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Protein expressions and genetic variations of SLC5A8 in prostate cancer risk and aggressiveness

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Abstract

OBJECTIVES—Previous studies suggest that *SLC5A8* may function as a tumor suppressor gene whose silencing by epigenetic changes may contribute to carcinogenesis. To understand a role in prostate cancer risk and aggressiveness, we investigated expression in prostate tumor and single nucleotide polymorphisms (SNPs) of *SLC5A8*.

METHODS—We constructed tissue microarrays (TMAs) from 183 prostate tumor tissues, 43 adjacent non-neoplastic tissues from the same prostate cancer patients, and 13 tissues from patients with benign prostatic hyperplasia (BPH) or prostate intraepithelial neoplasia (PIN). A semi-quantitative assessment of SLC5A8 protein expression was determined as the product of immuno-stain intensity and percentage of cells stained. In addition, we compared the frequencies of four SNPs (rs164365, rs1709189, rs1399236, and rs1681096) in *SLC5A8* between 668 prostate cancer cases and 385 controls.

RESULTS—SLC5A8 expression was significantly higher in tumor tissues than in paired non-neoplastic tissues ($p<0.0001$). In the Moffitt samples, we observed a borderline moderate risk increase in individuals with a genotype containing at least one ‘A’ allele of rs164365 (OR=1.35, 95%=1.00–1.80), especially among tall individuals (≥70 inches) (OR=1.80, 95%=1.20–2.68). However, these results were not confirmed in the CGEMS population.

Conclusions—These data suggest that expression pattern of SLC5A8 may be used as a diagnostic biomarker, and a larger study is required to assess the importance of SLC5A8 SNPs in prostate cancer.

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Keywords

Prostate cancer; SLC5A8; tissue microarray; polymorphism

INTRODUCTION

Solute carrier family 5 (SLC5) is a solute-linked carrier gene family that contains 12 sodium-coupled transporters for several chemicals[1]. SLC5A8 is a sodium-coupled transporter for nicotinate and analogues[2], lactate[3], and short-chain fatty acids[4–7]. Among these substrates, butyrate, propionate, and pyruvate function as histone deacetylase (HDAC) inhibitors. There is evidence that *SLC5A8* functions as a tumor suppressor gene that when silenced may contribute to carcinogenesis and progression of tumors[6,8]. For example, low expression of *SLC5A8* is associated with tumor status and poor prognosis in brain[9], colon[8], thyroid[10], gastric[11], breast[5], lung[12], pancreas[13], head and neck[14], acute myeloid leukemia[15] and prostate[16] cancer. Hypermethylation in the promoter region of *SLC5A8* is associated with disease progression features, including target tissue invasion, lymphangiogenesis, tumor multifocality and advanced tumor stage[17]. However, little is known about the significance of protein expression and inherited genetic variations of SLC5A8 in prostate tumors. In the present study we examined the potential role of SLC5A8 as a risk factor of prostate cancer risk and aggressiveness by assessing expression pattern of the SLC5A8 protein in prostate cancer and paired tissues, and comparing *SLC5A8* genotypes in prostate cancer patients and non-cancer controls.

PATIENTS AND METHODS

Tissue Microarray (TMA) Study Population

We investigated SLC5A8 protein expression analysis using immunohistochemistry and the TMA technique. The TMAs were constructed using prostate tumor tissues from 183 surgically resected prostate cancer patients who underwent a radical prostatectomy at Moffitt Cancer Center. For the TMAs from non-neoplastic prostate samples, 43 tissues (19 BPH and 24 PIN) were taken from areas adjacent to tumors of the 183 radical prostatectomy specimens, and 5 BPH tissues and 8 PIN tissues were taken from non-neoplastic patients[18].

Immunohistochemistry

TMA slide was immunostained using the avidin-biotin-peroxidase method, and after blocking with universal blocking serum (DAKO Diagnostic, Ontario, Canada) for 30min, they were incubated with a rabbit polyclonal anti-SLC5A8 antibody (dilution 1:600) at 4°C overnight. The characteristics of this antibody have been described previously[3,7]. Then biotin-labeled secondary antibody and streptavidin-peroxidase were applied for 30min each (DAKO Diagnostic). Tissues were developed with a 3, 3′-diaminobenzidine substrate (Vector Laboratories, Ontario, Canada) and counterstained with hematoxylin. Negative controls were created by omitting the anti-SLC5A8 antibody during primary antibody incubation. A single pathologist (DC), blinded to tissue origins, semi-quantitatively scored the stain considering intensity of SLC5A8 and percent of cells stained, as previously described[19]. In case of a disagreement between duplicate cores, the higher of the two scores was used as the measure of SLC5A8 protein expression.

Moffitt Case-Control Study Populations

Institutional Review Board approvals were obtained for the study protocol at each institution. Signed informed consent was obtained from all study participants. A total of 668 incident cases (587 Caucasians, and 81 African Americans) with primary adenocarcinoma of

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the prostate were recruited between 2002 and 2009 at the Moffitt Cancer Center and the James A. Haley VA Hospital (Tampa, FL). All cancer cases were histologically confirmed and were diagnosed no more than one year prior to being enrolled. Controls consisted of 385 subjects (347 Caucasians and 38 African Americans) who were visiting Moffitt's Lifetime Cancer Screening Center or the VA Hospital. All control subjects were male and had no previous diagnosis of cancer. For simplicity, we called this study population the Moffitt group.

Subjects were asked to provide a blood or buccal sample after the interview as a source of genomic DNA. DNA was extracted according to standardized protocols[20].

Genotyping Assays

In this study, we focused on four *SLC5A8* SNPs, two non-synonymous (codon 193 Val>Ile (rs1709189), codon 490 Met>Ile (rs164365)) and two common SNPs in the promoter region (rs1399236 and rs1681096) that may affect activity or expression. Assays for each SNP were based on a custom TaqMan 5′ allelic discrimination analysis (Applied Biosystems, Foster City, CA).

Quantitative Real-time Reverse Transcription-PCR

RNA was extracted from 10 tumor and adjacent non-neoplastic prostate tissues using the Optimum FFPE RNA isolation kit (Asuragen Inc., Austin, TX) as described previously[16]. Real-time quantitative RT-PCR was performed according to the manufacture's protocol (Applied Biosystems, Foster City, CA). The amplification plots of the PCR reaction were used to determine the threshold cycle (CT). The initial copy number of the target mRNA was calculated by a linear regression based on a plot of the CT against log-transformation of the input target quantity.

Cancer Genetic Markers of Susceptibility (CGEMS) Data

We obtained approval to access the individual genotype data from Phase I of the CGEMS prostate cancer genome-wide association study Phase I data[21] in an effort to replicate the *SLC5A8* genetic association findings. This allowed us to download genotype and phenotype data for 1,151 prostate cancer cases and 1,101 Caucasians controls from the prostate, lung, colon and ovarian (PLCO) cancer screening trial from the website (http://cgems.cancer.gov/data). In this database, 20 SNPs in *SLC5A8*, including 3 (rs164365, rs1709189, and rs1399236) of the 4 SNPs from the current study, age, family history and prostate cancer aggressive status were available.

Statistical Methods

The study participants' demographic and clinical characteristics by disease status were summarized using descriptive statistics. The characteristic differences between the case-control groups in each data source were compared using t-tests for continuous variables and Fisher's exact test for categorical variables.

Protein expression data analyses—Non-parametric methods were applied to test the group difference of SLC5A8 protein expression because of the ordinal expression scores. The expression differences between 43 pairs of prostate tumor and adjacent non-neoplastic tissues (19 BPH and 24 PIN) were compared using the Wilcoxon signed rank test. Comparing the SLC5A8 expression difference between 140 prostate tumor and independent non-neoplastic (24 BPH and 32 PIN) tissues, the Wilcoxon rank sum test was applied.

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Genotype data analyses—Four and 20 SNPs were evaluated in the Moffitt and CGEMS study populations, respectively. All of the following analyses were done separately for the three study groups (Moffitt Caucasians, Moffitt African Americans, and CGEMS). Hardy-Weinberg equilibrium was evaluated for all SNPs among the controls using chi-square tests. The individual SNPs associated with prostate cancer risk and aggressiveness were evaluated using logistic regression analysis adjusting for age and family history. Prostate cancer aggressiveness was defined as a Gleason score ≥7 or stage ≥III; those patients with a Gleason score <7 and stage <III were in the non-aggressive group. To evaluate whether the association between *SLC5A8* genotypes and prostate cancer risk varied by other genetic, clinical and environmental factors, we performed sub-group analyses based on family history, body mass index (BMI) and height in the Moffitt Caucasian group. We performed SNP-SNP interactions and haplotype analysis using Multivariate adaptive regression splines (MARS) method[23,24,25], and the expectation-maximization (EM) algorithm[26], respectively (Supplemental information). Statistical analyses were performed using SAS (SAS Institute, Cary, NC) and MARS (Salford Systems, San Diego, CA) software.

RESULTS

Table 1 provides descriptive characteristics of the cancer patients and controls. Cases tended to be older than controls in both the Moffitt Caucasian ($p<0.001$) and African American group ($p=0.022$). More men with prostate cancer than without reported having a first-degree family member with prostate cancer in the Moffitt Caucasian ($p<0.001$) and African American group ($p=0.019$) and the CGEMS group ($p<0.001$). No significant difference was observed between the cases and controls in BMI and height for both the Moffitt Caucasian and African-American groups. As expected, PSA levels of most patients were higher than 4ng/dl, and Gleason scores were 6 or higher.

SLC5A8 Protein Expression

SLC5A8 protein expression was seen predominantly in the cytoplasm of malignant prostate glands with rare expression in non-neoplastic tissues. Comparing 19 pairs of tumor and adjacent BPH tissues, SLC5A8 was over-expressed in 84% of tumor tissues as compared to their paired adjacent BPH tissues, not different in 11%, and under-expressed in 5%. The SLC5A8 expression was significantly higher in tumor tissues than in adjacent BPH tissues (median of expression difference=3, inter-quartile=1–6, and $p<0.0001$). Among 24 pairs of tumor and adjacent PIN tissues, 50%, 42% and 8% of tumor tissues were over-expressed, not different and under-expressed compared with adjacent PIN tissues, respectively. The SLC5A8 expression was significantly higher in tumor tissues than in adjacent PIN tissues (median of expression difference=2.1, inter-quartile=0–4, and $p=0.002$). For comparing independent tissues, the median (inter-quartile range) of SLC5A8 expressions was 4 (3–6), 2 (0.5–3) and 0 (0–1) for 140 tumor, 32 PIN and 24 BPH tissues, respectively. As shown in Figure 1, the expression difference among these three types of tissues was significant ($p<0.0001$). The expression in tumor tissue was significantly higher than in PIN tissues ($p<0.0001$), which was significantly higher than in BPH tissues ($p=0.002$).

The immunostained specimens shown in Figure 2 are examples of a prostate cancer expressing high levels of SLC5A8 protein (Figures 2C–2F), as compared to a sample of BPH or PIN tissues exhibiting loss of SLC5A8 expression (Figures 2A and 2B). We did not find any statistically significant association between SLC5A8 expression and histopathologic characteristics of prostate cancer (data not shown).

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mRNA Expression of *SLC5A8*

To assess SLC5A8 mRNA expression in prostate tumors and adjacent non-neoplastic tissues, quantitative RT-PCR was used in 10 tumor and non-neoplastic paired tissues from prostate cancer patients (Supplementary Figure 1). *SLC5A8* was down regulated in 6 of 10 of prostate tumor tissues (60%), while 4 tumor tissues were either up-regulated or not changed (Supplementary Figure 1). The median (inter quartile range) of mRNA expression of SLC5A8 in tumor tissues minus adjacent non-neoplastic tissues was −0.07 (95% CI= −0.12–0.01, p=0.084).

SLC5A8 Polymorphism and Prostate Cancer Susceptibility

Informative genotyping results on the SLC5A8 polymorphisms were obtained from 99.1% of the study population. The genotype distributions of SLC5A8 SNPs among controls were consistent with Hardy-Weinberg equilibrium. The allele frequencies of these polymorphisms among Caucasians were similar in the Moffitt and CGEMS data (Table 2). Racial difference in the C allele frequency of rs1681096 was observed among the Moffitt controls: 57% versus 29% for Caucasians versus African Americans, respectively (p<0.001), but not for any other measured SNPs (Table 2).

To determine whether these genetic variants were associated with increased risk for prostate cancer, we compared genotypes (Table 2) and haplotypes (supplementary Table 1) between prostate cancer cases and controls. Among the Moffitt Caucasian subjects, prostate cancer risk was increased for subjects with a genotype containing at least one ‘A’ allele of rs164365 (OR =1.35, p-value=0.047) after adjusting for age and family history. This association between rs164365 and prostate cancer risk was not statistically significant after correction for multiple comparisons (FDR adjusted p-value=0.187). None of the SLC5A8 polymorphisms were significantly associated with prostate cancer risk among Moffitt African-American subjects (Table 2). Next, we performed haplotype analyses of the four studied SLC5A8 SNPs among the Moffitt Caucasians. Five haplotypes with frequencies at least 5% were observed (see supplement Table 1). None of these five haplotypes were significantly associated with prostate cancer risk. To further explore the potential association of SLC5A8 SNPs with prostate cancer, we obtained access to the CGEMS data. All 20 SLC5A8 SNPs including three of the four SLC5A8 SNPs investigated in the Moffitt set, were not associated with prostate cancer risk (Table 2).

In the sub-group analyses based on family history, BMI or height for the Moffitt Caucasian subjects, there were statistically significant associations between two SLC5A8 polymorphisms (rs164365 and rs1709189) and prostate cancer risk among individuals with ≥ 70 inches height (for example, rs1709189 TT/CT vs. CC, OR=1.60, 95%CI=1.08–2.38) but not among shorter individuals (<70 inches, TT/CT vs. CC, OR=0.85, 95%CI=0.54–1.33, Supplement Table 2). These two associations were still significant after multiple comparison justification. The FDR adjusted-p values were 0.016 and 0.040 for rs164365 and rs1709189, respectively. We could not confirm these associations in the CGEMS group because neither BMI nor height data are available.

SLC5A8 Polymorphism and Prostate Cancer Aggressiveness

The Moffitt Caucasian patients with the GG genotype of rs1399236 were more likely to have aggressive prostate cancer (OR=5.5, raw p=0.041, Table 2, Supplementary Table 3). However, this result became non-significant after multiple comparison justification (FDR-adjusted p=0.16) and was not replicated in the CGEMS Caucasian patients. The Moffitt African-American patients with the AA/AC genotypes of rs164365 were less likely to have aggressive prostate cancer (OR = 0.24, raw p=0.019, FDR adjusted p=0.058). In the

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CGEMS study, the patients with the CC genotype of rs1877780 tended to have aggressive prostate cancer (OR=1.41, raw p=0.043, FDR-adjusted p=0.850).

SNP-SNP Interactions

Two-way SNP-SNP interactions were evaluated using the MARS and logistic models. Before performing SNP-SNP interactions, linkage disequilibrium was evaluated separately in each data set. In the Moffitt Caucasian group, rs164365 and rs1709189 had strong linkage disequilibrium. The r^2 for these two SNPs in the Moffitt Caucasian case-control data and case-only data was 0.94 and 0.93, respectively. Thus, rs1709189 was excluded from the SNP-SNP interaction analyses. For the Moffitt African-American group, the r^2 among the 4 testing SNPs were all less than 0.8 in both case-control and case-only datasets. For the CGEMS group, the linkage disequilibrium plot of the 20 SNPs was shown in Supplement Figure 2. A total of 14 SNPs were selected after excluding one SNP in each pair with strong linkage disequilibrium (r^2>0.8). In the CGEMS group, those with the genotype combination of rs2712623 GG and rs1877780 TT/TC were likely to have higher risk of prostate cancer (OR=1.43, 95% CI=1.11–1.85, p=0.006). For the prostate cancer patients in the CGEMS group, the interaction of rs1877780 and rs7962305 was significantly (p=0.013) associated with prostate cancer aggressiveness when the main effect of rs1877780 (p=0.005) was in the model. In the Moffitt Caucasian group without family history of prostate cancer, those with the genotype combination of rs1681096 CT/TT and rs164365 GT/TT tended to have higher risk of prostate cancer (OR=1.40, 95%CI=1.02–1.90, p=0.036). No significant 2-way SNP interactions were found to be associated with prostate cancer risk and aggressiveness in the Moffitt Caucasian and African-American group.

DISCUSSION

In the present study, we hypothesized that expression and genetic variations in SLC5A8 are associated with increased risk for prostate cancer and its progression. To test this, protein expression and four SNPs of SLC5A8 were examined for potential associations with prostate cancer risk and aggressiveness. We observed that the SLC5A8 protein is over-expressed in 84% of prostate tumor tissues in the BPH pairs and 50% of tumor tissues in the PIN pairs using the IHC analysis. However, only 30% of tumor tissues over-expressed SLC5A8 based upon RT-PCR analysis. Therefore, these results suggest that DNA methylation in the promoter region may not be the only regulator of SLC5A8 expression in prostate tissues.

SLC5A8 is a plasma membrane transporter that mediates the entry into the cell of various monocarboxylates (acetate, propionate, butyrate, pyruvate, lactate, nicotinate, and β-hydroxybutryate)[4,6]. This transporter has been suggested as a putative tumor suppressor for a variety of cancers[5,8–16]. The ability of SLC5A8 to transport butyrate, propionate and pyruvate that function as HDAC inhibitors underlies the tumor-suppressive role of this transporter[4,6]. Recently, Zhang et al.[27] elucidated the mechanism underlying the regulation of SLC5A8 transcription and identified a novel regulatory sequence associated with expression of SLC5A8.

Many previous studies reported that SLC5A8 is detected often in normal tissues but is silenced in tumor tissues through DNA methylation. Therefore, based upon previous studies, gene silencing of SLC5A8 was anticipated in prostate tumor tissues. However, SLC5A8 is over-expressed in 50–80% of prostate tumor based on IHC results. Results from RT-PCR analysis is consistent with our previous study, which show overexpression of SLC5A8 in ~30% of prostate tumors[16]. Interestingly, even though the level of SLC5A8 protein expression is higher in prostate tumors than in control tissues, the location of the protein appears to be cytoplasmic and not membranous, indicating that the increased expression

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does not necessary correlate with increased transport activity. A recent study showed that SLC5A8 interacts with the anti-apoptotic protein survivin[28]. This interaction must occur in the cytoplasm rather than at the plasma membrane in tumor tissues. Whether this has anything to do with the observed association between increased cytosolic levels of SLC5A8 protein and prostate cancer is not known. These are novel findings and warrant a larger study to investigate their significance.

We initially observed that the SLC5A8 SNP (rs164365) was associated with prostate cancer risk among Caucasian men and Caucasians of increased stature and rs164365 and rs1399236 were associated with aggressiveness, in African Americans and Caucasians, respectively. However, these associations were not confirmed in the CGEMS data, which includes only a Caucasian population. Moreover, an *in vitro* functional analysis of rs164365 variant did not show any difference in activity compared to the wild type (data not shown). Potential explanation for inconsistent results of individual SNP effects between the current study and CGEMS data may be due to population heterogeneity, linkage disequilibrium between the test SNPs and the true ones, or population-specific gene–gene or gene–environment interactions[29]. An advantage of this study is to explore SNP-SNP interactions in SLC5A8 associated with prostate cancer risk and aggressiveness. Several SNP interactions were observed in the study groups. Due to lack of testing SNPs in the CGEMS dataset, the validation test cannot be performed.

However, the observed association between greater height and elevated prostate cancer risk is consistent with previous studies[30]. One of the hypothesized biological mechanisms between height and prostate cancer is that height may be associated with higher levels of serum insulin-like growth factor-I (IGF-I) levels, which acts as a promoter of prostate cancer in men. It is unclear why the association between height and prostate cancer differs based upon SLC5A8 genotypes. A potential explanation is that change in SLC5A8 enzyme activity or expression modulate the level of growth factors, (such as IGF-1) and androgens available.

The main limitation of the current study is that only 16 patients were in both the case-control study and the TMA study. Therefore, a meaningful correlation analysis between protein expression and genotype was not feasible. Based upon available data, SLC5A8 expression in patients with the AC genotype of SNP rs164365 is not different as compared with those with the CC genotype (median=5 vs. 3, p=0.246). In summary, these data suggest that expression pattern of SLC5A8 may be used as a diagnostic biomarker.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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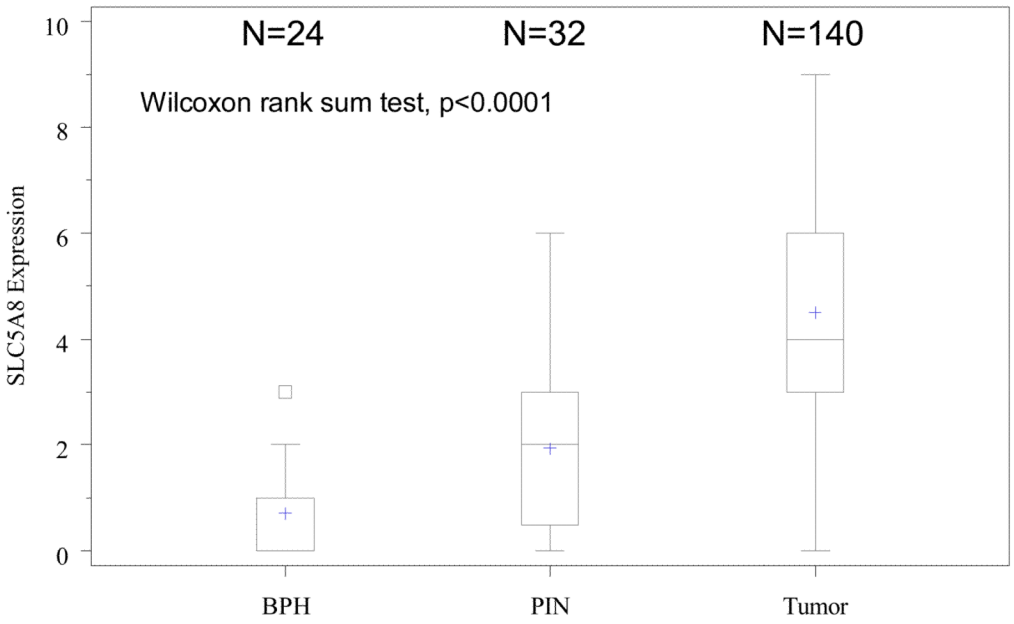

Figure 1.

Distribution of SLC5A8 expression levels in BPH, PIN, and prostate tumor tissues. SLC5A8 expression in prostate tumor tissues was significantly higher than in PIN ($p<0.0001$), which was significantly higher than in BPH ($p=0.002$). The median (inter-quartile range) of SLC5A8 expressions was 0 (0–1), 2 (0.5–3) and 4 (3–6) for the BPH, PIN and tumor tissues, respectively. Symbol in the box: group mean; horizontal line in the box: group median; length of the box: inter-quartile range (between 25th and 75th percentiles); vertical lines: group minimum and maximum values

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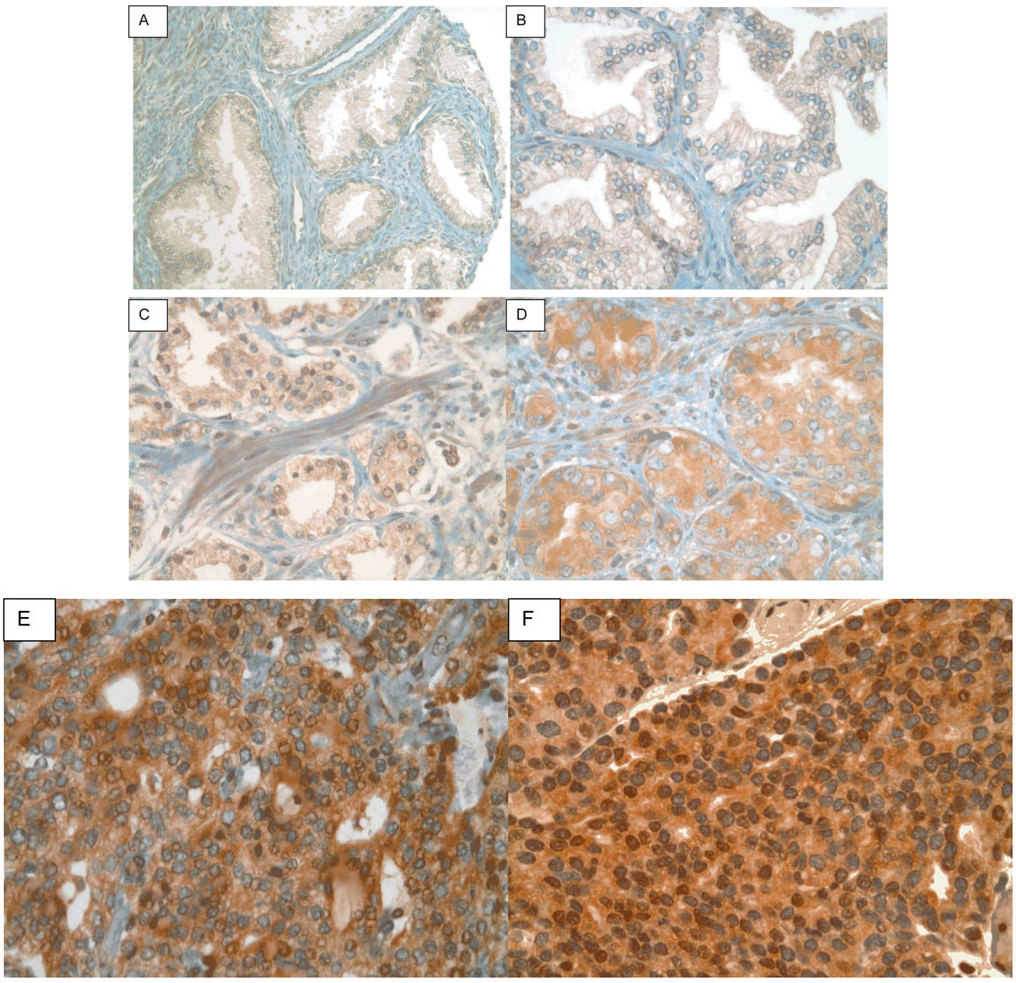

Figure 2.

Immunostain for SLC5A8 in non-neoplastic, BPH, and different grades of prostate adenocarcinoma. The immunostained specimen shown in 2A is an example of the low expression of SLC5A8 found in non-neoplatic prostate tissues, in contrast to the SLC5A8 over-expression generally seen in high grade (Gleason 10) of prostate tumor tissues (2F). 2B: BPH, 2C: prostate tumor tissue with Gleason score 7 (3+4), 2D: prostate tumor tissue with Gleason score 7 (4+3), 2E: prostate tumor tissue with Gleason score 8–9, 2F: prostate tumor tissue with Gleason score 10.

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Table 1

Characteristics of study subjects by disease status and data source

	Moffitt-Caucasian N (%)		Moffitt-African American N (%)		Moffitt-TMA N (%)		CGEMS (all Caucasians) N (%)	
	Cases (n=587)	**Controls (n=347)**	**Cases (n=81)**	**Controls (n=38)**	**Cases* (n=183)**	**Controls (n=13)**	**Cases (n=1151)**	**Controls (n=1101)**
Age								
Mean±SD	**65.0±8.5**‡	**59.9±7.9**	**62.1±8.7**‡	**57.7±10.7**	61.2± 8.3	62.2 ±7.1	-	-
Under 60	148(25.2)	157(45.4)	29 (36.7)	25 (65.8)	79 (43.2)	4 (30.8)	138 (12.0)	135 (12.3)
60–69	261(44.5)	152(43.9)	38 (48.1)	7 (18.4)	72 (39.3)	7 (53.8)	628 (54.6)	623 (56.6)
70 and up	178(30.3)	37(10.7)	12 (15.2)	6 (15.8)	32 (17.5)	2 (15.4)	385 (33.4)	343 (31.1)
Family History†								
No	**445 (75.8)**‡	**319 (91.9)**	**62 (76.5)**‡	**36 (94.7)**	-	-	**1018 (88.4)**‡	**1033 (93.8)**
Yes	**142 (24.2)**	**28 (8.1)**	**19 (23.5)**	**2 (5.3)**			**133 (11.6)**	**68 (6.2)**
Height inches								
Mean±SD	69.7± 2.8	70.0± 3.8	70.1± 3.1	69.6± 3.1	-	-	-	-
BMI (Kg/M^2)								
Underweight (<18.5)	0	1 (0.3)	1 (1.3)	0	-	-	-	-
Normal weight (18.5–24.9)	118 (21.2)	81(23.5)	6 (7.9)	5 (14.3)				
Overweight (25.0–29.9)	256 (46.0)	168(48.7)	29 (38.2)	18 (51.4)				
Obese (>=30)	182 (32.7)	95(27.5)	40 (52.6)	12 (34.3)				
PSA								
<4 ng/ml	97 (16.8)	N/A	13 (16.1)	N/A	-	N/A	-	N/A
4–10 ng/ml	401(69.5)		55 (67.9)					
>=10 ng/ml	79 (13.7)		13 (16.1)					
Gleason Score								
0–5	17 (2.9)	N/A	2 (2.5)	N/A	0	N/A	-	N/A
6	345 (59.7)		35 (43.7)		33 (19.9)			
7	178 (30.8)		34(42.5)		82 (49.4)			
8–10	38 (6.6)		9 (11.3)		51 (30.7)			
Aggressiveness								
No	355 (61.9)	N/A	35 (44.3)	N/A	135 (72.6)	N/A	492 (42.8)	N/A

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	Moffitt-Caucasian N (%)		Moffitt-African American N (%)		Moffitt-TMA N (%)		CGEMS (all Caucasians) N (%)	
	Cases (n=587)	**Controls (n=347)**	**Cases (n=81)**	**Controls (n=38)**	**Cases* (n=183)**	**Controls (n=13)**	**Cases (n=1151)**	**Controls (n=1101)**
Yes	219 (38.1)		44 (55.7)		51 (27.4)		659 (57.2)	

*9 minority individuals (7 African Americans and 2 others) included

†Men having at least one first-degree family member with prostate cancer

‡Values in bold: case-control group difference was significant ($p<0.05$).

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Table 2

Associations of genotypes with prostate cancer risk and aggressiveness

SNP	Study group	Locatio n	Allele	Control/Case			MAF*	OR of Risk (95 %CI)†	
			Major/Minor	Homo major	Hetero	Homo minor			OR of Aggressiveness (95 %CI)
rs2279834^{D}	CGEMS	3′ UTR	A/G	642/671	391/406	67/74	0.24	1.01 (0.86–1.20)	1.02 (0.81–1.30)
rs7969185^{R}	CGEMS	Intron12	A/G	771/813	306/302	24/36	0.16	1.41 (0.83–2.39)	1.07 (0.54–2.10)
rs2654993^{R}	CGEMS	Intron12	C/T	932/977	164/167	5/7	0.08	1.29 (0.40–4.11)	1.02 (0.23–4.63)
rs164365^{D}	Moffitt	Exon12	C/A	219/332	114/225	14/28	0.20	**1.35 (1.00–1.80)** ‡	1.15 (0.81–1.62)
	Moffitt (AA)	Exon12	C/A	28/59	9/20	1/2	0.14	1.13 (0.40–3.23)	**0.24 (0.07–0.8)** ‡
	CGEMS	Exon12	C/A	689/730	349/371	49/39	0.21	0.98 (0.83–1.17)	1.11 (0.87–1.42)
rs164364^{D}	CGEMS	Intron12	C/T	427/438	506/552	167/160	0.38	1.03 (0.86–1.22)	1.16 (0.91–1.48)
rs724344^{R}	CGEMS	Intron10	C/T	695/732	356/379	50/40	0.21	0.78 (0.51–1.20)	1.30 (0.68–2.50)
rs2671434^{D}	CGEMS	Intron9	C/T	970/1009	124/136	6/5	0.06	1.06 (0.82–1.37)	0.95 (0.66–1.36)
rs2625154^{R}	CGEMS	Intron5	C/T	691/724	355/377	50/40	0.21	0.78 (0.51–1.20)	1.16 (0.61–2.22)
rs1709189^{D}	Moffitt	Exon5	C/T	214/336	119/222	14/29	0.21	1.22 (0.91–1.63)	1.14 (0.81–1.61)
	Moffitt (AA)	Exon5	C/T	25/52	12/24	1/5	0.18	1.01 (0.40–2.59)	0.49 (0.18–1.33)
	CGEMS	Exon5	C/T	695/733	355/379	50/39	0.21	0.99 (0.83–1.17)	1.11 (0.87–1.41)
rs12305787^{R}	CGEMS	Intron4	G/A	768/807	306/302	24/36	0.16	1.42 (0.84–2.40)	1.07 (0.54–2.10)
rs189488^{D}	CGEMS	Intron3	G/A	377/386	515/563	208/199	0.42	1.02 (0.85–1.21)	1.16 (0.91–1.49)
rs12580634^{D}	CGEMS	Intron1	G/T	839/896	248/239	14/16	0.13	0.92 (0.76–1.12)	1.22 (0.92–1.63)
rs2712622^{R}	CGEMS	Intron1	T/C	345/359	522/569	234/222	0.45	0.89 (0.72–1.10)	0.98 (0.73–1.32)
rs7962305^{R}	CGEMS	Intron1	G/A	622/650	418/431	61/70	0.25	1.10 (0.77–1.58)	1.22 (0.74–2.01)
rs1399236^{R}	Moffitt	5′ near	A/G	241/411	93/157	8/8	0.16	0.48 (0.17–1.39)	**5.5 (1.08–28.1)** ‡
	Moffitt (AA)	5′ near	A/G	24/59	11/19	3/3	0.22	0.37 (0.06–2.45)	-
	CGEMS	5′ near	A/G	787/842	286/278	25/27	0.15	1.06 (0.61–1.84)	0.61 (0.28–1.33)
rs11110700^{D}	CGEMS	5′ near	T/C	695/710	344/377	59/59	0.21	1.07 (0.90–1.27)	1.05 (0.82–1.34)
rs2712623^{R}	CGEMS	5′ near	A/G	348/374	542/529	208/244	0.44	1.15 (0.94–1.42)	1.03 (0.77–1.37)
rs1681096^{D}	Moffitt	5′ near	C/T	109/174	170/291	62/113	0.43	1.08 (0.79–1.47)	0.86 (0.59–1.25)

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SNP	Study group	Locatio n	Allele	Control/Case			MAF*	OR of Risk (95 %CI)†	
			Major/Minor	Homo major	Hetero	Homo minor			OR of Aggressiveness (95 %CI)
	Moffitt (AA)	5′ near	T/C	22/32	10/37	6/12	0.29	1.76 (0.72–4.30)	1.38 (0.52–3.64)
rs2062167*R*	CGEMS	5′ near	C/T	548/574	456/474	97/103	0.30	1.04 (0.78–1.39)	0.95 (0.63–1.43)
rs1631980*D*	CGEMS	5′ near	T/C	899/965	193/177	9/9	0.10	0.87 (0.70–1.09)	1.00 (0.73–1.37)
rs1877780*R*	CGEMS	5′ near	T/C	414/410	499/559	188/182	0.40	0.92 (0.73–1.15)	**1.41 (1.01–1.96)** ‡

*Minor allele frequency estimated from the controls.

†Adjusted for family history and age (Moffitt Caucasian: <55, 55–59, 60–64, 65–69, 70–74, 75+, and Moffitt African American and CGEMS <60, 60–69, 70+)

‡Values in bold: case-control group difference was significant (p<0.05).

D dominant model;

R recessive model