · Smoking · Selenium · Age · Single nucleotide polymorphisms · Glutathione peroxidase activity · Thioredoxin reductase activity

Abstract
Background: Prostate cancer is a leading public health burden worldwide, and in New Zealand it is the most commonly registered cancer and the third leading cause of cancer deaths among males. Genetic variability and its associations with diet, demographic and lifestyle factors could influence the risk of this disease. ***Methods:*** The single nucleotide polymorphisms (SNPs) within a group of antioxidant genes and related markers were tested between patient and control cohorts, adjusted for significant differences between basic lifestyle and demographic characteristics. ***Results:*** Increasing age, smoking and low serum selenium levels were significantly associated with an increased risk for prostate disease. Alcohol consumption increased the glutathione peroxidase (GPx) activity. A significant reduction in alcohol consumption was recorded with prostate disease. Three SNPs, namely *GPx1* rs1050450, *SEL15* rs5845 and *CAT* rs1001179, were significantly associated with prostate disease risk. A cumulative risk of prostate cancer was noted with 6 risk alleles. A lower GPx activity was recorded with prostate disease compared to the controls. However, the *GPx1* rs1050450 allele T in association with prostate cancer recorded a significantly higher GPx activity compared to the controls. ***Conclusions:*** These data point to a possibility of identifying individuals at risk of prostate cancer for better management purposes.

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Introduction

Prostate cancer is a leading public health burden worldwide, and in New Zealand it is the most commonly registered cancer among males, making up 28% of all male cancer registrations, and the third leading cause of cancer deaths among males [1]. Some hereditary risk loci have been linked to the disease [2–7]; however, they are not as aggressive or as predictive as the breast cancer type 1 susceptibility protein *(BRCA1)* or the adenomatous polyposis coli gene *(APC)* in colon cancer. Due to its high prevalence, long latency, hormonal dependency and the ability to monitor with markers such as PSA, prostate cancer is a good candidate for studies on primary prevention [8]. Dietary modification of the disease including disease initiation, progression and severity has also been reported [9–11].

The well-known Nutritional Prevention of Cancer study showed beneficial effects of 200 μg/day selenium (Se) as selenised yeast against several cancers, including prostate cancer, in a group of US men [9]. A meta-analysis of 11 cohort studies and 5 case-control studies carried out by Etminan et al. [12] indicated a decrease in the pooled relative risk of prostate cancer among those with moderate Se intakes compared with those with low intakes. However, a large study with over 32,000 US men failed to show beneficial effects of 200 μg/day Se supplemented as selenomethionine [13]. High baseline serum Se levels and the use of selenomethionine instead of selenised yeast are considered the major causes of this discrepancy, that may have caused a lack of cancer protection/reduction effects. Among the other possible reasons could be the degree of genetic variability among different cohorts analysed. The prostate disease risk variability among selenogenome in humans, with a range of functional differences, is thus worth investigating. The *GPx1* rs1050450 C>T single nucleotide polymorphism (SNP) occurs within exon 1 of the *GPx1* gene and forms structurally different subunits containing either prolein or leucine at codon 198 [14]. Located in the 3′ UTR near the selenocysteine (Sec) insertion sequence element, the *GPx4* rs713041 C>T polymorphism can modulate GPx4 activity by altering Sec insertion and the protein binding to the 3′ UTR [15]. Selenoprotein P is presented in two isoforms in human plasma where the production of the Se-rich 60-kDa isoform is favoured by the major allele G of *SEPP1* rs3877899 while allele A favours the 50-kDa isoform [16, 17]. This is probably an evolutionary adaptation for survival in low Se environments. The *SEL15* gene is involved in protein folding pathways, and differential Se incorporation among *SEL15* rs5845 C and T alleles has been reported [18]. According to Curran et al. [19], the G allele of the *SELS* rs28665122 SNP is required to produce sufficient promoter activity in the presence of a stress stimulator, whereas the A allele shows significantly lower activity. It therefore seemed appropriate to look at the prostate cancer risk modulation with the level of serum Se, body mass index (BMI), smoking status and alcohol consumption. This analysis further investigates the effect of SNPs related to Se metabolism and antioxidant defence in modulating prostate disease risk as well as the effects on activity levels of two major antioxidant selenoenzymes – glutathione peroxidase (GPx) and thioredoxin reductase (TR) – among patient and control cohorts. The Se metabolism-related SNPs considered here are *GPx1* rs1050450, *GPx4* rs713041, *SEPP1* rs3877899, *SEL15* rs5845, *SELS* rs28665122 and *SELS* rs4965373. Among the other antioxidant pathway-related SNPs considered here are catalase *(CAT)* rs1001179 and manganese superoxide dismutase *SOD2* rs4880.

Methods

Study Population

Prostate cancer patient recruitment was carried out with the informed consent of the patients, and the recruitment took place through the Department of Urology, Auckland Hospital, covering Auckland, North Shore and Manukau centres (ethics ref. NTY/05/06/037). The patients identified from the registry of the Department

of Urology were sent an invitation of participation for the study. Approximately 25% of the patients responded to the invitation. Similarly, patients with benign prostate hyperplasia and with negative biopsies for prostate cancer were also recruited with their informed consent. The control subjects were from the general public in the Auckland region, who responded to public advertisements made through media, New Zealand Blood Services and e-mails circulated through the University of Auckland. Participants who have self-reported that they do not carry urological problems or have no history of cancers except for skin cancers are considered here as the control group. They are part of the volunteers recruited with informed consent for the Se supplementation trial carried out by the Discipline of Nutrition, University of Auckland (ethics ref. NTY/06/07/060).

Our patient and control database consisted of 868 subjects within the age range of 40–81 years. A total of 838 (96.5%) of them self-reported a European ancestry. Additionally, the database included 11 patients (1.3%) reporting a Maori and Pacific ethnicity, 6 patients (0.7%) of Asian and 13 (1.5%) of Indian/Middle Eastern ethnicities. According to a meta-analysis performed by Chen et al. [20], Asian populations show a significantly lower mean frequency for minor allele T of *GPx1* rs1050450 (0.06) compared to Europeans (0.30). The Asian population in our database had a zero T allele frequency, while the Maori and Pacific populations showed a frequency of 0.04, similar to that of Asian populations reported by Chen et al. [20]. The Indian and Middle Eastern group had a T allele frequency of 0.25, which is within the range reported for Europeans [20]. Therefore, the current subject selection for the analysis was based on the *GPx1* rs1050450 T allele frequency distribution (similar to that of Europeans reported by Chen et al.) and included subjects self-reporting as Indian sub-continent and Middle Eastern origins, along with those reporting a European origin. A total of 851subjects, 275 with malignant disease, 135 with benign prostate disease and 441 healthy controls, are included in this study.

Collection of Demographic and Lifestyle Data

The heights and weights of the patients were measured during their clinical appointments, while the same was done with the control population at their recruitment to the Se supplementation study. Both patients and controls were asked to complete a lifestyle questionnaire that was used to collect information on tobacco smoking habits and alcohol intake.

Blood Collection and Processing

At the entry to the study, blood samples from each volunteer were collected in each of an EDTA, heparin and plain Vacutainer® tube from Becton Dickinson. An aliquot of the EDTA sample was subsequently used for DNA extraction. Total genomic DNA was extracted from blood with the QIAamp DNA Blood Mini Kit (Qiagen) according to the manufacturer's instructions, using a fully automated procedure on the QIAcube.

SNP Genotyping

The TaqMan® SNP Genotyping Assay from Applied Biosystems was used for the SNP genotyping of the panel of genes selected in this study. The assays were obtained either pre-designed from Applied Biosystems or custom-made through Assay-by-Design service by ABI. TaqMan assays were optimized with 24 samples consisting of 20 reference Centre d'Etude du Polymorphisms Humain (CEPH) samples with known genotypes from the HapMap project [21] and 4 blanks. A total of 8 HapMap controls were also included with each reaction plate. The call rate for each SNP genotype determination was over 95%. The reactions were prepared using 2× TaqMan Universal Master Mix, 40× SNP Genotyping Assay Mix, DNase-free water and 10 ng genomic DNA in a final volume of 5 μl per reaction. The PCR amplification was performed using the ABI Prism 7900 HT Fast Real-Time PCR sequence detector machine under the following conditions for both test samples and HapMap controls: 10 min 95°C enzyme activation followed by 40 cycles at 92°C for 15 s and 60°C for 1 min (annealing/extension). The allelic discrimination results were determined after the amplification by performing an end point reading.

Serum Se

The blood collected in the plain Vacutainer tube was spun at 2,000 *g* for 10 min at 4°C to separate the serum. Serum Se levels were assayed at Gribbles Veterinary Pathology, Hamilton, using a modified semi-automated fluorometric assay based on methods reported by Rongpu et al. [22], Watkinson [23] and Watkinson and Brown [24]. The fluorescence of the final benzopiazselenol extracted into cyclohexane was measured with an excitation wave length of 360 nm and an emission wave length of 518 nm. A pooled human control serum sample was administered with each batch of samples tested for Se levels. Se measurement on this sample showed an interassay coefficient of variation of 9.9%.

Selenoenzyme Activities

GPx and TR activities were measured in a subset of samples following procedures given in Karunasinghe et al. [25]. Briefly, erythrocyte lysates were prepared from 100 µl aliquots of EDTA blood, as described for the Calbiochem Cellular glutathione peroxidase assay kit (catalog No. 354104). The conversion of the haemolysates to cyanomethemoglobin using a transformation solution was also carried out before the enzyme activities (both GPx and TR) were measured. GPx activity was assayed using the protocol of Wendel [26], modified to suit a 96-well plate format, and the samples were assayed in duplicate. The spectrophotometric measurements were carried out for 4 min at 366 nm using the kinetic protocol on a Multiskan spectrophotometer (Thermo Scientific). For GPx activity assay, each well contained an equivalent of 250 mg haemoglobin. One unit of GPx activity is defined as 1 mmol NADPH oxidized per minute at 37°C. A standard solution of glutathione peroxidase from bovine erythrocytes (Sigma catalog No. G6137), diluted 400-fold according to the manufacturer's instructions, was used as a positive control to assure the reaction was working and to estimate inter- and intra-experiment variation. The inter- and intra-assay coefficients of variation were 8.62 and 3.16%, respectively. TR activity was assayed using the protocol of Smith and Levander [27], with 20 mmol of aurothiomalate (Sigma Aldrich 157201) instead of aurothioglucose as the suppressor of TR for the measurement of non-TR related-activities. Each well contained an equivalent of 125 mg haemoglobin, and the samples were assayed in duplicate. Data collection was commenced 1 min after the initiation of the reaction to allow for a non-enzymatic reduction of 5,5′-dithiobis(2-nitrobenzoic acid) to go to completion. One unit of TR activity is defined as 1 mmol 5-thio-2-nitrobenzoic acid formed per minute at 37°C. All spectrophotometric measurements were carried out for 4 min at 412 nm using the kinetic protocol on a Multiskan spectrophotometer (Thermo Scientific). Rat recombinant TR from IMCO, Sweden (catalog No. TR03), diluted 100-fold in milliQ water, was used as a positive control to ensure the reaction was working and to estimate inter- and intra-experiment variation. The inter- and intra-assay coefficients of variation were 5.11 and 4.88%, respectively.

Statistical Analysis

Since the serum Se concentrations were the log-transformed values, the estimated actual values were determined by utilizing the exponential (anti-log) function and will be hereafter referred to as serum Se level. The differences among the confounding variables, including age, BMI, alcohol consumption, smoking status and serum Se levels, were tested between the prostate cancer patient group, patients with benign urology disease and/or controls (table 1). These confounding variables, with the exception of BMI (as the BMI was not significantly different among these three groups (table 1), were adjusted for the SNP analyses. There was no significant difference between the odds ratios (ORs) from current and former smokers on either malignant ($p = 0.7364$) or benign ($p = 0.3531$) disease compared to controls. Therefore, these two groups were combined and considered as ever-smokers for further SNP analysis. SNPs were analysed using the additive model (count of the number of the tested allele: 0, 1 and 2). The assessment of the risk of incident prostate cancer association with SNPs was carried out by a logistic regression model with adjustments for the confounding variables. The genetic risk score (GRS) was calculated using 7 of the candidate SNPs studied in this study. The missing genotype rate of *SELS* rs28665122 was 8.7%; therefore, it was excluded from the GRS construction. The GRS ranged from 0 to 14 on the basis of the number of tested alleles. All analyses were carried out using R statistical software [28] and SAS (V9.2 SAS Institute, Cary, N.C., USA).

Results

Demographic characteristics of the study group and their associations with prostate disease are given in table 1. Tobacco smoking was found to be a lifestyle factor significantly related to an increased risk of prostate cancer (OR = 1.79) when patient lifestyle factors were compared to those of healthy controls ($p = 0.0065$). The reported alcohol consumption produced a significant risk reduction in both the benign (OR = 0.48, p = 4.83E-06) and malignant (OR = 0.37, p = 8.84E-04) disease groups compared to controls, while alcohol intake showed a marginal non-significant increase in BMI across all subjects (data not shown). However, no significant increase in prostate cancer or benign prostate disease risk was observed with BMI ($p = 0.534$ and $p = 0.916$, respectively). We also observed individuals with

Table 1. Demographic and lifestyle characteristics of the study group and their association with prostate disease

	Malignant (n = 275, 32.3%)	Benign (n = 135, 15.9%)	Healthy controls (n = 441, 51.8%)
Current alcohol consumption			
No, n (%)	75 (46.0)	31 (19.0)	57 (35.0)
Yes, n (%)	189 (28.1)	100 (14.9)	384 (57.0)
OR (95% CI)	0.37 (0.25–0.55)	0.48 (0.29–0.78)	1.00
p value	8.84E-04	4.83E-06	
Past or current smoking status*			
No, n (%)	127 (26.9)	73 (15.4)	273 (57.7)
Yes, n (%)	140 (37.9)	61 (16.5)	168 (45.5)
OR (95% CI)	1.79 (1.32–2.44)	1.36 (0.92–2.01)	1.00
p value	0.0065	0.9395	
Se level, ng/ml			
Mean ± SE	101.2±1.01	100.7±1.02	112.9±1.01
Range	52.1–206.9	62.0–165.8	63.2–304.8
Estimate (95% CI)	0.90 (0.87–0.92)	0.89 (0.86–0.93)	1.00
p value	2.49E-12	4.27E-09	
BMI			
Mean ± SE	27.2±0.25	27.0±0.36	27.0±0.19
Range	16.7–40.8	16.8–39.1	17.2–44.8
Estimate (95% CI)	0.20 (–0.41–0.81)	–0.04 (–0.83–0.74)	0
p value	0.5335	0.9163	
Age at recruitment, years			
Mean ± SE	66.3±0.48	66.6±0.60	57.4±0.49
Range	45.4–80.9†	41.7–80.9†	40.0–81.0
p value	<0.0001		

* Current (n = 34) and past (n = 335) smokers were combined due to no significant difference in prostate disease risk between the two groups (p = 0.4941).

† The mean difference between the age at recruitment and the date of diagnosis with the disease was 0.31 years (SD = 0.72).

either benign or malignant disease having a significantly (p = 4.27E-09 and p = 2.49E-12, respectively) lower serum Se level compared to controls (table 1).

Although the patient and control cohorts were selected according to an age range of 40–81 years, the controls were significantly younger than the patients (p < 0.001) (table 1). Therefore, we adjusted the data for age in the subsequent analyses along with the adjustments for other confounding variables.

Association of Confounding Variables and Antioxidant Enzyme Levels

Alcohol consumption was positively and significantly (p = 0.002) associated with GPx activity in the overall study cohort, although age, BMI and smoking status showed no such association (table 2).

Association of Health Status and Antioxidant Enzyme Levels

Patients with malignant and benign urology diseases showed a significantly lower level of GPx activity compared to controls (p = 0.033 and p = 0.011, respectively) before data were adjusted for alcohol consumption. This association remained significant among those with benign urology disease after the data were adjusted for alcohol consumption (p = 0.027) (table 3).

Table 2. Variation of selenoenzyme activities with demographic and lifestyle factors in the overall study group

	GPx activity		TR activity	
	estimate (95% CI)	p	estimate (95% CI)	p
Age	0.033 (–0.017 to 0.082)	0.194	0.003 (–0.003 to 0.009)	0.293
BMI	0.092 (–0.048 to 0.232)	0.197	0.003 (–0.014 to 0.019)	0.735
Current alcohol consumption				
Yes	2.16 (0.79 to 3.52)	0.0019	0.056 (–0.103 to 0.215)	0.490
No	0		0	
Ever smoked				
Yes	–0.21 (–1.27 to 0.85)	0.693	0.037 (–0.085 to 0.160)	0.554
No	0			

Table 3. Variation of selenoenzyme activities in a subset of subjects with malignant and benign urology diseases compared to controls

		Mean ± SE	Before adjustment		After adjustment*	
			estimate (95% CI)	p	estimate (95% CI)	p
GPx	Benign (n = 86)	13.0 ± 0.61	–0.011 (–3.609 to –1.475)	0.0107	–1.180 (–3.352 to –0.207)	0.0266
	Malignant (n = 136)	13.6 ± 0.48	–0.033 (–2.722 to –0.112)	0.0334	–1.133 (–2.450 to 0.184)	0.0916
	Control (n = 431)	15.0 ± 0.35	0.00		0.00	
TR	Benign (n = 84)	1.12 ± 0.09	0.576 (–0.131 to 0.236)	0.5763		
	Malignant (n = 132)	1.12 ± 0.06	0.037 (–0.117 to 0.190)	0.6389		
	Control (n = 427)	1.08 ± 0.04	0.00			

* Adjustments were made for alcohol consumption.

Se and Anti-Oxidant Metabolism-Related Genes

The SNP genotype frequencies recorded among malignant and benign patients and healthy controls are given in table 4, and the risk associations between SNP genotypes are given in table 5. Individuals with *GPx1* rs1050450 minor allele T had a significantly higher prostate cancer risk as compared to controls (OR = 1.38, p = 0.02) after adjustment for the confounding factors. The risk of malignant disease was also significant compared to benign disease among those carrying the *CAT* rs1001179 minor allele T before (OR = 1.55, p = 0.022) and after (OR = 1.61, p = 0.015) adjustment for the confounding variables. Individuals with the minor allele T of the *SEP15* rs5845 were at significant risk of having benign disease compared to controls (OR = 1.77, p = 0.0003), and this remained significant after adjustment for the confounding factors (OR = 1.98, p = 0.0001). Those with this allele also showed a lower risk of malignancies compared to benign disease (OR = 0.63, p = 0.006), and this remained significant after adjustment for the confounding variables (OR = 0.62, p = 0.005). The estimated GRS indicated a significantly increased risk of malignancies due to the cumulative effect of the tested alleles compared to controls (OR = 1.16, p = 0.0074) (table 5).

Table 4. Frequency of SNPs associated with Se and other anti-oxidant metabolisms assessed by patient and control subjects

Gene	SNP	Genotype	Malignant	Benign	Controls
SELS	rs28665122	A/A	4 (1.7)	4 (3.2)	7 (1.7)
		A/G	57 (24.6)	28 (22.1)	102 (24.4)
		G/G	171 (73.7)	95 (74.8)	309 (73.9)
SELS	rs4965373	A/A	29 (11.2)	14 (11.1)	44 (10.0)
		A/G	116 (44.6)	53 (42.1)	186 (42.4)
		G/G	115 (44.2)	59 (46.8)	209 (47.6)
SEPP1	rs3877899	A/A	18 (7.0)	9 (6.8)	19 (4.4)
		A/G	88 (34.0)	46 (34.9)	162 (37.2)
		G/G	153 (59.1)	77 (58.3)	255 (58.5)
SEP15	rs5845	C/C	160 (62.0)	67 (49.6)	270 (62.8)
		C/T	84 (32.6)	52 (38.5)	145 (33.7)
		T/T	14 (5.4)	16 (11.9)	15 (3.5)
GPx1	rs1050450	C/C	122 (46.6)	62 (46.3)	216 (49.7)
		C/T	110 (42.0)	60 (44.8)	186 (42.8)
		T/T	30 (11.5)	12 (9.0)	33 (7.6)
GPx4	rs713041	C/C	84 (32.3)	44 (32.8)	144 (32.8)
		C/T	129 (49.6)	68 (50.8)	210 (47.8)
		T/T	47 (18.1)	22 (16.4)	85 (19.4)
CAT	rs1001179	C/C	144 (55.8)	92 (69.2)	258 (59.5)
		C/T	99 (38.4)	35 (26.3)	160 (36.9)
		T/T	15 (5.8)	6 (4.5)	16 (3.7)
SOD2	rs4880	C/C	70 (26.8)	32 (23.9)	108 (24.6)
		C/T	138 (52.9)	71 (53.0)	222 (50.5)
		T/T	53 (20.3)	31 (23.1)	110 (25.0)

Association of Health Status and Gene Polymorphisms with Activity of Selenoenzymes

The *GPx1* rs1050450 T allele showed a significantly higher GPx activity among those with prostate cancer compared to controls (OR = 2.14, p = 0.034 before adjustment and OR = 2.13, p = 0.034 after adjustment) (table 6). None of the other gene polymorphisms in association with health status showed any effect on GPx activity. There was also no significant effect on TR activity as a consequence of the polymorphisms studied in association with health status.

Discussion

A recent review has found that age, race and family history of prostate cancer are established variables in the consideration of prostate cancer risks, while behavioural and lifestyle factors have only weak associations [29]. However, our evaluations have shown that smoking is a lifestyle factor that shows an association with prostate cancer in the current study group. This could be related to tobacco smoke being an established carcinogen that could cause many human cancers [30, 31].

The reported alcohol intake showed a significant association with a reduced risk of prostate disease. The lower alcohol intake among those with prostate disease could also be a lifestyle adaptation since diagnosis. A meta-analysis covering over 50 published studies has shown no evidence of an increased prostate cancer risk, even with the highest doses of alcohol intake [32].

Table 5. Prostate cancer and benign urology disease risk association among the subjects based on Se and anti-oxidant pathway-related genes

Gene	SNP	Status	Tested allele	Tested allele frequency	Before adjustment		After adjustment	
					OR (95% CI)	p	OR (95% CI)	p
Malignant vs. control								
SELS	rs28665122	Malignant	G	0.860	0.99 (0.71–1.38)	0.9465	1.00 (0.69–1.45)	0.9987
		Control		0.861	1.00		1.00	
SELS	rs4965373	Malignant	A	0.335	1.11 (0.88–1.40)	0.3848	1.14 (0.88–1.49)	0.3129
		Control		0.312	1.00		1.00	
SEPP1	rs3877899	Malignant	A	0.239	1.06 (0.82–1.37)	0.6682	1.15 (0.86–1.54)	0.3396
		Control		0.229	1.00		1.00	
SEP15	rs5845	Malignant	T	0.217	1.09 (0.83–1.42)	0.5472	1.23 (0.91–1.67)	0.1736
		Control		0.203	1.00		1.00	
GPx1	rs1050450	Malignant	T	0.324	1.18 (0.93–1.49)	0.1703	1.38 (1.05–1.80)	0.0196
		Control		0.290	1.00		1.00	
GPx4	rs713041	Malignant	C	0.571	1.02 (0.82–1.26)	0.8859	0.98 (0.77–1.25)	0.8866
		Control		0.567	1.00		1.00	
CAT	rs1001179	Malignant	T	0.250	1.18 (0.91–1.54)	0.2082	1.29 (0.97–1.74)	0.0853
		Control		0.221	1.00		1.00	
SOD2	rs4880	Malignant	C	0.533	1.16 (0.93–1.44)	0.2012	1.11 (0.87–1.42)	0.3956
		Control		0.498	1.00		1.00	
GRS based on 7 SNPs (excluding rs28665122)					1.10 (1.00–1.20)	0.0558	1.16 (1.04–1.29)	0.0074
Benign vs. control								
SELS	rs28665122	Benign	G	0.858	0.98 (0.65–1.46)	0.9044	1.00 (0.64–1.57)	0.9957
		Control		0.861	1.00		1.00	
SELS	rs4965373	Benign	A	0.321	1.04 (0.78–1.41)	0.7799	1.04 (0.75–1.45)	0.8032
		Control		0.312	1.00		1.00	
SEPP1	rs3877899	Benign	A	0.242	1.08 (0.78–1.49)	0.6554	1.08 (0.75–1.55)	0.6841
		Control		0.229	1.00		1.00	
SEP15	rs5845	Benign	T	0.311	1.77 (1.30–2.41)	0.0003	1.98 (1.40–2.81)	0.0001
		Control		0.203	1.00		1.00	
GPx1	rs1050450	Benign	T	0.313	1.13 (0.83–1.52)	0.4469	1.30 (0.92–1.84)	0.1342
		Control		0.290	1.00		1.00	
GPx4	rs713041	Benign	C	0.582	1.06 (0.81–1.40)	0.6680	1.07 (0.79–1.46)	0.6633
		Control		0.567	1.00		1.00	
CAT	rs1001179	Benign	T	0.177	0.75 (0.52–1.07)	0.1144	0.74 (0.50–1.09)	0.1285
		Control		0.221	1.00		1.00	
SOD2	rs4880	Benign	C	0.504	1.03 (0.78–1.35)	0.8619	0.97 (0.72–1.32)	0.8620
		Control		0.498	1.00		1.00	
GRS based on 7 SNPs (excluding rs28665122)					1.07 (0.94–1.22)	0.2894	1.07 (0.94–1.22)	0.2894
Malignant vs. benign								
SELS	rs28665122	Malignant	G	0.860	1.01 (0.66–1.57)	0.9521	0.90 (0.58–1.42)	0.6617
		Benign		0.858	1.00		1.00	
SELS	rs4965373	Malignant	A	0.335	1.06 (0.77–1.46)	0.7163	1.09 (0.79–1.51)	0.6023
		Benign		0.321	1.00		1.00	
SEPP1	rs3877899	Malignant	A	0.239	0.98 (0.70–1.38)	0.9271	1.00 (0.71–1.41)	0.9961
		Benign		0.242	1.00		1.00	
SEP15	rs5845	Malignant	T	0.217	0.63 (0.46–0.88)	0.0057	0.62 (0.44–0.87)	0.0053
		Benign		0.311	1.00		1.00	
GPx1	rs1050450	Malignant	T	0.324	1.05 (0.77–1.44)	0.7555	1.07 (0.77–1.47)	0.6944
		Benign		0.313	1.00		1.00	
GPx4	rs713041	Malignant	C	0.571	0.96 (0.71–1.29)	0.7663	0.99 (0.72–1.35)	0.9330
		Benign		0.582	1.00		1.00	
CAT	rs1001179	Malignant	T	0.250	1.55 (1.06–2.25)	0.0222	1.61 (1.10–2.36)	0.0150
		Benign		0.177	1.00		1.00	
SOD2	rs4880	Malignant	C	0.533	1.13 (0.83–1.53)	0.4283	1.12 (0.82–1.53)	0.4662
		Benign		0.504	1.00		1.00	
GRS based on 7 SNPs (excluding rs28665122)					1.02 (0.89–1.17)	0.0558	1.05 (0.91–1.20)	0.5313

Table 6. Association of tested alleles of *GPx1* rs1050450 and disease condition with the activity level of selenoenzymes GPx and TR

	No. of T allele rs1050450	n	Mean ± SE	Before adjustment		After adjustment*	
				estimate (95% CI)	p	estimate (95% CI)	p
Benign	0	41	13.26±0.76	0.81 (–1.52 to 3.14)	0.4949	1.08 (–1.25 to 3.40)	0.3622
	1	34	12.67±1.14				
	2	10	12.55±1.71				
Malignant	0	62	13.14±0.64	2.14 (0.16 to 4.11)	0.0343	2.13 (0.16 to 4.10)	0.0340
	1	58	14.02±0.70				
	2	15	14.95±1.87				
Control	0	213	15.77±0.51	0.0		0.0	
	1	181	14.43±0.52				
	2	32	13.46±1.26				

Alleles of other genotypes were not significantly associated with the disease condition and GPx activity, while no associations were recorded with TR activity.

* Adjustments were made for alcohol consumption.

Serum Se levels were lower in both the malignant and benign prostate disease groups as compared with controls. Although there is controversy over the effect of Se supplementation as a prostate cancer preventative [9, 13], our study has shown a significant difference in serum Se level among both prostate cancer patients (101.2 ng/ml) and those with benign prostate disease (100.7 ng/ml) compared to controls (112.9 ng/ml). According to Meyer et al. [33], both serum Se and selenoprotein levels are lower among those with prostate cancer, while Steinbrecher et al. [34] have shown that the OR for prostate cancer is 0.89 for each 10-μg/l increment in serum Se. According to Marshall et al. [35], Se supplementation for those with high-grade prostatic intraepithelial neoplasia by way of 200 μg/day selenomethionine for 3 years has produced a non-significant decrease in prostate cancer incidence only among those in the lowest tertile of plasma Se equivalent to 106 ng/ml. We have previously shown that DNA damage, a possible precursor for cancer, showed an inverse relationship with increasing serum Se level up to a level of 100 ng/ml. Beyond this level, there was no correlation between serum Se and DNA damage in a group of men at high risk for prostate cancer [36]. A recent study by our group has also shown benefits on DNA integrity between serum Se levels of 116 and 149 ng/ml, varying with genotype, among a healthy male population [37]. Serum Se levels have been shown to positively correlate to prostate tissue Se levels [38], and therefore serum Se levels could indicate the 'seleno-nutrient health' in prostate tissue. Serum Se level is an indicator of red blood cell GPx and TR activities that are well known for their antioxidant potential [37]. Therefore, the lower levels of serum Se reported with prostate disease indicate that the disease condition accompanies oxidative stress. The current data on GPx activity levels also indicate that generally both prostate cancer patients and those with benign urology disease have significantly lower levels of GPx activity as compared to controls. This is similar to the observations made by Arsova-Sarafinovska et al. [39], indicating an increased oxidative stress in both groups. However, whether the decrease in serum Se and the accompanying oxidative stress is due to or a cause of the condition is not fully known.

Both increased and decreased levels of GPx activity with alcohol consumption have been previously reported [40–42], while the current evaluation indicates an increased GPx activity with alcohol consumption. It is possible that the increased GPx activity level recorded here

among alcohol consumers is an adaptation to overcome the possible oxidative stress associated with this lifestyle, as suggested by Apte et al. [43].

The current study indicates that the *GPx1* rs1050450 minor allele T carries a significant risk for prostate cancer. This allele was also associated with an increased GPx activity among those with prostate cancer. We have previously recorded that the GPx activity has no correlation with serum Se levels among those carrying the variant *GPx1* rs1050450 allele [37]. It is possible that the increased GPx activity among *GPx1* rs1050450 allele T is a feature dependent on the prostate cancer pathway. A meta-analysis carried out in 14,372 cases with different tumour types and 18,081 controls derived from 31 published case-control studies has indicated that individuals with *GPx1* rs1050450 CT and TT genotypes are at high risk for overall cancer incidence [20].

The current study has produced evidence that the *CAT* rs1001179 minor T allele is associated with a significant increase in prostate cancer risk as compared to benign prostate disease, before and after adjusting for confounding factors. Studies have indicated that the *CAT* rs1001179 T allele is associated with lower catalase activity [44–46]. Here again the T allele, with lower catalase activity, could be related to increased oxidative stress and instability in the cellular environment, leading to lesions and tumours. Ahn et al. [44] have reported that the difference in catalase activity between CC and CT+TT genotypes are further distanced with increased fruit and vegetable consumption, with those having the CC genotype recording increased levels compared to the those with the TT genotype.

Our study also reports that the *SEL15* rs5845 minor allele T carries a higher risk for benign prostate disease compared to controls, while recording a lower OR of developing malignant disease compared to benign disease. The lower OR of prostate cancer as compared to benign disease associated with this allele could be due to undetected prostate cancers among the benign group. Men with urological diseases related to inflammation and subsequent bladder outlet obstruction are subjected to transurethral or open prostatectomy to relieve bladder outlet obstruction. A study of surgical specimens from such procedures has indicated that 4–16% of such patients have prostate cancer which would have gone unnoticed if surgical specimens were not available [47]. In addition, both prostate cancer and benign prostate hyperplasia have inflammation as a common denominator [48]. Therefore, identifying those with malignancies among those presenting with benign prostate disease is important in disease management. Monitoring alleles such as *SEL15* rs5845 T could be a useful tool in this regard. Our studies have previously reported that the rs5845 TT genotype has a significant correlation between serum Se level and red blood cell TR activity, as well as producing higher levels of TR activity compared to the CC and CT genotypes [37]. Prostate cancer cells are in a state of redox imbalance, and, as a consequence, TR and thioredoxins are reportedly up-regulated [49–51], probably as a cancer cell-protective mechanism. It is possible that the higher TR activity level produced by rs5845 TT homozygotes supports an increased protection of cancer cells. However, Shan et al. [52] have indicated that TR activity in prostate cells diminishes with progression of prostate cancer, forming increased oxidized thioredoxin levels that get localised to the nucleus. This localisation could affect nuclear transcription factors such as p53, NFκB and NRF2 [53–55]. Kumaraswamy et al. [18] have reported that mouse liver tumours have less SEL-15, whereas mouse prostate adenocarcinoma cells have no expression compared to normal tissue. The *SEL-15* gene is located in the chromosome 1p.31 region [18], where deletions or mutations are common among many cancers, with a suggested effect on a tumour suppressor gene [56, 57].

Overall, 7 alleles (*GPx1* rs1050450 allele T, *GPx4* rs713041 allele C, *SEPP1* rs3877899 allele A, *SEL15* rs5845 allele T, *SELS* rs4965373 allele A, *CAT* rs1001179 allele T and *SOD2* rs4880 allele C) tested for the GRS pointed to a significant cumulative effect on prostate cancer risk association compared to controls. Our study has not recorded significant GRS

between prostate cancer and benign urology disease patients, or between benign urology disease patients and controls. This may be again due to the benign groups carrying undetected cancers and affecting the statistics.

We have therefore recorded the possible lifestyle factors and gene polymorphisms that may adjust the risk of prostate cancer and benign prostate disease in a cohort from Auckland, New Zealand. These risk factors mostly point to deficiencies in oxidative stress management with lifestyle factors that could get enhanced by genetic factors. Such risk factors could be used to identify subjects who could develop prostate cancer lesions among those with benign prostate disease and could enable close monitoring of at-risk subjects for better prostate cancer management practices.

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