Environmental factors and risk of aggressive prostate cancer among a population of New Zealand men - a genotypic approach

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Abstract: Prostate cancer is one of the most significant male health concerns worldwide. Numerous researchers carrying out molecular diagnostics have indicated that genetic interactions with biological and behavioral factors play an important role in the overall risk and prognosis of this disease. Single nucleotide polymorphisms (SNPs) are increasingly becoming strong biomarker candidates to identify susceptibility to prostate cancer. We carried out a gene x environment interaction analysis linked to aggressive and non-aggressive prostate cancer (PCa) with a number of SNPs. By this method, we identified the susceptible alleles in a New Zealand population, and examined the interaction with environmental factors. We have identified a number of SNPs that have risk associations both with and without environmental interaction. These indicate that, certain SNPs have been associated with disease vulnerability based on behavioral factors. The list of the genes with SNPs identified as risk of PCa in a New Zealand population is mentioned in the pictorial abstract.

1. Introduction

Prostate cancer (PCa) is one of the most significant male health concerns worldwide, due to its high prevalence and a risk of around 1 in 6 patients developing the aggressive form of this disease\textsuperscript{1}. Understanding of the risk of the disease becoming aggressive is important for the appropriate management of PCa\textsuperscript{2}. PCa may start as an indolent disease of the prostate gland, followed by non-castrate increase in prostate-specific antigen (PSA), followed by a non-castrate metastatic stage and finally progression to the lethal castration-resistant, aggressive disease\textsuperscript{3}.

In 2002, PCa was identified as the third-most prevalent cancer among adult males worldwide\textsuperscript{4} and, after a further ten years, PCa, as the second-most common cancer among men and the sixth leading cause of death due to cancer among males worldwide\textsuperscript{5}. The highest recorded rate of men with PCa, relative to the population of healthy men, is observed in the Oceania region\textsuperscript{6,7}.

Although a heredity aspect is known for PCa\textsuperscript{8}, studies have also shown that genetic interactions...
between biological and behavioral factors play an important role in the overall risk and prognosis of PCa\textsuperscript{9-11}. Since decisions of clinical management are also based on identifying the risk of aggressive PCa\textsuperscript{3}, extensive research is being carried out worldwide to identify the role played by single nucleotide polymorphisms (SNPs), their association in the development and progression of PCa and their potential use as biomarkers\textsuperscript{12,13}. SNPs are increasingly becoming strong biomarker candidates to identify PCa susceptibility\textsuperscript{12,14,15}. Due to the increasing number of cases of PCa, identifying one or more biomarkers for early detection and proper management of PCa to avoid disease progression into the aggressive state is becoming an urgent priority internationally\textsuperscript{16}.

It is recognized that one form of defense against cancer development involves a series of genes whose role is to metabolize and excrete potentially toxic compounds and to repair subtle mistakes in DNA such as the mismatch repair genes. Particular environmental exposures can exacerbate the genetic influence on PCa through gene x environment interaction\textsuperscript{17}.

This paper explores the use of SNP genotypes as biomarkers for aggressive PCa. Here we present the data obtained following the genotype analysis of 138 SNPs, located in 60 genes and 10 chromosomal locations using SEQUENOM MassArray technology and the TaqMan SNP genotyping procedure. The cohort includes New Zealand men (of European descent) with different grades/stages of PCa, and age matched male controls. We seek to detect the association of SNPs with both aggressive and non-aggressive disease as well as the influence of external factors in risk modification. This, we believe, is the first such study on genetic and environmental risk association and interaction analysis leading to aggressive PCa in a New Zealand cohort.

2. Materials and Methods

2.1 Study population:

Patients with proven diagnosis for prostate cancer from the Auckland Regional Urology Registry (Auckland, Middlemore, and North Shore hospitals), and private practices in the Waikato region, New Zealand were invited to take part in this study between 2006-2014 (ethics reference NTY05/06/037). Patient recruitment was carried out with their informed consent. Initially, patients were recruited within one year of diagnosis, if they have not undergone any treatment for PCa. In 2008, the criterion was relaxed to include all patients with malignancies, regardless of treatment but within 1 year of diagnosis. In September 2010, the time frame for recruitment was removed altogether. From those that took part in this study, a total of 197 men were identified with aggressive PCa, and 57 with non-aggressive PCa. Additionally, 369 healthy males took part in our studies (ethics reference NTY/06/07/AM04), who were considered as healthy controls. The age of patients varied from 40-81 years at the time of recruitment and those with a self-reported European ancestry were included in the study.

2.2 Data collection for demography, and lifestyle details:

Each patient completed a demographic and lifestyle questionnaire at entry into the study. The questionnaire included details about the individual’s history of smoking tobacco, consumption of alcohol, body mass index (BMI) at time of recruitment, and age at diagnosis of PCa. Current smokers and individuals who reported a history of smoking tobacco were jointly considered as smokers.

The clinical history of each patient was extracted from hospital databases and transferred to a central study database. The age at which PCa was diagnosed in patients and the age at recruitment for the controls were considered as age for analysis.

2.3 Collection and processing of blood samples:

Blood samples from each volunteer were collected in Vacutainer\textsuperscript{®} tubes (Becton Dickinson) containing Ethylene Diamine Tetra Acetic acid (EDTA). An aliquot of the sample collected was then used for genomic DNA extraction. Each patient’s DNA was extracted using a QIAamp genomic DNA kit (Qiagen, Hilden, Germany) following the manufacturers’ protocol with the aid of a fully automated QIAcube (Qiagen, Hilden, Germany). The DNA samples were diluted to 5.0ng/μl as per requirement of the SEQUENOM MassARRAY iPLEX\textsuperscript{®} assay protocol.
2.4 Selection of SNPs:

A total of 135 SNPs, located in 66 genes and some undefined chromosomal locations (Supplementary Table 1) were identified by a literature search of the published genome-wide association studies (GWAS) for both PCa and its aggressive form.

The PubMed database was screened for research articles using key terms “prostate cancer” and/or “aggressive prostate cancer”, along with “SNP genotyping sequenom massarray iplex”, and/or “genome-wide association studies”. Only research articles published on or after the year 2000 were considered for this study to maintain the current trend of research. Since the sample population for this research is of European ancestry, attention was given to research papers which reported statistically significant findings among patients with such backgrounds.

The research team has also used their own knowledge and discretion regarding which SNPs to be genotyped using SEQUENOM MassARRAY® assay, or the TaqMan SNP genotyping assay. Various metabolic pathways such as selenium metabolism and androgen metabolism pathway were included, for instance, as was, screening for chromosomal regions such as 8q24, 10q11, 17q12, and Xp11 all known for PCa risk association.

Additionally certain putative oncogenes such as MYEOV (Myeloma Overexpressed), and DNA mismatch repair genes such as MLH1 (MutL homolog 1); as well as certain genes with an established role in the PSA metabolism pathway7,10,18 were also considered in this assessment.

Our approach uses genome wide association analysis (GWAA) to provide a comprehensive evaluation of multiple genes with polymorphisms that interact in the same pathway/s, in line with proposals made by Hsing et al., (2008)9 and Kwon et al., (2012)20, who claim that studies on GWAS are a better way to study multifactorial diseases such as PCa. Also, Hsing et al., (2008), have suggested that research pertaining to the AR pathway should include co-regulators along with important genes involved in androgen metabolism and biosynthesis19. A similar approach was followed in our selection of genes with the SNPs studied below.

2.5 SNP genotyping of candidate genes:

2.5.1 SNP genotyping by Sequenom multiplexing:

Genotyping for the candidate SNPs was carried out in the Auckland UniServices Sequenom Facility at Liggins Institute, Auckland, and AgResearch Limited, Mosgiel, New Zealand, using custom-designed multiplex gene panel and iPlex chemistry. Genotype calling was performed using the standard post-processing calling parameters in SEQUENOM Type 4.0 software. Each 384-well plate prepared for genotyping contained known HAPMAP control samples, negative controls (Water) and repeats of samples used in different locations in the 384-well plate for validation of the genotyping procedure.

2.5.2 SNP genotyping by TaqMan® assay:

SNP genotyping using TaqMan® SNP genotyping (Applied Biosystems) was carried out on a panel of genes. The primers used were either obtained pre-designed from Applied Biosystems or were custom-made using Assay-by-Design service by ABI7,10,18. The protocol provided by the manufacturers (Applied Biosystems) was used to carry out the SNP genotyping7,10,18.

2.6 Collection of clinical characteristics of patients:

The total serum PSA level and Gleason score of the tumour at biopsy or post-surgery or both were obtained from the patients’ clinical records at the time of recruitment or subsequent to recruitment. Post-surgery Gleason score was given priority over biopsy Gleason score. Staging data was collected from clinical records during the years 2013-2014, but was not available for all the patients.

2.7 Definition of aggressiveness:

The aggressiveness of PCa for this study is based on the classification used by the American Urological Association21 and first proposed by D’Amico et al. (1998) for defining high-risk or aggressive PCa as clinical T stage ≥T2c, or Gleason score ≥8, or serum PSA level ≥20ng/ml22.

2.8 Statistical analysis:
2.8.1 SNP data cleaning:
A total of 39 SNPs were removed from the list before data analysis after checking the genotype for compliance with Hardy Weinberg Equilibrium (HWE) and in order to avoid linkage disequilibrium using PLINK. 13 SNPs were reduced for not complying with HWE and an additional 26 SNPs were removed for being in linkage. The entire list of the SNPs removed from the final analyses is colour-coded in the Supplementary table 1. We therefore analysed the data using a reduced list of 99 SNPs.

Analysis of the data for SNP association with PCa based on aggressiveness and gene x environment interaction for risk of PCa were both carried out using PLINK- a tool set for whole genome association. Correction for multiple testing was applied to significance value where appropriate otherwise, statistical significance was set at p≤0.05.

2.8.2 SNP association analysis for risk of Prostate Cancer:
A structured association approach similar to the one proposed by Arya et al. in 2009, working on rheumatoid arthritis, was followed. This provides a simple but powerful method, to detect population stratification, and is implemented in the PLINK. PLINK’s clustering approach is based on the genome-wide average proportion of alleles shared identical-by-state (IBS) between two individuals SNPs, i.e., pairing up the SNPs based on genetic identity. The IBS clustering is used to test whether the SNPs of two individuals belong to the same population. Following the stratification analysis, we performed a standard case-control association test using a Cochran-Mantel-Haenszel statistic (1 df (degree of freedom)) that tests for SNP-disease association conditional on the clustering. This accounts for stratification effects, as has been reported by Arya et al. (2009).

To avoid the possibility of false positives with multiple SNP testing, statistical significance was restricted by the most conservative Bonferroni correction (BONF) along with the less conservative Benjamini and Hochberg false discovery rate (BH-FDR) for multiple testing corrections. The complete set of results is provided in Supplementary Table 2. However, as the tested SNPs were already shown statistically to be significantly associated with PCa risk by other researchers, variations that demonstrated significant association to risk of PCa before BONF and BH-FDR were considered for discussion in our study.

2.8.2 Significance of demographic factors:
The variation of lifestyle characteristics between pathology was tested using the Fisher exact test.
The BMI data were not normally distributed; therefore comparisons between pathologies were carried out using the Mann Whitney test.

2.8.3 Gene-environment interaction for risk of PCa:
The covariates included the three environmental factors- BMI record, tobacco smoking and alcohol consumption history. Also three genotypic models: additive (ADD), dominant deviation (DOMDEV), and general_2 df joint test of both additive and dominant deviation (GENO_2DF) were generated. An additive model represents the additive effects of SNPs i.e., the effect of each additional minor allele as represented by the direction of the regression coefficient. For example, a positive regression coefficient indicates that the minor allele increases risk. A DOMDEV model represents a separate test of the dominance component, and a general model represents the joint test of both ADD and DOMDEV components. However, in contrast to a dominance model, ADD refers to a variable coded in such a way (0, 1, 0 for three genotypes AA, Aa, aa) that it represents the dominance deviation from additivity without specifying whether a particular allele is dominant or recessive. Effects of genotype x environmental (BMI, tobacco smoking and alcohol consumption) interactions was tested and eventually corrected for.

3. Results
3.1 Gene x environment effects and prostate cancer risk in a New Zealand population:
The tables show the results of the statistically significant SNPs associated between non-aggressive PCa and healthy controls (Table 1.1), between patients with aggressive and non-aggressive PCa
(Table 1.2), and patients with aggressive PCa and healthy controls (Table 1.3), all assessed before the use of BONF.

The results obtained after a detailed analysis of the association of SNPs as risk of PCa can be broadly classified into two categories—expected, and unique with respect to the results reported by other groups.

Certain SNPs present near genes such as MYEOV-a putative oncogene (Table 1.3); TLR4 (Toll-like receptor 4) (Tables 1.1 and 1.2) and MMP9 (Matrix metallopeptidase 9) (Table 1.2)-involved with the inflammatory pathway; and KLK3 (Kallikrein-3) (Table 1.2 and 1.3) and MSMB (Microseminoprotein Beta) (Table 1.3)—both involved in the PSA metabolism pathway were understandably identified as statistically significant in our study, due to their proven risk association to PCa.

The number of SNPs present near various genes associated with obesity and diabetes mellitus such as FADS2 (Fatty acid desaturase 2) (Table 1.3), LEP (Leptin) (Tables 1.2 and 1.3), PPAR-γ (Peroxisome Proliferator-Activated Receptor gamma) (Table 1.3) were associated with the risk of aggressive PCa vs healthy controls as recorded in our analysis.

### Table 1.1: Statistically significant Case/Control SNP association between patients with non-aggressive prostate cancer and healthy controls

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>SNP ID</th>
<th>Gene name</th>
<th>Gene location</th>
<th>Tested allele</th>
<th>Odds Ratio</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>rs2292884</td>
<td>MLPH</td>
<td>2q37.2</td>
<td>G</td>
<td>1.774</td>
<td>0.02375</td>
</tr>
<tr>
<td>2</td>
<td>rs4965373</td>
<td>SEPS1</td>
<td>15q26.3</td>
<td>A</td>
<td>1.801</td>
<td>0.02413</td>
</tr>
<tr>
<td>3</td>
<td>rs11536889</td>
<td>TLR4</td>
<td>9q33.1</td>
<td>C</td>
<td>2.198</td>
<td>0.02727</td>
</tr>
<tr>
<td>4</td>
<td>rs3735035</td>
<td>PODXL</td>
<td>7q32</td>
<td>C</td>
<td>1.572</td>
<td>0.03493</td>
</tr>
</tbody>
</table>

### Table 1.2: Statistically significant Case/Control SNP association between patients with aggressive prostate cancer and non-aggressive prostate cancer

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>SNP ID</th>
<th>Gene name</th>
<th>Gene location</th>
<th>Tested allele</th>
<th>Odds Ratio</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>rs632148</td>
<td>SRD5A2</td>
<td>2p23.1</td>
<td>C</td>
<td>1.799</td>
<td>0.01731</td>
</tr>
<tr>
<td>2</td>
<td>rs887391</td>
<td>SLC26A6</td>
<td>19q13</td>
<td>C</td>
<td>1.793</td>
<td>0.02063</td>
</tr>
<tr>
<td>3</td>
<td>rs11536889</td>
<td>TLR4</td>
<td>9q33.1</td>
<td>G</td>
<td>2.303</td>
<td>0.02251</td>
</tr>
<tr>
<td>4</td>
<td>rs2292884</td>
<td>MLPH</td>
<td>2q37.2</td>
<td>A</td>
<td>1.801</td>
<td>0.02614</td>
</tr>
<tr>
<td>5</td>
<td>rs3735035</td>
<td>PODXL</td>
<td>7q32</td>
<td>T</td>
<td>1.621</td>
<td>0.03126</td>
</tr>
<tr>
<td>6</td>
<td>rs10244329</td>
<td>LEP</td>
<td>7q33</td>
<td>T</td>
<td>2.062</td>
<td>0.03222</td>
</tr>
<tr>
<td>7</td>
<td>rs17632542</td>
<td>KLK3</td>
<td>19q13.33</td>
<td>T</td>
<td>3.394</td>
<td>0.04647</td>
</tr>
<tr>
<td>8</td>
<td>rs3918256</td>
<td>MMP9</td>
<td>20q13.12</td>
<td>A</td>
<td>1.555</td>
<td>0.04959</td>
</tr>
</tbody>
</table>
Table 1.3: Statistically significant Case/Control SNP association between patients with aggressive prostate cancer and healthy controls

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>SNP ID</th>
<th>Gene name</th>
<th>Gene location</th>
<th>Tested allele</th>
<th>Odds Ratio</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>rs17793693</td>
<td>PPAR-γ</td>
<td>3p25</td>
<td>A</td>
<td>4.534</td>
<td>0.000173</td>
</tr>
<tr>
<td>2</td>
<td>rs7931342</td>
<td>MYEOV</td>
<td>11q13</td>
<td>T</td>
<td>1.565</td>
<td>0.0007423</td>
</tr>
<tr>
<td>3</td>
<td>rs10896438</td>
<td>MYEOV</td>
<td>11q13.3</td>
<td>T</td>
<td>1.498</td>
<td>0.002322</td>
</tr>
<tr>
<td>4</td>
<td>rs887391</td>
<td>SLC26A6</td>
<td>19q13</td>
<td>C</td>
<td>1.594</td>
<td>0.005094</td>
</tr>
<tr>
<td>5</td>
<td>rs5945619</td>
<td>NUDT11</td>
<td>Xp11</td>
<td>T</td>
<td>1.694</td>
<td>0.005749</td>
</tr>
<tr>
<td>6</td>
<td>rs17632542</td>
<td>KLK3</td>
<td>19q13.33</td>
<td>T</td>
<td>1.998</td>
<td>0.008268</td>
</tr>
<tr>
<td>7</td>
<td>rs7920517</td>
<td>MSMB</td>
<td>10q11</td>
<td>A</td>
<td>1.400</td>
<td>0.01227</td>
</tr>
<tr>
<td>8</td>
<td>rs11228565</td>
<td>MYEOV</td>
<td>11q13</td>
<td>G</td>
<td>1.433</td>
<td>0.02189</td>
</tr>
<tr>
<td>9</td>
<td>rs10244329</td>
<td>LEP</td>
<td>7q33</td>
<td>T</td>
<td>1.557</td>
<td>0.02344</td>
</tr>
<tr>
<td>10</td>
<td>rs6983561</td>
<td></td>
<td>8q24</td>
<td>A</td>
<td>1.885</td>
<td>0.02883</td>
</tr>
<tr>
<td>11</td>
<td>rs130067</td>
<td>CCHCR1</td>
<td>6p21.3</td>
<td>A</td>
<td>1.383</td>
<td>0.03656</td>
</tr>
<tr>
<td>12</td>
<td>rs2727270</td>
<td>FADS2</td>
<td>11q13</td>
<td>C</td>
<td>1.525</td>
<td>0.04184</td>
</tr>
<tr>
<td>13</td>
<td>rs12529</td>
<td>AKR1C3</td>
<td>10p15</td>
<td>C</td>
<td>1.294</td>
<td>0.04685</td>
</tr>
<tr>
<td>14</td>
<td>rs2659122</td>
<td>KLK3</td>
<td>19q13.33</td>
<td>A</td>
<td>1.345</td>
<td>0.04748</td>
</tr>
</tbody>
</table>

Tables 1.1-1.3 colour legends risk association:

- Common SNPs between (Agg PCa vs Healthy Control) and (Agg PCa vs Non-Agg PCa)
- Common SNPs between (Non-Agg PCa vs Healthy Control) and (Agg PCa vs Non-Agg PCa)

3.2 Pathology, BMI and lifestyle:

Owing to the established role of environmental or non-genetic aspects in the expression of gene\textsuperscript{20} in PCa\textsuperscript{30}, we compared the variation in demographics and lifestyle factors such as age, alcohol consumption, smoking tobacco, and levels of obesity among the patients recruited for our study. The disease association with BMI (based on the classification by World Health Organization\textsuperscript{31}) at the time of recruitment, tobacco smoking status, and alcohol consumption (at the time of recruitment in this study) are provided in tables 2.1 to 2.3 respectively. In these tables the controls are compared to non-aggressive and aggressive PCa patients.

Table 2.1: The association between BMI and risk and/or aggressiveness of prostate cancer

<table>
<thead>
<tr>
<th>Compared groups</th>
<th>Pathology (N &amp; UW)</th>
<th>Pathology (OW &amp; O)</th>
<th>Total</th>
<th>Median (75\textsuperscript{th} percentile)</th>
<th>Mann-Whitney U test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aggressive</td>
<td>36</td>
<td>161</td>
<td>197</td>
<td>27.00 (30.50)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table legends: N= men with normal weight (18.50 kg/m\(^2\)-24.99 kg/m\(^2\)); UW= men who were under-weight (<18.50 kg/m\(^2\)); OW= men who were over-weight (25.00 kg/m\(^2\)-29.99 kg/m\(^2\)); O= men who were obese (≥30.00 kg/m\(^2\)); N’= number of men.

**Table 2.2**: Association between tobacco smoking status and risk and/or aggressiveness of prostate cancer

<table>
<thead>
<tr>
<th>Compared groups</th>
<th>Pathology</th>
<th>N’</th>
<th>Percentage (ever smokers)</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>Yes</td>
<td>Total</td>
<td>(95% CI)</td>
<td></td>
</tr>
<tr>
<td>Aggressive vs Control</td>
<td>Aggressive</td>
<td>89</td>
<td>108</td>
<td>197</td>
<td>54.82%</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>225</td>
<td>144</td>
<td>369</td>
<td>39.02%</td>
</tr>
<tr>
<td>Aggressive vs Non-Aggressive</td>
<td>Aggressive</td>
<td>89</td>
<td>108</td>
<td>197</td>
<td>54.82%</td>
</tr>
<tr>
<td></td>
<td>Non-Aggressive</td>
<td>23</td>
<td>34</td>
<td>57</td>
<td>59.64%</td>
</tr>
<tr>
<td>Non-Aggressive vs Control</td>
<td>Non-Aggressive</td>
<td>23</td>
<td>34</td>
<td>57</td>
<td>59.64%</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>225</td>
<td>144</td>
<td>369</td>
<td>39.02%</td>
</tr>
</tbody>
</table>

Table legends: N’= number; 95% CI= 95% confidence interval, No= never smokers, Yes= ever smoker.

**Table 2.3**: Comparison of alcohol consumption and risk and/or aggressiveness of prostate cancer

<table>
<thead>
<tr>
<th>Compared groups</th>
<th>Pathology</th>
<th>N’</th>
<th>Percentage (alcohol consumers)</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No (1)</td>
<td>Yes (0)</td>
<td>Total</td>
<td>59.89%</td>
<td>0.256</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>54</td>
<td>315</td>
<td>369</td>
<td>85.36%</td>
</tr>
<tr>
<td>Aggressive vs Non-Aggressive</td>
<td>Aggressive</td>
<td>79</td>
<td>118</td>
<td>197</td>
<td>59.89%</td>
</tr>
<tr>
<td></td>
<td>Non-Aggressive</td>
<td>25</td>
<td>32</td>
<td>57</td>
<td>56.14%</td>
</tr>
<tr>
<td>Non-Aggressive vs Control</td>
<td>Non-Aggressive</td>
<td>25</td>
<td>32</td>
<td>57</td>
<td>56.14%</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>54</td>
<td>315</td>
<td>369</td>
<td>85.36%</td>
</tr>
</tbody>
</table>

Table legends: N’= number; 95% CI= 95% confidence interval.

### 3.3 Correction for the effect of covariants to identify the genic risk of aggressive PCa:

The statistically significant results obtained using logistic models after adjustments for multiple covariates are presented in tables 3.1 and 3.2 (Aggressive PCa vs non-aggressive PCa and aggressive PCa vs healthy control are shown in tables 3.1 and 3.2 respectively). The data obtained for non-aggressive PCa vs healthy control was not statistically significant and will not be further addressed in this paper.

The results obtained after the interaction analysis can also be broadly classified into two
categories - expected, and unique to the New Zealand population studied. The New Zealand aspect of gene x environment interaction was much better evident after analyzing these results.

Certain SNPs present near genes such as MYEOV - a putative oncogene (Table 3.2); KLK3 and MSMB (both, Table 3.2) - involved in the PSA metabolism pathway; MMP9 (Table 3.1) - involved with the inflammatory pathway; and MLH1 (Table 3.2), which has a role in DNA mismatch repair were identified as statistically significant in our study as expected. However, we had some novel findings as well.

A SNP present near the gene LEP (Tables 3.1 and 3.2), associated with obesity is identified as statistically significant risk for both aggressive and non-aggressive PCa; and the SNP present near the gene SEP15 (Seloprotein 15kDa) - involved with quality control of protein folding in the endoplasmic reticulum (ER) may reveal new knowledge about the changes of risk for aggressive PCa with local environmental conditions and its effects on the New Zealand Caucasian men studied here.

Table 3.1: Logistic model of SNPs risk for aggressive prostate cancer vs non-aggressive prostate cancer after correcting for interaction with multiple covariates (BMI, tobacco smoking, and alcohol consumption)

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>SNP ID</th>
<th>Gene name</th>
<th>Gene location</th>
<th>Tested allele</th>
<th>Odds Ratio</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>rs632148</td>
<td>SRD5A2</td>
<td>2p23.1</td>
<td>C</td>
<td>2.144</td>
<td>0.01196</td>
</tr>
<tr>
<td>2</td>
<td>rs10244329</td>
<td>LEP</td>
<td>7q33</td>
<td>T</td>
<td>2.395</td>
<td>0.02893</td>
</tr>
<tr>
<td>3</td>
<td>rs3918256</td>
<td>MMP9</td>
<td>20q13.12</td>
<td>A</td>
<td>1.873</td>
<td>0.03109</td>
</tr>
<tr>
<td>4</td>
<td>rs3735035</td>
<td>PODXL</td>
<td>7q32</td>
<td>T</td>
<td>1.625</td>
<td>0.03561</td>
</tr>
</tbody>
</table>

Table 3.2: Logistic model of SNPs risk for aggressive prostate cancer vs healthy controls after correcting for interaction with multiple covariates (BMI, tobacco smoking, and alcohol consumption)

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>SNP ID</th>
<th>Gene name</th>
<th>Gene location</th>
<th>Tested allele</th>
<th>Odds Ratio</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>rs7931342</td>
<td>MYEOV</td>
<td>11q13</td>
<td>T</td>
<td>1.705</td>
<td>0.000423</td>
</tr>
<tr>
<td>2</td>
<td>rs10896438</td>
<td>MYEOV</td>
<td>11q13</td>
<td>T</td>
<td>1.699</td>
<td>0.000421</td>
</tr>
<tr>
<td>3</td>
<td>rs7920517</td>
<td>MSMB</td>
<td>10q11</td>
<td>A</td>
<td>1.590</td>
<td>0.001734</td>
</tr>
<tr>
<td>4</td>
<td>rs5845</td>
<td>SEP15</td>
<td>1p22.3</td>
<td>C</td>
<td>1.845</td>
<td>0.01333</td>
</tr>
<tr>
<td>5</td>
<td>rs2659122</td>
<td>KLK3</td>
<td>19q33.33</td>
<td>A</td>
<td>1.702</td>
<td>0.01516</td>
</tr>
<tr>
<td>6</td>
<td>rs1799977</td>
<td>MLH1</td>
<td>3p21</td>
<td>G</td>
<td>1.53</td>
<td>0.0202</td>
</tr>
</tbody>
</table>
4. Discussion

It is well established that there are three major risk factors for PCa, namely, advancing age, ethnicity, and familial history. Various studies have indicated that alterations in genetic and epigenetic make-up are predominantly the basis for the development of various malignancies. In this study, SNP genotype data was used to identify risk association with aggressive PCa while the effect of non-genetic or environmental factors was also considered.

4.1 Effect of environment and SNP genotype with risk of prostate cancer:

Parts of the panel of SNPs used in this analysis have previously been considered to assess PCa risk. The approach here is to assess their significance to aggressive PCa also. Out of the 99 SNPs studied, 4 SNPs were significantly associated with non-aggressive PCa when compared with healthy controls, and 8 SNPs were identified to be significantly associated with aggressive PCa compared to non-aggressive PCa. Also, 14 SNPs were identified to be significantly associated with aggressive PCa compared to non-aggressive PCa. The majority of the SNP associations lost significance after correcting for multiple testing using the BONF, an overly conservative approach, and the BH-FDR, a less conservative correction that tolerates more false positives.

Large scale GWAS have previously shown direct SNP associations with aggressive PCa. However, SNP interactions with demographic and lifestyle factors could also add to the allelic effect producing a modified risk of a disease. These SNPs could be indicating a unique situation for New Zealand men- who are an example of isolated men of European origin, with PCa. Of particular interest in the New Zealand context are, aspects such as obesity, higher intake of red meat and dairy products, and possible deficiency in selenium intake. All of these could impact on genetic mechanisms in ways that may lead to a higher risk of aggressive PCa.

The three broad classifications under which the data were analyzed are SNP associations between patients with aggressive PCa and healthy controls, between patients with non-aggressive PCa and healthy controls and between patients with aggressive and non-aggressive PCa. The results for these groups are discussed below.

4.1.1 SNP genotype analysis of non-aggressive prostate cancer vs healthy controls:

Of the 4 SNPs identified as significant risk for non-aggressive PCa vs healthy controls, 3 SNPs were identified to be commonly associated to risk of the progression of this disease (aggressive PCa vs non-aggressive PCa). These SNPs include rs2292884 in chromosomal region 2q37 near the gene MLPH (Melanophilin), rs3735035 present in chromosomal region 7q32 in the gene POXL (Podocalyxin-like), rs478089984 in chromosomal region 8q24 and rs11536889 present in chromosomal region 9q33 near the gene TLR4.

The TLR4 gene is responsible for activating innate immunity in humans. TLR4 is one of 13 transmembrane receptors found in the troll-like receptors family which plays an important role in chronic infection and inflammation pathways, in turn controlling the incidence of development of...
cancer. The expression of the TLR4 protein in PCa is already well established.

SNP rs2292884 close to chromosomal region 2q37 near the gene MLPH, was also identified to be statistically significantly associated with non-aggressive PCa compared to controls. Very little is known about the possible functional impact of MLPH in carcinogenesis and/or tumor progression. Dysregulation of the protein MLPH has recently been found in several types of tumors in lung cancer, meningiomas, and of breast cancers. A recent study found an association of expression of the gene MLPH with nearby SNPs in prostate tissue. In non-small cell lung cancer, MLPH mRNA was identified as a target of differentially expressed miRNAs.

Interestingly, the protein MLPH was also found to be significantly overexpressed in estrogen receptor (ER) positive breast cancer, suggesting a regulation of this protein by estrogen hormones.

SNP rs3735035 present in chromosomal region 7q32 in the gene PODXL was also identified to be significantly associated with non-aggressive PCa (before the use of BONF) in our study. PODXL is a cell-adhesion glycoprotein and stem cell marker that has been associated with an aggressive tumour phenotype and poor prognosis in several forms of cancer. It has been previously identified as a candidate biomarker for PCa aggressiveness. Since rs3735035 present in this gene came up as statistically significantly associated with non-aggressive PCa (before the use of BONF) in our population, we feel that this SNP may play a role from the point of disease initiation. SNP rs4965373 present in chromosomal region 15q26.3 in the gene SEPS1 (selenoprotein S) was also identified to be statistically significantly associated with non-aggressive PCa (before the use of BONF) in our population. The human gene SEPS1, encodes selenoprotein S which participates in the retro-translocation of misfolded proteins from the ER to the cytosol for their degradation. This ER membrane protein functions in stress responses to prevent the deleterious consequences of accumulation of misfolded proteins, that has been linked to immune and inflammatory processes.

The SEPS1 gene was first suggested to be related to the stress response process including immune and inflammatory processes through the study of Curran et al., in 2005. In addition, several diseases, including inflammatory disorders such as insulin-dependent diabetes mellitus, and gerontological disorders such as Alzheimer’s disease, have been shown to be linked to this gene region. The A allele of this rs4965373 SNP has previously shown positive associations with serum Selenium levels in a healthy male population from New Zealand. Comparing the current findings with those of Ferguson et al. (2012) indicates a possibility for retention of excess selenium as a cause of non-aggressive PCa risk. These direct us to the relation between functional defects, inflammation, immunity, and its depletion due to ageing as responsible for initiation of diseases such as PCa.

4.1.2 SNP genotype analysis of aggressive prostate cancer vs non-aggressive prostate cancer:

Non-aggressive PCa can be considered as an intermediary between healthy and aggressive PCa states. We identified a number of SNPs to be common between the categories of SNP association between aggressive PCa and healthy controls and non-aggressive PCa and healthy controls.

Among these common SNPs are those in genes that have been identified to play roles in immunity (rs11536889), development and progression of PCa (rs2292884, and rs3735035). The SNPs common to be associated with aggressive PCa compared to controls and the SNPs identified to be associated with the progression of this disease (aggressive PCa vs non-aggressive PCa) are rs10244329 in 7q33.3 chromosomal region near the gene LEP, rs887391 and rs17632542, both present in chromosomal region 19q13. Of the SNPs that are common to be associated to non-aggressive PCa compared to controls and the SNPs identified to be associated with the progression of this disease (aggressive PCa vs non-aggressive PCa), there are SNPs in genes that have been identified to play roles in the expression of PSA (rs17632542), development and progression of PCa (rs887391), and morbid obesity.

SNPs rs632148 in the gene SRD5A2 (Steroid 5a-reductase type 2) on chromosomal region 2p23, and rs3918256 in the gene MMP9 have also been associated with aggressive PCa when compared to non-aggressive PCa patients. The SNPs rs632148
and rs3918256 have been previously reported by groups working on various aspects related to and causing PCa in Caucasian populations and not restricted to studies pertaining to quality of sperms (rs632148)\(^6\), and also contribute to the invasive and metastatic properties of malignant tumors, including those of the prostate (rs3918256)\(^6\). These SNPs are of considerable importance in understanding the overall progression of PCa to aggressive stage.

4.1.3 SNP genotype analysis of aggressive prostate cancer vs healthy controls:

A link between SNPs in the 11q13 region and PCa has been previously identified by various GWAS\(^66,69\). Three SNPs- rs10896438, rs7931342, rs11228565, present in MYEOV and one SNP- rs2727270 present in FADS2 are identified to be statistically significant before the use of BONF when comparing the controls to those with aggressive PCa. Frequent rearrangements are observed in human cancers in the area 11q13\(^71\),72 and rs6983561 present to have a statistically significant risk association with aggressive PCa, compared to controls among our population before the use of BONF. These are rs7920517 present near the gene MSMB, and rs5945619 present in the gene NUDT11 (Nucleoside Diphosphate-linked Moiety X Motif 11) respectively. These SNPs knit a very tight story and are of considerable interest.

MSMB is one of just three predominant proteins, along with PSA and prostatic acid phosphatase secreted by a normal human prostate gland\(^71\). The PSA level in men is usually measured to estimate the health of a prostate gland, and MSMB, owing to its varied expression levels in cancer cells compared to the prostate epithelial cells in the benign stage, is implicated as a potential PCa biomarker\(^86\). MSMB is also known to modulate immunoglobulin levels in hypoxic tumour cores\(^86\).

The SNP rs5945619\(^96\) present in the Xp11 region also has been identified to be significantly (before BONF were implemented) associated with aggressive PCa to controls. This SNP is present near the paralogous human gene NUDT11, predominantly expressed in the testes, and may play a role in signal transduction\(^88\). A number of GWAS have also suggested that the susceptibility locus at NUDT11 may have involvement with risk of PCa\(^86,87,90,91\).
The SNP rs17793693 present in the PPAR-γ gene was also identified to be significantly associated with aggressive PCa in our population. The significance, however, was lost after the BONF was implemented. PPAR-γ ligands induce growth arrest in cells through apoptosis, in both macrophages and endothelial cells. Among other roles, the PPAR-γ protein has also been identified to be having a role in immunity. The PPAR-γ gene, present in the 3p25 region of the human genome has been suggested to have an association with PCa by various researchers. PPARs (comprising of PPAR-α, PPAR-β and PPAR-γ genes) are members of the nuclear hormone receptor superfamily of ligand-activated transcription factors including receptors for steroid hormones, Vitamin D3, retinoid and thyroid hormones. The PPAR-γ gene has been identified to be mainly present in adipose tissues, wherein it plays a vital role in the pre-adipocytes to adipocytes transition. The PPAR-γ gene has also been identified to be involved in the pathology and progression of various diseases including obesity and diabetes mellitus. Diabetes mellitus has previously been associated with PCa, such that patients with diabetes have been suggested to be at a lower risk of PCa. Certain metabolic factors including reduced testosterone levels may affect blood glucose levels.

SNPs rs130067 present in 6p21.3 chromosomal region near the gene CCHCR1 (coiled-coil alpha-helical rod protein 1), rs10244329 present in 7q33.3 chromosomal region near the gene LEP and rs12529 present in chromosomal region 10p15 in the gene AKR1C3 (Aldo-keto reductase family 1 member C3) have also been identified to be statistically significantly associated with aggressive PCa in this study. The AKR1C3 rs12529 G allele has been previously associated with PCa risk when interacting with age and lifestyle habits including tobacco smoking. It could be that although the G allele of this SNP is producing a risk for PCa, it is the C allele that produces aggressiveness of the disease. A study by Yu et al., (2013) has shown that this C allele is responsible for prostate cancer-specific mortality among those receiving androgen deprivation therapy. The same allele has also shown retention of cancer-specific symptoms, if managed only with non-androgen deprivation treatment methods. The SNPs rs130067, rs12529, and rs12529 have been previously reported by groups working on various diseases related to and causing PCa in Caucasian populations such as rheumatoid arthritis, obesity, and sex hormone metabolic pathways, respectively. The rs12529 SNP is also related to lung cancer and bladder cancer.

4.2 Age and risk of prostate cancer:

Age is a major risk factor for PCa. However, in the data presented in our present study we did not consider the role of ageing, as it is the only external factor which cannot be reversed, but the other aspects such as high (or, low) BMI, smoking tobacco and alcohol consumption habits can be altered by individuals. Moreover, we wanted to see the effect of gene x environment aspects prevalent in our local population in the expression and progression of PCa.

4.3 BMI, smoking tobacco, and alcohol consumption at recruitment and risk of prostate cancer:

Our previous research showed that BMI is not statistically different in age matched controls and those with benign urology disease. However, with the current stratification BMI was shown to be significantly higher (27.00kg/m²) among those with aggressive PCa compared to controls (26.36kg/m²). The mean BMI at recruitment of all our groups (cases as well as healthy controls) was calculated to be in the category of overweight for adults. However, Vidal et al. (2014) and Haque et al. (2014) discuss that men with aggressive PCa have a higher risk of mortality due to the disease if they are overweight or obese.

Smoking tobacco has been identified as a risk factor for PCa, and our results support smoking tobacco being a major risk factor in individuals developing aggressive as well as non-aggressive PCa. Our results suggest that more than half of our patient cohort identified themselves as ever smokers, and this is very similar to the results discussed by Huncharek et al. (2010) and Braithwaite et al. (2012). In another study carried out by Pantarotto et al. (2007), previous and present tobacco smokers have been associated with a greater risk of PCa metastasizing. Tobacco smoking, has previously also been reported as a risk factor for PCa as a whole, compared with age and ethnicity matched healthy controls by our group.
A systematic review in 2014 indicated that tobacco smoking is a risk factor for fatal prostate cancer but not a risk factor for prostate cancer incidence\textsuperscript{13}. Adding to the published work by Islami \textit{et al.} (2014), Sclatmann and Blanker (2015) indicated that the mortality risk due to tobacco use was higher in the pre-PSA era and not in the post-PSA era\textsuperscript{13,14}. Tobacco use being a risk factor for both aggressive and non-aggressive PCs in our New Zealand cohort is concerning as it indicates a scenario similar to that of the pre-PSA era from other western countries. The possibility of former tobacco smokers changing their lifestyle after being identified as having PCs, or being in a high risk population, has also been reported by Blanchard \textit{et al.} (2003). However, unlike the alcohol consumption changes observed in our study cohorts, the frequency of men ever exposed to tobacco smoking in both aggressive (54.8\%) and non-aggressive (51.06\%) groups has not declined (53.1\% in 2012)\textsuperscript{107}.

Alcohol consumption, in spite of being well-documented as a potential cause of cancer\textsuperscript{15,16}, is yet to be proven as a risk factor for PCs\textsuperscript{17,18}. Unlike the results of Zuccolo \textit{et al.} (2013), who have shown a moderate risk of aggressiveness with alcohol consumption\textsuperscript{18}, our study has found inverse significance related to alcohol consumption when comparing data obtained from groups of aggressive PCs vs controls as well as non-aggressive PCs vs controls. Additionally, previous publications from our group with the initial 264 PCs patients recruited within one year of PCs diagnosis, has recorded that 72\% of patients were alcohol consumers\textsuperscript{7}. The current decline in alcohol consumption rate to 59\% in patients with aggressive PCs and 56\% in patients with non-aggressive PCs could well be due to PCs survivors changing their behavior and consuming less alcohol.

4.4 Correction for gene x environment interaction and risk of prostate cancer:

Knowledge of gene–environment interaction is important for risk prediction and the identification of certain high-risk populations to inform public health strategies for targeted prevention\textsuperscript{119}. We associated the environmental factors with the genotypes of the men in our study to identify the risk alleles for specific modifiable factors such as BMI, smoking tobacco and alcohol consumption. Since these factors play an important role in the risk association of PCs and yet can be controlled by individuals, they therefore are of much importance to understand and limit this disease.

4.4.1 Genotype and the outcome of non-aggressive prostate cancer (vs healthy controls):

Many malignancies have been linked to specific environmental exposures\textsuperscript{120}. Several environmental and occupational factors such as farming and use of pesticides, exposure to sunlight/ultraviolet radiation, as well as trace minerals which are commonly used in tyre and battery manufacturing have been studied for an association with PCs risk\textsuperscript{120}. The factors, however, that initiate PCs, unfortunately, still remain to be identified as an essential prelude to strategies designed to reduce disease occurrence\textsuperscript{121}.
Figure 1: Individual aggressive PCa risk association with SNPs, environmental factors as well as in combination of both compared to non-aggressive disease

In our study, we have certain indicators in terms of the external or environmental factors such as tobacco smoking, alcohol consumption, or high BMI associated with the initiation of this disease, i.e.
with non-aggressive PCa. However, when the data was corrected for these aspects using PLINK and reanalyzed, not one of the SNPs were identified as significantly associated with the disease. This may be because mammalian body is designed to fight unfavorable conditions and it is the external factors which influence the initiation of non-aggressive PCa.

4.4.2 Genotype and the outcome of aggressive prostate cancer (vs non-aggressive prostate cancer):

We corrected for the effect of three prominent risk factors for the prognosis and progression of PCa- BMI, tobacco smoking and alcohol consumption as risk factors for aggressive PCa (compared to non-aggressive PCa) using PLINK and then analyzed the data to identify a pure genic risk for the disease and are mentioned in Table 3.1. A wholistic approach of these external factors was taken into consideration while looking into the gene-environment interaction in our study.

Only few SNPs were identified to have statistically significant risk for aggressive PCa risk compared to that of non-aggressive disease (before the use of BONF). They have been illustrated in Figure 1. SNPs rs632148, rs10086908, rs887391, and rs16987929 were identified to be common both with genotypic risk as well as interacting with environmental risk. Common SNPs between these states indicate the crucial role of gene x environment interactions in both initiation and the progression of this disease.

4.4.3 Genotype and the outcome of aggressive prostate cancer (vs healthy controls):

We continued to analyze the effect of the three aforementioned risk factors on the genotype in our population for the prognosis of PCa by statistically correcting it using PLINK. The same wholistic approach as previously mentioned, in terms of the external factors were taken into consideration while looking into the gene-environment interaction in our study.

A higher number of SNPs were identified to have statistically significantly increased interactions with external factors in producing aggressive PCa compared to that of healthy controls (before the use of BONF) than the SNPs which potentially had a genic effect on the disease. They have been summarized in Figure 2.

Of the 9 SNPs that came up significantly associated with aggressive PCa after correcting for external factors in our population, only 2 SNPs were identified not to be repeats from the results already discussed in section 4.1.3. The higher number of SNPs identified in the gene x environment interaction category compared to in this category, provides statistical proof that gene x environment interactions play a crucial role in the progression of this disease. Of the SNPs that are identified in the genes, the expression of which may be independent of the environmental, or external factors (Table 3.2), were rs5845 and rs1799977.

The SNP rs5845 present in the gene SEP15 encoding for selenoprotein containing selenocysteine is involved in the quality control of protein folding. The A allele of this SNP has a unique effect on PCa risk even compared to benign urology disease. The SNP rs1799977 present in the gene MLH1, plays a major role in mismatch repair. The SNP rs10896469 present near MYEOV, a putative oncogene, as previously mentioned to be frequently amplified in a number of tumours.

Interestingly, certain SNPs showing significant associations as risk for aggressive PCa did not show significant associations when interacting with environmental factors. In contrast, the SNPs that showed the strongest evidence for interactions with environmental factors did not show significant in pure SNP effect associations.

This finding reveals that in a study like ours, it is very important to not only consider the SNPs identified by a GWAS, but also consider genotype (additive or dominant) by environmental (local to the population studied) interaction effects on aggressive PCa in addition to established associations. If this is avoided, such variants may be ignored. Therefore, the list of SNPs to be studied and eventually followed up for replication or confirmation changes with the genotype x environmental interaction effects should be extensive and not limited to just the ones reported in certain populations ignoring the local factors which may be pivotal for the final expression of genes.
We would also like to point out that, SNPs with statistically significant genotype x environmental interaction did not necessarily have a significant (or even suggestive) association with aggressive PCa.

Thus, limiting interaction tests to markers with significant main SNP effects would likely find different results, and will aid in identifying a much better localized cause of diseases, as has also been mentioned by Arya et al., (2009). Since the genetics of aggressive PCa is still largely unknown, we cannot comment whether this approach would lose power or would protect against false positives because the underlying genetic architecture of aggressive PCa is largely unknown and more research needs to be done in this field of study, but we believe this is the right direction to move forward.

**Figure 2:** Individual aggressive PCa risk association with SNPs, environmental factors as well as in combination of both compared to controls

5. Conclusions

There is an urgent need to develop a biomarker for PCa with high sensitivity, and specificity to an...
individual's risk of developing the aggressive form of the disease. A considerable number of patients with aggressive PCa are being under-treated. The results obtained are suggestive of certain genes being associated with aggressive PCa. The data generated, suggest that SNP genotyping as a screening tool, along with other prevalent diagnostic tools, has the potential to help identify men heading towards lethal aggressive PCa at an earlier stage.

Although epidemiological studies have shown an association with PCa, such that patients with diabetes mellitus have been suggested to be at a lower risk of PCa, or an inverse association, we did not find any direct and/or indirect effect of BMI on SNPs identified to have a risk association with non-aggressive PCa, but with aggressive PCa in our population. It has been proven that BMI has a strong association with diabetes mellitus and insulin resistance and with PCa. We believe that obesity is linked with diabetes mellitus and PCa alike, and it is not diabetes mellitus which has a direct relation with PCa, but obesity.

The fact that a number of SNPs in various genes were identified to be associated with a risk of prostate cancer calls for detailed work in this aspect for risk of PCa. We observed that the SNPs vulnerable to environmental conditions discussed here do not play a role in the initiation of the disease, and as the progression of PCa was mapped, we found an increasing role of environmental factors. It is also worthy of mention that the various external factors described here, such as increasing BMI, and wide-spread tobacco smoking in New Zealand, and deficiency of trace elements such as selenium in the New Zealand soil may be playing a much more important role in the expression and progression of PCa in our population than they have been previously credited for and need to be further looked into. These factors may be very crucial for the progression of the disease and our gene x environment logic is strengthened by the findings in our unique population.

We identified that the SNPs rs17793693 in the gene PPARγ and rs2727270 in the gene FADS2 were risk for aggressive PCa before correction for environmental factors was carried out. However, after correcting for multiple factors, these SNPs did not figure as a risk for aggressive PCa. We also identified the SNP rs4965373 in the gene SEPS1 to be significantly associated with risk for non-aggressive PCa (vs healthy controls), but, again, when corrected for, did not come up as a risk association for the disease. Interestingly, though, we identified another SNP rs5845 in another selenoprotein gene - SEP15 when we corrected the SNP association for aggressive PCa (vs healthy controls) for multiple factors. This is very unique to the environmental conditions in New Zealand, where a selenoprotein SNP may trigger the start of the disease, but is eventually controlled due to the lack of selenium in the New Zealand soil, and is effectively checked in the eventual progress of the disease, unlike if it is the case when proper intake of selenium is not practiced.

These curious associations and links leading to aggressive and non-aggressive PCa are better explained in the pictorial conclusion provided herewith (Figure 3).

Figure 3: Pictorial conclusion illustrating the various associations and links leading to aggressive and non-aggressive PCa.

Figure legends - Non-Agg: Non-aggressive PCa patients; Agg: Aggressive PCa patients; Con: Healthy Controls

Supplementary Materials:

Supplementary Table 1: Selected SNPs for present study.

Supplementary Table 2: Gene x environment association analysis of SNPs as risk for PCa.
Supplementary Table 3: SNP association with risk of PCa after correcting for environmental factors

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**Author Contributions:** V.V., N.K., and K.B., planned the experiment. V.V. wrote the manuscript. V.V., V.N., M.K., and A.N. conceived the idea for the results section (statistics). V.V., V.N. and A.N. did the data cleaning and statistical analysis. I.M. and M.G. carried out patient recruitment. S.Z. managed the database. V.V., C.H.-J.K., A.W., N.K., K.B., and P.S. did parts of the experiment. C.H.-J.K. did the graphical representations. V.V., N.K., A.J., G.M., R.P. and L.R.F. conceived the idea of the discussion chapters. All authors edited and proofread the manuscript.

**Conflicts of Interest:** The authors declare no conflict of interest.

**Abbreviations**

The following abbreviations (alphabetically) are used in this manuscript:

- ADD: additive
- AKR1C3: Aldo-keto reductase family 1 member C3
- BH-FDR: Benjamini and Hochberg false discovery rate
- BMI: body mass index
- BONF: Bonferroni correction(s)
- CCHCR1: coiled-coil alpha-helical rod protein1
- DOMDEV: dominant deviation
- df: degree of freedom
- ER: endoplasmic reticulum
- FADS2: Fatty acid desaturase 2
- GWAA: Genome-wide association analysis
- GWAS: Genome-wide association studies
- IBS: identical-by-state
- KLK3: Kallikrein-3
- LD: linkage disequilibrium
- LEP: Leptin
- MLH1: MutL homolog 1
- MLPH: Melanophilin
- MMP9: Matrix metalloproteinase 9
- mRNA: messenger-ribonucleic acid
- MSMB: Microsemninoprotein Beta
- MYEOV: Myeloma Overexpressed
- ng/μl: nanogram per microliter
- NUDT11: Nucleoside Diphosphate-linked Moiety X Motif 11
- PCA: prostate cancer
- PODXL: Podocalyxin-like
- PPAR-γ: Peroxisome proliferator-activated receptor gamma
- PSA: prostate-specific antigen
- SE15: Seleoprotein 15kDa
- SEPS1: Selenoprotein S
- SLC26A6: Solute carrier family 26 member 6
- SRD5A2: Steroid 5α-reductase type 2
- TLR4: Toll-like receptor 4

**References**


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