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Evaluation of a Variant in the Transcription Factor 7-like 2 (*TCF7L2*) Gene and Prostate Cancer Risk, Progression, and Mortality in a Population-Based Study

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Abstract

Transcription factor 7-like 2 (*TCF7L2*) is a high mobility group -box containing protein that is a critical member of the Wnt/ β -catenin signaling pathway. In addition to its recently recognized role in diabetes, aberrant *TCF7L2* expression has been implicated in cancer through regulation of cell proliferation and apoptosis by *c-MYC* and *cyclin D*. It has been hypothesized that germline variants within the *TCF7L2* gene previously associated with diabetes may affect cancer risk through the Wnt/ β -catenin signaling pathway. Specifically, the same risk allele of single nucleotide polymorphism (SNP) rs12255372 that is associated with diabetes (T allele) has recently been associated with an increased risk of breast cancer.

Here, we investigated associations between rs12255372 and prostate cancer risk among 597 cases and 548 controls from a population-based study. We also evaluated prostate cancer progression/recurrence and prostate-specific mortality among these cases under long-term surveillance. The risk allele was associated with cancer progression/recurrence after primary treatment [hazard ratio (HR) = 1.48, 95% confidence interval (CI) = 1.01–2.19], but this association was attenuated when the model was further adjusted for Gleason score and tumor stage. There were no associations between this SNP and the risk of developing disease or prostate cancer-specific mortality. Our findings suggest that this variant in the *TCF7L2* gene may enhance tumor progression or metastasis, but it does not contribute significantly to tumor initiation. These findings are of clinical importance for identification of patients that may develop more aggressive/recurrent disease after primary treatment.

Introduction

Prostate cancer is the most common solid tumor in men in the United States and the second most common cause of death related to cancer (1). Studies of selected hospital-based patient populations (2,3), population-based case control studies (4–7), and cohort studies (7,8) all

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demonstrate that a family history of disease increases an individual's risk. If the affected family member is a first-degree relative (i.e., brother or father, or son), the risk increases substantially from 1.7 to 3.7-fold. Younger ages at diagnosis and multiple affected relatives with the disease tend to be associated with even higher relative risk (2). The disease has a complex etiology, with genetic studies suggesting that multiple genes are important in the onset, progression, and treatment response (9-14).

The Wnt signaling pathway plays a critical role in normal developmental and processes as well as in human carcinogenesis (15). Activation of this pathway originates when secreted Wnt ligands bind a transmembrane protein complex consisting of low density lipoprotein receptor related protein (LRP) and frizzled (Fz), which in turn induces activation of the intracellular protein disheveled (DSH). This in turn leads to a series of molecular events involving β -catenin, axin, adenomaous polyposis coli (APC), and glycogen synthase kinase 3 β (GSK-3 β), that in the absence of Wnt stimulation target β -catenin for degradation. Aberrant activation of this pathway due to mutations in key components, such as the *APC* or *β -catenin* genes, has been described in colorectal (16,17), breast (18), head, neck (19), and oral (20) cancers, as well as in melanoma (21). Similar mutations have also been detected in a subset of prostate cancers (22-24). However, such mutations were found in only a small fraction of prostate tumors studied.

Transcription factor 7-like 2 (TCF7L2) is an important component of the Wnt signaling pathway. β -catenin combines with TCF7L2 to form an active nuclear complex that binds and induces the expression of target genes involved in cellular proliferation, evasion of apoptosis, and tissue invasion and metastasis. In addition, it was recently shown that the androgen receptor (*AR*) gene is also a target for transcriptional activation of the TCF7L2- β -catenin complex (25). As a mediator of the steroid hormones testosterone and dihydrotestosterone, AR is an essential regulator of prostate growth and function. It has also been demonstrated that β -catenin, as well as TCF7L2 protein, can induce the expression of androgen regulated genes by binding to the AR (26,27). Considering the numerous interactions between Wnt and androgen signaling pathways, together with the growth regulatory role of TCF7L2 protein, we hypothesize that variation in the *TCF7L2* gene can influence the development and progression of prostate cancer.

Recently the *T*, as opposed to *G* allele, of a single nucleotide polymorphism (SNP), rs12255372, which is located within intron four of the *TCF7L2* gene was found to be significantly overrepresented in familial breast cancer cases (28). This variant likely plays a role in several cellular pathways as it has been shown, together with a strongly linked microsatellite marker termed DG10S478, to associate with type 2 diabetes (29-33). Some studies have suggested that diabetes may decrease the risk of developing prostate cancer (34-37). Here, we investigated the relationships between the rs12255372 SNP and prostate cancer risk in 597 prostate cancer cases and 548 age frequency-matched controls from a population-based case control study of middle-aged men, as well as with cancer progression/recurrence and prostate-specific mortality among these cases with long-term surveillance.

Materials and Methods

Study population

Five hundred and ninety-seven Caucasian prostate cancer cases, aged 40 to 64 years, and 548 Caucasian population-based frequency aged-matched controls, residing in King County, Washington, served as study subjects for this analysis. These Caucasian men represent a subset of 753 cases and 703 controls who participated in a population-based case control study of risk factors for prostate cancer that we have previously described (38), and who provided a blood sample at interview. Incident cases were diagnosed between January 1st, 1993 and December

31st, 1996 and identified via the population-based Seattle-Puget Sound Surveillance Epidemiology and End Results (SEER) cancer registry. Controls were men without a self-reported history of prostate cancer that were recruited via random digit dialing during the same ascertainment period, and frequency matched to cases by 5-year age groups. Background information including demographic and lifestyle factors, medical history, prostate cancer screening history, and family history of prostate cancer was collected from the participants at interview. Clinical information such as Gleason score, tumor stage, and serum prostate specific antigen (PSA) levels at diagnosis, as well as primary treatment was obtained from the SEER cancer registry.

Prostate cancer cases are under long-term surveillance for mortality via the Seattle-Puget Sound SEER cancer registry. The patient file is linked to the registry on a quarterly basis to ascertain vital status and primary cause of death. For each deceased patient, a death certificate is requested from the state where the patient died to confirm the underlying cause of death. The date of last follow-up for vital status as it pertains to this work was December 1st, 2006. In addition, in January 2004, a follow-up survey about prostate cancer progression/recurrence and secondary therapies was sent to consenting cases. The follow-up questionnaire asked about quality of life and secondary therapies received after prostate cancer diagnosis and initial treatment, follow-up PSA results, including the date of the most recent test, and requested information about any physician's diagnosis or diagnostic work-up for detection of prostate cancer progression/recurrence. The follow-up survey was completed by 82.4% of cases, 7.2% refused to participate, 9.0% were lost to follow-up, and 1.4% of men alive in January 2004 when the survey was mailed, had recently died. Men who did not complete a follow-up survey were more likely to be younger (<50 years) at prostate cancer diagnosis, but there were no differences between responders and non-responders with regard to clinical characteristics such as tumor stage, Gleason score, or primary therapy of cancer (data not shown). The institutional review boards of the Fred Hutchinson Cancer Research Center and the National Human Genome Research Institute approved all study procedures and materials, and study subjects gave written informed consent prior to participation.

Genetic analysis

Genotypes of the *TCF7L2* rs12255372 SNP were determined using the TaqMan allelic discrimination assay (Applied Biosystems, Foster City, CA). Ten ng of genomic DNA was amplified in a reaction volume of 10 μ l in the presence of TaqMan Universal Master Mix with no AmpErase UNG, PCR primers, and fluorescently labeled allele specific probes obtained from Applied Biosystems (Foster City, CA). The amplification was carried out using the following conditions: 95°C for 10 minutes, then 40 cycles of 15 s at 95°C and 1 minute at 60°C. An endpoint plate read was performed using the 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA) and the alleles were automatically called using Sequence Detection System software. There was 100% agreement for the rs12255372 genotypes based on 60 blind duplicates randomly distributed across genotyping batches.

Prostate cancer risk analysis

Deviation of genotype frequencies from Hardy-Weinberg Equilibrium (HWE) among Caucasian controls was assessed by a χ^2 -test (39). Unconditional logistic regression was used to examine the relationship between the *TCF7L2* SNP (rs12255372) and prostate cancer risk among Caucasian men, to compute odds ratios (OR) and 95% confidence intervals (CI) (40). First, we examined the association between this SNP and prostate cancer risk adjusting for age at diagnosis; then analyses were further adjusted for first-degree family history of prostate cancer, prostate cancer screening history, and self-reported history of diabetes. Goodness-of-fit was assessed by likelihood ratio statistics of nested models. In addition, the association between the *TCF7L2* SNP (rs12255372) and prostate cancer risk was examined among strata

defined by first-degree family history of prostate cancer (yes vs. no), body mass index (BMI) (obese: BMI ≥ 30 kg/m² vs. non-obese: BMI < 30 kg/m²), and self-reported history of diabetes (yes vs. no).

We also examined the association between the *TCF7L2* SNP (rs12255372) and prostate cancer risk according to strata defined by Gleason score, stage of cancer, and a composite measure of disease aggressiveness. Gleason scores were obtained from biopsy reports (29.5%) or from surgical pathology reports (70.5%). For these analyses prostate cancer cases were grouped into two strata: those with Gleason scores of 2-6 or 7=3+4, and those with Gleason scores of 7=4+3 or 8-10. For cancer stage, we grouped cases with regional and distant stage together and compared them to men with localized stage. The definition of more aggressive prostate cancer included a Gleason score of 7=4+3 or 8-10 or regional or distant stage or a serum PSA ≥ 20 ng/ml at diagnosis; tumors of cases with a Gleason score of 2-6 or 7=3+4, and localized stage and a serum PSA < 20 ng/ml were defined as less aggressive. The frequency of genotypes for the rs12255372 in each group of cases was compared to that of controls using polytomous logistic regression (41).

Prostate cancer-specific mortality and progression/recurrence analyses

The outcomes evaluated in these analyses were deaths from prostate cancer and prostate cancer progression/recurrence. For prostate cancer mortality, the survival time, i.e., time elapsed from diagnosis until death, was the time dependent variable used in the analysis. Living cases were censored as of December 1st, 2006 since this was the most recent date that participants were matched with the cancer registry database. The definition of prostate cancer progression/recurrence included several criteria with information obtained from the self-administered survey: a physician's diagnosis of prostate cancer progression/recurrence, a positive bone scan, biopsy or magnetic resonance imaging (MRI) showing prostate cancer after primary treatment for the disease, or a PSA value of > 0.2 in men who received radical prostatectomy as primary treatment. Some men fulfilled two or more of these criteria. Patients who completed a survey, but were initially diagnosed with distant stage disease, were excluded from the analyses of progression/recurrence. Time elapsed from diagnosis until progression/recurrence was calculated as the difference between the date of reported evidence of progression/recurrence and the date of diagnosis. A patient was also classified as having progression/recurrence if he died of metastatic prostate cancer prior to the follow-up survey and if he was initially diagnosed with localized or regional stage disease; no date of prostate cancer progression/recurrence was available for these men. In order to include them in analyses, we used a method developed by Robins and colleagues (42) that predicts the probability of missing time to prostate cancer progression/recurrence based on baseline data regressors. After this procedure, for the prostate cancer progression/recurrence analyses, weighted Cox's proportional hazard models were fit where the weights were the inverse of the probability of having a recurrence date (for men with progression/recurrence) and a weight of one was assigned for those men who did not have the event of interest (42).

Cox's proportional hazard models were used to examine associations between the rs12255372 SNP and prostate cancer outcomes and to estimate hazard ratios (HR) and 95% CI (43). First, we examined associations between the SNP and prostate cancer outcomes (specific mortality, progression/recurrence) adjusting for age at diagnosis. Analyses were also adjusted for other variables related to survival and progression/recurrence such as tumor stage, Gleason score, and co-morbid conditions (diabetes, cirrhosis, heart attack, and high blood pressure). SAS Version 9.1 was used for the statistical analyses.

Results

Prostate cancer risk

Table 1 shows the characteristics of prostate cancer cases and controls that were genotyped for the *TCF7L2* SNP (rs12255372). The reported prevalence of diabetes was about 5% in this population and was similar between cases and controls; 15.7% of cases and 17.0% of controls were obese (BMI ≥ 30 kg/m²). The allele frequency of the SNP rs12255372 among controls was consistent with HWE ($\chi^2=0.08$, $P=0.78$). We found no association between this SNP and overall prostate cancer risk (Table 2). A total of 11.5% of cases who had a first-degree family member with prostate cancer had a TT genotype, as opposed to 6.0% of cases without a family history of prostate cancer. However, no association was found between this SNP and prostate cancer risk in analyses stratified by first-degree family history of prostate cancer. Since previous reports have suggested an association between the T allele of the rs12255372 SNP and diabetes (29-33), we also stratified the analyses by self-reported history of diabetes as well as BMI (obese vs. non-obese). Among prostate cancer cases with a history of diabetes (n=31), the frequency of the TT genotype was 3.2% in comparison to 7.4% among controls, resulting in an OR of 0.26 (95% CI=0.02-3.46). Among men with no history of diabetes we observed a slightly lower OR of 0.76 (95% CI=0.46-1.27) among men with the TT genotype, however as with the subgroup with diabetes, the sample sizes were small and the differences were not significant. When the analysis was stratified by BMI, we observed no difference between the two subgroups according to genotype and risk for prostate cancer.

Next we examined associations between the *TCF7L2* SNP and prostate cancer risk by clinical characteristics of disease (Table 3). A total of 9.2% and 6.8% of cases with high and low Gleason score, respectively, carried the TT genotype. Furthermore, 11.0% of cases with either regional or distant cancer stage had the TT genotype, compared to only 5.5% of those with a local disease. The definition of more aggressive prostate cancer included a Gleason score of 7=4+3 or 8-10 or regional or distant stage or a serum PSA ≥ 20 ng/ml at diagnosis. Within the aggressive disease group, 8.9% of the cases had the TT genotype, compared to 6.1% from the less aggressive disease group. The proportion of controls with the TT genotype was 8.1%, resulting in no statistically significant increases in odds ratios for the associations between this SNP and clinical features of prostate cancer (Table 3). However, there was a borderline inverse association between the TT genotype and localized stage cancer (OR=0.69, 95% CI=0.47-1.01) after adjusting for age, first-degree family history of prostate cancer, prostate cancer screening, and history of diabetes.

Prostate cancer progression/recurrence and mortality

Table 4 highlights the distribution of demographic and clinical characteristics of prostate cancer cases by vital status and progression/recurrence status. There were 37 prostate cancer-specific deaths and 112 prostate cancer progression/recurrence events during the average 10.1 years of follow-up after diagnosis. Patients who died of prostate cancer were younger at diagnosis and were more likely to have more aggressive disease characteristics (35.1% with distant stage, 59.5% with a Gleason score of 7=4+3 or 8-10). They were also more likely to have had no prostate cancer screening in the five years before diagnosis (21.6%) and to have had non-surgical treatment (37.8% androgen deprivation therapy only). Men with prostate cancer progression/recurrence were also more likely to be younger at diagnosis and to have been diagnosed with regional stage disease (41.1%) or to have a Gleason score of 7=4+3 or 8-10 (29.5%) at diagnosis. There was no difference in BMI compared to those who had no evidence of prostate cancer progression/recurrence ($P=0.7$).

Table 5 shows results of Cox's proportional hazards models for prostate cancer-specific death and prostate cancer progression/recurrence and rs12255372 genotypes. There was some

evidence for an association between the *T* allele and prostate cancer progression/recurrence after adjusting for age at diagnosis (HR=1.48, 95% CI=1.01-2.19). However, when the model was additionally adjusted for Gleason score, stage of cancer, and co-morbid conditions, the association between the GT or TT genotype and prostate cancer progression/recurrence was attenuated and was not statistically significant (HR=1.15, 95% CI=0.77-1.72). By contrast, men carrying any *T* allele had a 58% reduction in prostate cancer-specific mortality (HR=0.42, 95% CI=0.19-0.89) after adjusting for age, Gleason score, tumor stage, and co-morbid conditions.

Discussion

We found no evidence that prostate cancer risk is associated with carrying the *T* allele of the *TCF7L2* rs12255372 SNP. However, the *T* allele was found to associate with cancer progression or recurrence after primary treatment. These results suggest that the variant may increase proliferation of transformed cells and the tumor metastatic potential or/and decrease apoptosis of malignant cells, rather than contribute to the tumor initiation. This association disappeared when the analysis was further adjusted for Gleason score, stage of cancer, and co-morbid conditions. This clearly indicates that Gleason score and tumor stage contribute independently to the observed elevated risk of prostate cancer progression/recurrence; however, we cannot exclude the possible relationship between the *T* allele and cancer grade or stage. Indeed, the proportion of cases with the TT genotype was greater in the subset of patients with high Gleason scores (9.2%), regional/distant stage (11.0%), or more aggressive disease (8.9%) relative to those with low Gleason scores (6.8%), localized stage (5.5%), and less aggressive disease (6.1%). However, the proportion of controls with the TT genotype was 8.1%, resulting in no statistically significant increases in odds ratios for the associations between this SNP and clinical features of prostate cancer. The global *P*-values for the associations between SNP (rs12255372) and Gleason score was *P*=0.53, for tumor stage was *P*=0.19, and for cancer aggressiveness was *P*=0.51.

These findings suggest that the *T* allele of this SNP (rs12255372) may favor the progression of prostate cancer after primary treatment. The definition of prostate cancer progression/recurrence used in the present analysis included several different criteria obtained from the follow-up survey on the basis of different diagnostic and treatment procedures. To explore the association between this SNP and subgroups of progression or recurrence we stratified the analysis in different ways. When the analysis was restricted to men who had radical prostatectomy as primary treatment for the cancer (n=317), 14.5% of patients showed evidence of recurrent prostate cancer based on PSA failure (PSA >0.2 ng/ml) after the surgery. There was no association between the *T* allele and PSA failure after a surgical removal of the entire prostate, suggesting that the genotype may not affect prostate cancer recurrence. In contrast, when the analysis was restricted to men who reported to have had a positive bone scan, biopsy or MRI showing prostate cancer after primary treatment (36 men out of 422 receiving a treatment), there was an increased risk of metastasis associated with carrying the *T* allele (HR=1.88, 95% CI=1.06-3.42). However, the carriers of *T* allele had a lower risk of dying of prostate cancer compared to non-carriers was inversely associated with risk of prostate cancer-specific mortality (HR=0.42, 95% CI=0.19-0.89) after adjusting for age, Gleason score, tumor stage, and co-morbid conditions.

This is the first study to investigate the relationships between the *TCF7L2* rs12255372 polymorphism and prostate cancer. Burwinkel and colleagues (28) reported a modest increase in risk of familial breast cancer (OR=1.19, 95% CI=1.01-1.42, *P*=0.04) and the *T* allele of the same variant in the *TCF7L2* gene. In addition to the difference in malignancy type (breast vs. prostate), there are additional differences between these two studies. The cases in the study of Burwinkel et al. were German patients with a higher proportion (92%) of positive family history

of breast cancer. In contrast, prostate cancer cases in the present study were ascertained from a population-based SEER cancer registry and most of them (81%) did not have a family history of prostate cancer. Further studies examining associations between *TCF7L2* gene and hereditary prostate cancer (HPC) in high-risk HPC families are needed and the precise mechanism of action of this genetic variant in the process of tumorigenesis and metastasis remains to be clarified.

In conclusion, our findings do not support the hypothesis that the *TCF7L2* rs12255372 SNP increases susceptibility to sporadic prostate cancer. However, there was some suggestion that the *T* allele may be associated with prostate cancer progression and metastasis. More detailed investigation in a much larger study is needed to confirm this observation and to further understand the cellular mechanisms behind this observation.

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Table 1

Characteristics of prostate cancer cases and controls

Characteristics	Cases (n=597)		Controls (n=548)	
	n	%	n	%
Age at reference date, (years)				
40 - 49	36	6.0%	48	8.8%
50 - 54	125	20.9%	110	20.1%
55 - 59	201	33.7%	206	37.6%
60 - 64	235	39.4%	184	33.6%
First-degree family history of prostate cancer				
No	482	80.7%	491	89.6%
Yes	115	19.3%	57	10.4%
Body mass index at diagnosis, kg/m ²				
<25	212	35.5%	183	33.4%
25.0 - 29.9	291	48.7%	272	49.6%
≥30	94	15.7%	93	17.0%
Smoking status at diagnosis				
Non-smoker	215	36.0%	209	38.1%
Former smoker	287	48.1%	253	46.2%
Current smoker	95	15.9%	86	15.7%
Self-reported history of diabetes				
No	565	94.6%	521	95.1%
Yes	32	5.4%	27	4.9%
Prostate cancer screening history [*]				
None	21	3.5%	31	5.7%
DRE only	137	22.9%	327	59.7%
PSA & DRE or PSA only	439	73.5%	190	34.6%
Gleason score				
2 - 4	63	10.6%		
5 - 6	307	51.4%		
7=3+4	147	24.6%		
7=4+3	25	4.2%		
8 - 10	52	8.7%		
Missing	3	0.5%		
Stage of cancer				
Local	433	72.5%		
Regional	140	23.5%		
Distant	18	3.0%		
Missing	6	1.0%		
Prostate cancer aggressiveness [†]				
Less aggressive	390	65.3%		
More aggressive	207	34.7%		

Abbreviations: DRE - digital rectal examination; PSA - prostate specific antigen.

^{*} Prostate cancer screening history in the 5 years before prostate cancer diagnosis.[†] Less aggressive prostate cancer includes Gleason scores 2-6 or 7 (3+4) and local stage and PSA <20 ng/ml at diagnosis; More aggressive prostate cancer includes Gleason Scores 7 (4+3) or 8-10 or regional or distant cancer stage or a serum PSA ≥20 ng/ml at diagnosis.

Table 2 Association between *TCF7L2* SNP (rs 12255372) and prostate cancer risk*

Overall									
Cases (n=582)		Controls (n=540)		OR ¹		95% CI		OR ²	
Genotype	n	n	%	OR ¹	95% CI	OR ²	95% CI	OR ²	95% CI
GG	306	271	50.2%	1.00	ref	1.00	ref	1.00	ref
GT	235	225	41.7%	0.92	0.72-1.17	0.94	0.72-1.23		
TT	41	44	8.1%	0.82	0.52-1.30	0.73	0.44-1.20		
First-degree family history of prostate cancer									
Cases (n=113)		Controls (n=57)		OR ¹		95% CI		OR ²	
Genotype	n	n	%	OR ¹	95% CI	OR ²	95% CI	OR ²	95% CI
GG	53	46	9%	1.00	ref	1.00	ref	1.00	ref
GT	47	22	38.6%	1.10	0.55-2.21	1.15	0.64-3.12		
TT	13	7	12.3%	0.98	0.35-2.73	0.88	0.26-2.83		
No first-degree family history of prostate cancer									
Cases (n=469)		Controls (n=483)		OR ¹		95% CI		OR ²	
Genotype	n	n	%	OR ¹	95% CI	OR ²	95% CI	OR ²	95% CI
GG	253	243	50.3%	1.00	ref	1.00	ref	1.00	ref
GT	188	203	42.0%	0.89	0.68-1.16	0.90	0.67-1.20		
TT	28	37	7.7%	0.73	0.43-1.23	0.75	0.42-1.32		
Body mass index <30 kg/m ²									
Cases (n=488)		Controls (n=450)		OR ¹		95% CI		OR ²	
Genotype	n	n	%	OR ¹	95% CI	OR ²	95% CI	OR ²	95% CI
GG	259	226	50.2%	1.00	ref	1.00	ref	1.00	ref
GT	196	185	41.1%	0.92	0.70-1.21	0.97	0.72-1.30		
TT	33	39	8.7%	0.74	0.45-1.22	0.67	0.39-1.16		
Body mass index >30 kg/m ²									
Cases (n=94)		Controls (n=90)		OR ¹		95% CI		OR ²	
Genotype	n	n	%	OR ¹	95% CI	OR ²	95% CI	OR ²	95% CI
GG	47	45	50.0%	1.00	ref	1.00	ref	1.00	ref
GT	39	40	44.4%	0.91	0.50-1.67	0.83	0.41-1.66		
TT	8	5	5.6%	1.52	0.46-5.01	1.13	0.29-4.44		
Men with a history of diabetes									
Cases (n=31)		Controls (n=27)		OR ¹		95% CI		OR ²	
Genotype	n	n	%	OR ¹	95% CI	OR ²	95% CI	OR ²	95% CI
GG	14	9	33.3%	1.00	ref				
GT	16	16	59.3%	0.58	0.19-1.78				
TT	1	2	7.4%	0.26	0.02-3.46				
Men with no history of diabetes									
Cases (n=551)		Controls (n=513)		OR ¹		95% CI		OR ²	
Genotype	n	n	%	OR ¹	95% CI	OR ²	95% CI	OR ²	95% CI
GG	292	262	51.1%	1.00	ref	1.00	ref	1.00	ref
GT	219	209	40.7%	0.93	0.73-1.20	0.95	0.72-1.25		
TT	40	42	8.2%	0.86	0.54-1.37	0.76	0.46-1.27		

Abbreviations: OR - odds ratio; CI - confidence interval.

* 15 cases and 8 controls have missing data for the *TCF7L2* SNP (rs 12255372).

¹ OR - model is adjusted for age.

² OR - model is adjusted for age, first-degree family history of prostate cancer, prostate cancer screening history, and history of diabetes.

³ OR - model is adjusted for age, prostate cancer screening history, and history of diabetes.

⁴ OR - model is adjusted for age and prostate cancer screening history.

Table 3
Association between *TCF7L2* SNP (rs 12255372) and prostate cancer risk by clinical characteristics*

Gleason score	Controls (n=540)		Cases: Gleason 2-6, 7=3+4 (n=503)				Cases: Gleason 7=4+3, 8-10 (n=76)			
	n	%	n	%	OR [†]	95% CI	n	%	OR [†]	95% CI
Genotype										
GG	271	50.2%	270	53.7%	1.00	ref	36	47.4%	1.00	ref
GT	225	41.7%	199	39.6%	1.01	0.81-1.22	107	140.86-1.34	0.99	0.68-1.45
TT	44	8.1%	34	6.8%	0.88	0.65-1.20	7	9.2%	1.10	0.63-1.92
Stage of cancer										
Controls (n=540)										
Genotype										
GG	271	50.2%	224	53.1%	1.00	ref	78	50.6%	1.00	ref
GT	225	41.7%	175	41.5%	1.11	0.89-1.39	59	38.3%	0.85	0.64-1.12
TT	44	8.1%	23	5.5%	0.75	0.53-1.07	17	11.0%	1.26	0.85-1.86
Disease aggressiveness [‡]										
Controls (n=540)										
Genotype										
GG	271	50.2%	205	54.1%	1.00	ref	101	49.8%	1.00	ref
GT	225	41.7%	151	39.8%	1.04	0.83-1.30	84	41.4%	0.97	0.75-1.25
TT	44	8.1%	23	6.1%	0.82	0.58-1.16	18	8.9%	1.06	0.73-1.56

Abbreviations: OR - odds ratio; CI - confidence interval.

* 15 cases and 8 controls have missing data for the *TCF7L2* SNP (rs 12255372).

[‡] Less aggressive prostate cancer includes Gleason scores 2-6 or 7 (3+4) and local stage and PSA <20 ng/ml at diagnosis; More aggressive prostate cancer includes Gleason Scores 7 (4+3) or 8-10 or regional or distant cancer stage or a serum PSA ≥20 ng/ml at diagnosis.

[†] OR - model is adjusted for age.

² OR - model is adjusted for age, first-degree family history of prostate cancer, prostate cancer screening history, and history of diabetes.

Selected demographic and clinical characteristics of prostate cancer patients by vital status and by prostate cancer progression/recurrence

Table 4

Characteristics	Vital status			Prostate cancer progression/recurrence*		
	Prostate cancer deaths (n=37) n(%)	Other deaths n=48 n (%)	Alive n=512 n (%)	P-value	Yes† n=112 n(%)	No n=344 n(%)
Age at diagnosis (years)						
40 - 49	8 (21.6)	2 (4.2)	26 (5.1)	0.0004	12 (10.7)	11 (3.2)
50 - 54	5 (13.5)	4 (8.3)	116 (22.6)		19 (17.0)	75 (21.8)
55 - 59	11 (29.7)	16 (33.3)	174 (34.0)		38 (33.9)	122 (35.5)
60 - 64	13 (35.1)	26 (54.2)	196 (38.3)		43 (38.4)	136 (39.5)
First-degree family history of prostate cancer						
No	35 (95.0)	39 (81.3)	408 (79.7)	0.08	98 (87.5)	265 (77.0)
Yes	2 (5.0)	9 (18.7)	104 (20.3)		14 (12.5)	79 (23.0)
Smoking status at diagnosis						
Non smoker	13 (35.1)	8 (16.7)	194 (37.9)	0.003	35 (31.3)	141 (41.0)
Former smoker	15 (40.6)	25 (52.1)	247 (48.3)		55 (49.1)	166 (48.3)
Current smoker	9 (24.3)	15 (31.2)	71 (13.8)		22 (19.6)	37 (10.8)
Body mass index at diagnosis (kg/m ²)						
<25	13 (35.1)	11 (22.9)	188 (36.7)	0.23	41 (36.6)	123 (35.8)
25.0-29.9	16 (43.2)	26 (54.2)	249 (48.6)		51 (45.5)	170 (49.2)
≥30	8 (21.6)	11 (22.9)	75 (14.7)		20 (17.9)	51 (14.8)
History of diabetes						
No	36 (97.3)	42 (87.5)	487 (95.1)	0.22	109 (97.3)	327 (95.1)
Yes	1 (2.7)	6 (18.5)	25 (4.9)		3 (2.7)	17 (4.9)
Prostate cancer screening history‡						
None	8 (21.6)	1 (2.1)	12 (2.3)	<0.001	5 (4.5)	9 (2.6)
DRE only	10 (27.0)	17 (35.4)	110 (21.5)		31 (27.7)	60 (17.4)
PSA & DRE or PSA only	19 (51.4)	30 (62.5)	390 (76.2)		76 (67.9)	275 (79.9)
Stage of cancer at diagnosis						
Local	7 (18.9)	31 (64.6)	395 (77.2)	<0.001	65 (58.0)	270 (78.5)
Regional	17 (46.0)	16 (33.3)	107 (20.9)		46 (41.1)	69 (20.1)
Distant	13 (35.1)	1 (2.1)	4 (0.8)		-	-
Missing	-	-	6 (1.2)		1 (0.9)	5 (1.5)
Gleason score at diagnosis						
2 - 4	-	8 (16.7)	55 (10.7)	<0.001	3 (2.7)	39 (11.3)
5 - 6	5 (13.5)	20 (41.7)	282 (55.1)		42 (37.5)	201 (58.4)
7=3+4	8 (21.6)	12 (25.0)	127 (24.8)		33 (29.5)	83 (24.1)
7=4+3	5 (13.5)	2 (4.2)	18 (3.5)		7 (6.3)	10 (2.9)
8 - 10	17 (46.0)	6 (12.5)	29 (5.7)		26 (23.2)	11 (3.2)
Missing	2 (5.4)	-	1 (0.2)		1 (0.9)	-
Primary treatment of cancer,						
Radical prostatectomy	10 (27.0)	25 (52.1)	386 (75.4)	<0.001	77 (68.8)	257 (74.7)
Radiation or radiation & ADT	13 (35.1)	13 (27.1)	73 (14.3)		23 (20.5)	53 (15.4)
ADT only	14 (37.8)	5 (10.4)	14 (2.7)		8 (7.1)	9 (2.6)
Other treatment	-	-	7 (1.4)		1 (0.9)	5 (1.5)
Watchful waiting	-	5 (10.4)	32 (6.3)		3 (2.7)	20 (5.8)

Abbreviations: ADT - androgen deprivation therapy; DRE - digital rectal examination; MRI - magnetic resonance imaging; PC - prostate cancer; PSA - prostate specific antigen.

* P-values based on the χ^2 -test.

[†] Category includes a self-reported physician's diagnosis of prostate cancer recurrence/progression, a positive bone scan, biopsy or MRI showing cancer after primary treatment, a PSA value >0.2 after radical prostatectomy; category also includes men diagnosed with localized or regional stage disease who died of prostate cancer prior to the follow-up survey.

[†]Prostate cancer screening in the 5 years before diagnosis.

Association between *TCF7L2* SNP (rs12255372) and prostate cancer-specific mortality and prostate cancer progression/recurrence

Table 5

Prostate cancer-specific mortality				
Genotype	Censored Events	HR ¹	95% CI	95% CI
GG	285	21	1.00	ref
GT	227	8	0.49	0.22-1.10
TT	36	5	1.73	0.65-4.58
GT or TT	263	13	0.67	0.34-1.35
Prostate cancer progression/recurrence				
Genotype	Censored Events	HR ¹	95% CI	95% CI
GG	184	52	1.00	ref
GT	126	48	1.62	1.09-2.40
TT	25	8	0.74	0.28-1.95
GT or TT	151	56	1.48	1.01-2.19

*15 cases have missing data for the *TCF7L2* SNP (rs12255372).

Abbreviations: HR - hazard ratio; CI - confidence interval.

¹ HR - model is adjusted for age at diagnosis.
² HR - model is adjusted for age at diagnosis, Gleason score, stage of cancer, and co-morbid conditions (diabetes, cirrhosis, high blood pressure, and heart attack).