Research Article

Expression of *SLCO* Transport Genes in Castration-Resistant Prostate Cancer and Impact of Genetic Variation in *SLCO1B3* and *SLCO2B1* on Prostate Cancer Outcomes

Jonathan L. Wright[1,2], Erika M. Kwon[3], Elaine A. Ostrander[3], R. Bruce Montgomery[4], Daniel W. Lin[1,2], Robert Vessella[1], Janet L. Stanford[2,5], and Elahe A. Mostaghel[4,6]

Abstract

Background: Metastases from men with castration-resistant prostate cancer (CRPC) harbor increased tumoral androgens versus untreated prostate cancers. This may reflect steroid uptake by OATP (organic anion transporting polypeptide)/*SLCO* transporters. We evaluated *SLCO* gene expression in CRPC metastases and determined whether prostate cancer outcomes are associated with single nucleotide polymorphisms (SNP) in *SLCO2B1* and *SLCO1B3*, transporters previously shown to mediate androgen uptake.

Methods: Transcripts encoding eleven *SLCO* genes were analyzed in untreated prostate cancer and in metastatic CRPC tumors obtained by rapid autopsy. SNPs in *SLCO2B1* and *SLCO1B3* were genotyped in a population-based cohort of 1,309 Caucasian prostate cancer patients. Median survival follow-up was 7.0 years (0.77–16.4). The risk of prostate cancer recurrence/progression and prostate cancer–specific mortality (PCSM) was estimated with Cox proportional hazards analysis.

Results: Six *SLCO* genes were highly expressed in CRPC metastases versus untreated prostate cancer, including *SLCO1B3* (3.6-fold; $P = 0.0517$) and *SLCO2B1* (5.5-fold; $P = 0.0034$). Carriers of the variant alleles *SLCO2B1* SNP rs12422149 (HR: 1.99; 95% CI: 1.11–3.55) or *SLCO1B3* SNP rs4149117 (HR: 1.76; 95% CI: 1.00–3.08) had an increased risk of PCSM.

Conclusions: CRPC metastases show increased expression of *SLCO* genes versus primary prostate cancer. Genetic variants of *SLCO1B3* and *SLCO2B1* are associated with PCSM. Expression and genetic variation of *SLCO* genes which alter androgen uptake may be important in prostate cancer outcomes.

Impact: *OATP/SLCO* genes may be potential biomarkers for assessing risk of PCSM. Expression and genetic variation in these genes may allow stratification of patients to more aggressive hormonal therapy or earlier incorporation of nonhormonal-based treatment strategies. *Cancer Epidemiol Biomarkers Prev; 20(4); 619–27. ©2011 AACR.*

Introduction

Androgens play a critical role in the development and progression of prostate cancer (1, 2). Although androgen deprivation therapy (ADT) remains the most effective treatment option for men with advanced disease, the clinical course following ADT is uniformly marked by progression to castration-resistant prostate cancer (CRPC). Many mechanisms proposed to confer a castration-resistant phenotype [e.g., androgen receptor (AR) overexpression, *AR* mutations with promiscuous ligand interactions, and enhanced AR signaling via coregulator alterations] still require, or are enhanced by, the presence of AR ligands (3–5). In this respect, we and others have shown that the tissue response to castration is characterized by the presence of residual prostatic androgens at levels capable of activating the AR and maintaining androgen-regulated gene expression (6–9). Moreover, tumor metastases from men with CRPC have been found to contain detectable testosterone levels that exceed androgen levels in the prostate tissue of eugonadal men (10).

The source of residual tissue androgens present despite ADT has not been elucidated but may reflect the uptake of adrenal androgens and intracellular conversion to testosterone or *de novo* androgen synthesis from

Authors' Affiliations: [1]Department of Urology, University of Washington; [2]Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, Washington; [3]Cancer Genetics Branch, National Human Genome Research Institute, Bethesda, Maryland; Departments of [4]Medicine and [5]Epidemiology, School of Public Health, University of Washington; and [6]Division of Clinical Research, Fred Hutchinson Cancer Research Center, Seattle, Washington

cholesterol or progesterone precursors. Accordingly, we and others have shown that soft tissue and bone CRPC metastases express genes mediating steroid biosynthesis and adrenal androgen utilization (10, 11).

Emerging data also suggest a potential role for steroid transport proteins encoded by the *SLCO* gene family in mediating the uptake of androgen into prostate cancer cells and thereby influencing the clinical response to androgen suppression (12, 13). The organic anion transporting polypeptides (OATP) are a superfamily of *SLCO*-encoded membrane transporters involved in the transport of bile acids, steroid conjugates, xenobiotics, and a variety of clinically important drugs (14). Several family members are known to mediate the uptake of steroids and steroidogenic precursors, including sulfated forms of pregnenolone, estrone, and DHEA (dehydroepiandrosterone). Among these are SLCO1B1 and SLCO1B3, primarily expressed in the liver, and SLCO1A2 and SLCO2B1, more broadly distributed in liver, kidney, intestine, and brain and in steroidogenic tissues such as testis, ovary, mammary epithelium, placenta, and adipose tissue (14–19).

Consistent with a physiologic role for SLCO transporters in providing a pool of intracellular precursors for steroidogenic tissues, several studies have shown SLCO transporter expression in human breast carcinomas, suggesting a role for SLCO-mediated activity in the estrogen-dependent pathophysiology of this tumor (15, 20). Notably, Al Sarakbi and colleagues observed a statistically significant association between the expression of SLCO2B1 and breast cancer grade (21), whereas Nozawa and colleagues found that inhibition of estrone-sulfate transporter activity led to suppression of MCF-7 breast cancer cell proliferation (22).

Numerous studies have shown that single nucleotide polymorphisms (SNP) in *SLCO* genes can markedly alter substrate-specific transport efficiency, although few studies have specifically evaluated androgen transport (23). Hamada and colleagues recently showed that a nonsynonymous SNP (rs4149117) in *SLCO1B3* influenced testosterone uptake in transfected cells and was correlated with prostate cancer outcomes (13). Cells transfected with 2 copies of the *G* allele in rs4149117 showed impaired testosterone transport. Among 180 men with CRPC, those homozygous for the *GG* haplotype had a longer median survival (8.5 vs. 6.4 years; $P = 0.02$) and significantly improved 10-year survival (42% vs. 23%; $P = 0.023$). The authors also showed increased OATP1B3 staining in primary prostate cancers compared with faint staining in samples of normal tissue or benign prostatic hyperplasia. Sharifi and colleagues showed that in 68 men treated with hormone suppression for biochemical relapse or metastatic disease, those with 2 copies of the *G* allele in this *SLCO1B3* SNP had a longer time to androgen-independent progression (1.2 vs. 1.6 years; $P < 0.05$; ref. 12).

The identification of *SLCO* genes in breast cancer cells and the association of an *SLCO* genotype with response to ADT in men with advanced prostate cancer suggest that transport genes mediating cellular steroid uptake may influence the growth and/or progression of hormone-dependent tumors. The extent to which *SLCO* family gene members are expressed in untreated primary tumors or in CRPC metastases has not been previously reported. Moreover, whether genetic variation in these genes is associated with prostate cancer survival in men with less advanced disease is unknown. We sought to determine expression of *SLCO* gene family members in untreated primary prostate cancer and in CPRC metastases and to determine the association of allelic variation in *SLCO2B1* and *SLCO1B3* with prostate cancer outcomes in a large population-based cohort of men with prostate cancer.

Methods

Tissue analysis

Tissue acquisition. All procedures involving human subjects were approved by the Institutional Review Board (IRB) of the University of Washington Medical Center. Matched samples of benign and tumor prostate tissues were obtained from 8 untreated patients undergoing radical prostatectomy (RP) for localized prostate cancer. Castration-resistant tumors from 14 men with CRPC were obtained by rapid autopsy under the aegis of the University of Washington Prostate Cancer Donor Autopsy Program as previously described (24). Autopsies were done within 4 to 10 hours of death. Fresh tissue was embedded in freezing media (Tissue-Tek OCT Compound; Sakura Finetek) immediately after harvesting, snap frozen in liquid nitrogen, and maintained at −80°C. Tissues utilized in this study included distant or local soft tissue metastases acquired from surgically or medically castrated patients (the latter with anorchid serum testosterone levels documented at <50 ng/dL) and included 2 to 4 metastatic lymph node, liver, bladder, or lung tumor deposits per patient. Bone metastases were not included because of inconsistent RNA quality.

RNA isolation. Areas consisting of more than 85% tumor tissue were grossly macrodissected from 8 prostatectomy specimens and 22 metastases from 5 CRPC patients. Benign prostate samples were macrodissected after being examined by hematoxylin and eosin staining to ensure the absence of tumor tissue and homogenized in TRIzol for isolation of total RNA and conversion to cDNA as previously described (10). Laser capture microdissection was carried out on an additional 69 metastases from 14 CRPC patients. All RNA samples were DNase treated and amplified using oligo(dT) priming for first-strand cDNA synthesis (to minimize priming off residual genomic DNA). Yield, purity, and integrity of total and amplified RNA were determined by optical density measurements at wavelengths of 260 and 280 nm ($OD_{260/280}$) and gel electrophoresis. The quality of cDNA conversion was assessed in studies showing the amplification of genes encoding abundant [prostate specific antigen

(PSA), AR] versus rare transcripts (SRD5A2, steroidogenic enzymes) as previously described (10).

Quantitative RT-PCR.   Reactions were done in triplicate using an Applied Biosystems 7700 sequence detector with approximately 5 ng of cDNA, 1 μmol/L of each primer pair, and SYBR Green PCR master mix (Applied Biosystems). Primers specific for genes of interest were designed using the Web-based primer design service Primer3 provided by the Whitehead Institute for Biomedical Research (25). Sequences are provided in Supplementary Table S1. The specificity of amplification in each reaction was assessed on the basis of the melting point of the dissociation curve, and reactions with cycle threshold (C_t) values of more than 35 were considered undetectable for that transcript. The mean C_t obtained for each gene was normalized to the expression of the housekeeping gene *RPL13A* in the same sample (the ΔC_t). Samples with C_t above 35 were incorporated into the data analysis by using a C_t value of 36 for purposes of calculation. The percentage of samples that were undetectable varied for each gene (from 0% in *SLCO2A1*, *SLCO2B1*, *SLCO3A1*, and *SLCO4A1* to 8% in *SLCO1B3*; 13% in *SLCO4C1* and *SLCO5A1*; 28% in *SLCO1A2*, and 39% in *SLCO1B1*) and did not represent a unique population. The Wilcoxan rank-sum test was used to compare the mean ΔC_ts for each gene between the primary prostate cancers ($n = 8$) and metastatic autopsy samples ($n = 16–22$). The values of $P < 0.05$ were considered significant. The fold change was calculated from the difference in mean ΔC_ts between the sample groups ($\Delta\Delta C_t$ method; fold $= 2^{\Delta\Delta C_t}$).

Genotype analysis

Study population.   The analyses of *SLCO2B1* and *SLCO1B3* genetic variation and prostate cancer outcomes utilized data and DNA from patients (residents of King County, Washington) in one of 2 population-based studies of prostate cancer risk factors. Incident cases (histologically confirmed prostate cancer ascertained from the Seattle-Puget Sound SEER cancer registry) were diagnosed between January 1, 1993, and December 31, 1996 (study I), or between January 1, 2002, and December 31, 2005 (study II). A total of 2,244 eligible cases were identified and 1,754 (78%) participated in the study interview. Only Caucasian patients with DNA available ($n = 1,309$) were included in these analyses. All study procedures and protocols were approved by the IRB of the Fred Hutchinson Cancer Research Center. In addition, genotyping was approved by the IRB of the National Human Genome Research Institute.

Genotyping.   DNA was isolated from peripheral blood samples and stored at −80°C. SNPs in *SLCO1B3* and *SLCO2B1* were selected using publicly available data from the Genome Variation Server (26). Haplotype tagging SNPs (tagSNP) with a minor allele frequency of greater than 5.0% were selected to maximize coverage of the transcript of interest (+5 kb upstream and downstream). *SNP* genotype was determined using the Applied Biosystems (ABI) SNPlex Genotyping System,

and allele calling was done with GeneMapper software (27). A total of 7 (*SLCO2B1*) and 5 (*SLCO1B3*) SNPs were selected. Genotyping was successful in 98.0% to 99.4% of SNPs. Genotyping of blind duplicate samples ($n = 141$) was used for quality control with 100% agreement. The SNPs rs2306168 (*SLCO2B1*) and rs10743397 (*SLCO1B3*) had no genetic variability in our population and were excluded. For *SLCO2B1*, the SNPs analyzed included 4 tagSNPs, 1 SNP from the 5′-untranslated region (rs2851069), and a coding nonsynonymous SNP (rs12422149). For *SLCO1B3*, all SNPs were tagSNPs, including 2 coding nonsynonymous SNPs (rs7311358 and rs4149117) in perfect linkage disequilibrium (for which only the rs4149117 data are reported).

Data collection.   Study patients completed standardized in-person interviews providing information on demographic and lifestyle factors, medical and family history, and number of PSA tests within the 5 year before diagnosis. Clinical information on prostate cancer cases was obtained from the SEER (Surveillance, Epidemiology, and End Results) cancer registry, including Gleason score, tumor stage, diagnostic PSA, and primary therapy.

Prostate cancer outcomes.   All living cases who consented to future contact from study I ($n = 631$) were sent a follow-up survey in January 2004. Information on physician diagnoses of prostate cancer recurrence/progression, secondary therapy use, follow-up PSA values, and procedures such as subsequent biopsies and scans was collected along with dates and results. Outcomes were confirmed with review of medical records. Five hundred twenty men completed the survey (83%). Those not completing the survey were younger at diagnosis (<50 yrs), African American, and had less education than responders (P values <0.05). No significant differences existed between tumor stage, Gleason score, or primary treatment.

Disease recurrence/progression events were determined by multiple criteria. Date of recurrence was imputed for 13 patients who died from prostate cancer but had unknown recurrence date, with a multiple imputations methodology as previously described (28, 29). PSA progression definitions varied by primary treatment: a follow-up PSA value of 0.2 or greater in men who underwent RP; nadir PSA + 2 ng/mL (Phoenix criteria; ref. 30) for men treated with radiation (XRT); or any PSA increase in men treated with primary ADT. The date of last follow-up for recurrence/progression events was December 31, 2005. Prostate cancer–specific mortality (PCSM) was determined from the SEER registry, which links quarterly with the Washington State mortality database. Underlying cause of death was verified by a review of death certificates, with 99% agreement for prostate cancer–specific deaths. The date of last follow-up for mortality was December 1, 2009.

Statistical analysis

SNP alleles were in Hardy–Weinberg equilibrium by the Fisher exact test except for rs4944993 (*SLCO2B1*),

which was then excluded from the analyses. Multivariate Cox proportional hazards models were created to estimate the HRs and 95% CIs for prostate cancer recurrence/progression and PCSM adjusting for age, PSA level at diagnosis, Gleason score, stage, primary treatment, body mass index (BMI), and smoking status. All statistical analyses were conducted using STATA software (Version 11; Stata, Inc.).

Results

Increased expression of *SLCO* genes in CRPC metastases versus primary prostate cancers

To determine whether *SLCO* gene expression and therefore OATP transport activity might be present in prostate tumors, we used quantitative RT-PCR to evaluate the expression of these 11 genes in untreated prostate tumors and in advanced CRPC metastases. Transcripts encoding 9 of the 11 genes were reproducibly detected in prostate tumor samples and the CRPC metastases (Table 1). We did not find a significant difference in expression between the matched benign and cancer prostate samples for any of the *SLCO* genes (data not shown; of note, despite careful macrodissection, contamination of cancer samples with benign epithelium cannot be ruled out). However, compared with the untreated primary prostate cancers, CRPC metastases showed significantly increased expression of genes encoding 6 *SLCO* family members (Table 1). Dot plots depicting the expression level of these 6 transcripts in the CRPC metastases and in the untreated prostate tissues are shown in Figure 1. Interestingly, *SLCO1B1* was detected in only 3 of the benign prostate samples (at $C_t < 35$) but was detectable in a majority of the CRPC metastases.

Prevalent expression of multiple *SLCO* gene members in primary and metastatic CRPC tumors

To more carefully examine the range and distribution of *SLCO* gene expression in advanced prostate tumors, we expanded the initial cohort of CRPC tumors (isolated by gross macrodissection) to include 69 tumor samples (isolated by laser capture microdissection) from 14 men with CRPC. For each subject, we evaluated 2 to 10 soft tissue metastases and 1 to 2 primary samples (prostatic tumor simultaneously resected with the metastases at time of autopsy) to assess whether expression differed in the primary versus metastatic environment.

We found widespread but variable expression of multiple *SLCO* gene members across the CRPC samples (Fig. 2), with certain patients consistently expressing multiple *SLCO* transporters in their tumor samples (e.g., patients 1–4) and others showing low levels of *SLCO* gene expression (e.g., patients 11–14). Grouping of samples by patient of origin showed that gene expression was generally consistent across the metastatic samples of an individual patient, although not necessarily concordant between the primary tumor and metastases (paired analyses did not show consistent differences in primary vs. metastatic tumor deposits; data not shown). The primary and metastatic samples from individual patients occasionally showed strikingly different patterns of *SLCO* gene expression, consistent with the known heterogeneity that characterizes prostate cancer metastases (24, 31). For example, patients 6 and 7 had relatively low *SLCO* expression in the prostatic tumor samples harvested at autopsy but abundant expression of multiple *SLCO* genes in their metastases. In contrast, patients 10 and 11 expressed multiple *SLCO* gene members in their prostatic samples without marked expression of these genes in their metastases. These data are consistent with the hypothesis that in patients whose tumors have strong

Table 1. Expression of *SLCO* genes in castration resistant prostate cancer

Family	Prior name	Gene	Protein	Fold[a]	P[b]
OATP1	OATP-A, OATP1	*SLCO1A2*	OATP1A2	1.1	ns
	OATP-C, OATP2	*SLCO1B1*	OATP1B1	13.8	0.0053
	OATP8	*SLCO1B3*	OATP1B3	3.6	0.0517
	OATP-F	*SLCO1C1*	OATP1C1	nd	nd
OATP2		*SLCO2A1*	OATP2A1	3.2	0.0158
	OATP-B	*SLCO2B1*	OATP2B1	5.5	0.0034
OATP3	OATP-D	*SLCO3A1*	OATP3A1	5.4	0.0053
OATP4	OATP-E	*SLCO4A1*	OATP4A1	30	0.0001
	OATP-H	*SLCO4C1*	OATP4C1	1.9	ns
OATP5	OATP-J	*SLCO5A1*	OATP5A1	1.1	ns
OATP6	OATP-I	*SLCO6A1*	OATP6A1	nd	nd

Abbreviations: nd, not detected; ns, not significant.
[a]CRPC metastases versus untreated prostate cancer.
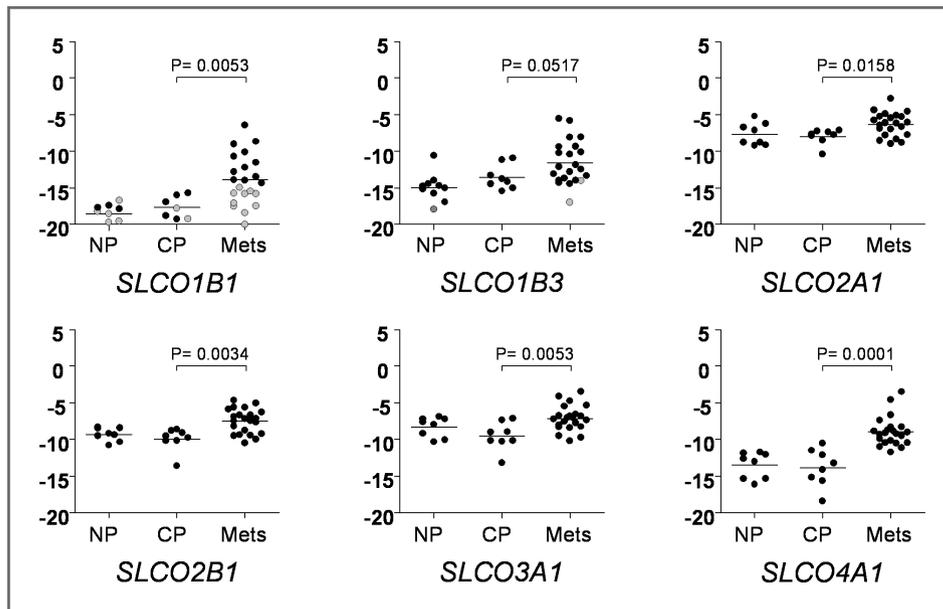[b]Wilcoxan rank-sum test.

Figure 1. Transcript levels for the indicated *SLCO* genes in matched normal prostate (NP) tissue and prostate cancer tissue (CP) from untreated men and from CRPC metastases (Mets). C_t for each gene was normalized to the housekeeping gene *RPL13A* in the same sample. The *y*-axis is the *RPL13A*-normalized C_t; more positive numbers reflect higher transcript abundance. The fold change was calculated by the $\Delta\Delta C_t$ method (fold = $2^{\Delta\Delta C_t}$). The Wilcoxan rank-sum test was used to compare the mean C_ts for each gene between the prostate cancer and metastatic tumor (Mets) samples. Values of $P < 0.05$ were considered significant. Significant differences in expression between the CRPC Mets and prostate cancer samples were not observed for *SLCO1A2*, *SLCO4C1*, and *SLCO5A1* (data not shown). Gray circles are samples for which a Ct value of 36 was used for purposes of calculation.

SLCO gene expression, steroid uptake proteins may play a role CRPC progression.

Association of *SLCO2B1* and *SLCO1B3* polymorphisms with prostate cancer risk and PCSM

A cohort of 1,309 Caucasian prostate cancer cases from the population-based studies described earlier had DNA available for genotyping, with selected characteristics shown in Table 2. Most cases were diagnosed with localized disease (78%), had Gleason score of (3 + 4) or less (85%) and a PSA value at diagnosis of less than 10.0 ng/mL (69%). For the recurrence/progression analysis, 469 Caucasian men had genotyping results available for analysis. Of these, 30% had a recurrence/progression event ($n = 143$). The median follow-up time for recurrence/progression was 8.9 years (range: 0.1–12.8 years). For PCSM, the median follow-up time was 7.0 years (range: 0.8–16.4 years).

Tables 3 and 4 show the results for the multivariate Cox analysis for recurrence/progression and PCSM, respectively. None of the SNPs were associated with the risk of recurrence/progression. Death due to prostate cancer was observed in 66 men (5% of cases). A total of 115 men who died of other causes were censored at time of death. Excluding these 115 from the analysis did not alter the results and thus they were included in the final models. Having 1 or 2 copies of the variant *A* allele in *SLCO2B1* SNP rs12422149 was associated with a 2-fold increased risk of PCSM (HR: 1.99; 95% CI: 1.11–3.55).

Similarly, carriers of the variant *T* allele in *SLCO1B3* SNP rs4149117 had a 76% increased risk of PCSM (HR: 1.76; 95% CI: 1.00–3.08). None of the other SNPs was associated with PCSM. There was no evidence for effect modification in those who received primary ADT.

Discussion

The source of residual androgens present in prostate cancer tissues despite ADT has not been elucidated but may reflect the uptake of adrenal androgens and intracellular conversion to testosterone or *de novo* androgen synthesis from the uptake of cholesterol or progesterone precursors. Although the uptake of steroid hormones is generally attributed to free diffusion across the lipid membrane, emerging data suggest a role for steroid transport proteins in actively mediating androgen uptake into prostate cells and thereby potentially influencing prostate cancer outcomes.

In this study, we evaluated expression of *SLCO*-encoded membrane transporters both in untreated primary prostate cancer and in CPRC metastases and evaluated genetic variation of *SLCO2B1* and *SLCO1B3* in association with prostate cancer outcomes. We found significantly increased expression of many *SLCO* family members in CRPC metastases compared with untreated primary prostate cancer. Moreover, individuals could be identified whose tumors were characterized by widespread or minimal transporter gene expression. An
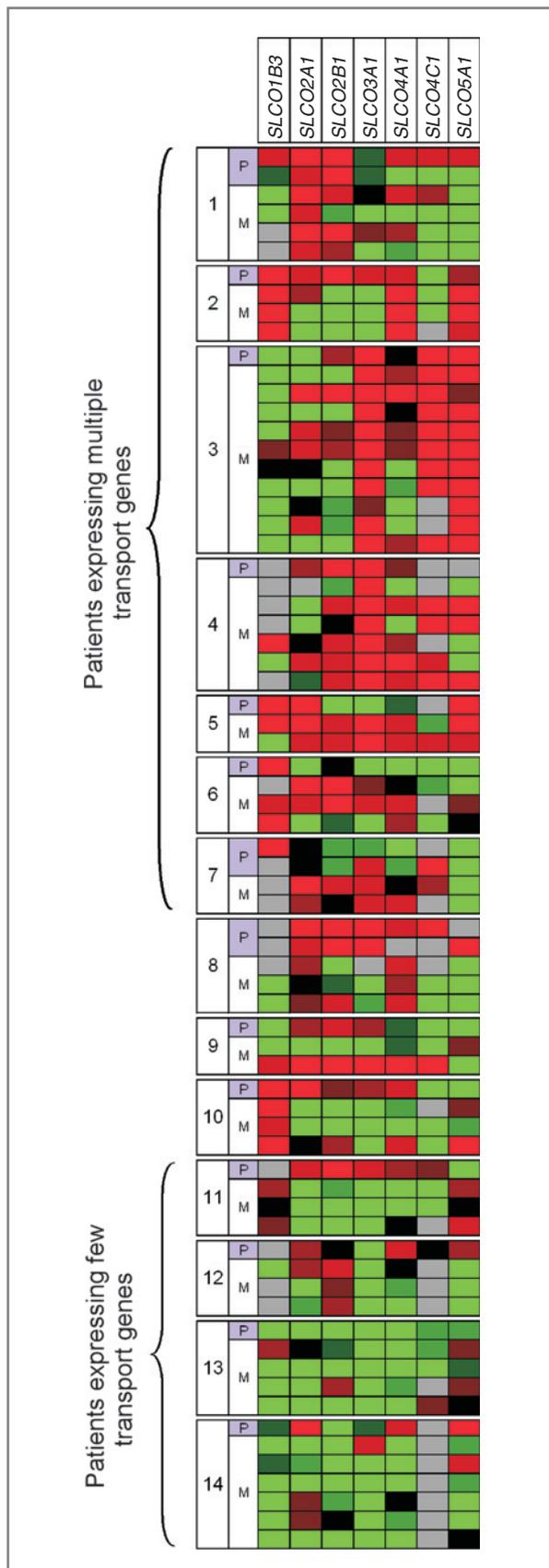
Table 2. Distribution of selected characteristics of Caucasian population-based prostate cancer cases used for SLCO2B1 and SLCO1B3 SNP genotype analyses

Total cases	1,309
Age, y	
40–49	102 (7.8)
50–54	189 (14.4)
55–59	325 (24.8)
60–64	395 (30.2)
65–69	153 (11.7)
70–74	145 (11.1)
Family history of prostate cancer[a]	
Negative	1,026 (78.4)
Positive	283 (21.6)
PSA tests within 5 y prior to diagnosis	
None	288 (22.1)
1–2	320 (24.5)
3+	638 (48.7)
Unknown	63 (4.8)
BMI (weight/height^2)	
<25.0	429 (32.8)
25.0–29.9	638 (48.7)
≥30.0	242 (18.5)
Smoking status	
Never	523 (40.0)
Former	631 (48.2)
Current	155 (11.8)
Diagnostic PSA, ng/mL	
0.0–3.9	178 (13.6)
4.0–9.9	722 (55.2)
10.0–19.9	191 (14.6)
≥20	118 (9.0)
Missing	100 (7.6)
Gleason score	
2–6, 3 + 4	1103 (84.5)
4 + 3, 8–10	202 (15.5)
Tumor stage	
Local	1,023 (78.2)
Regional	254 (19.4)
Distant	32 (2.4)
Treatment	
RP	770 (58.8)
XRT ± hormones	359 (27.4)
AD	61 (4.7)
Other	4 (0.3)
Watchful waiting	115 (8.8)

[a]First-degree family history of prostate cancer.

Figure 2. Expression of SLCO genes by quantitative RT-PCR in primary prostatic samples (P) and in metastatic (M) tumors obtained from 14 men with CRPC. Primary and metastatic samples were simultaneously harvested at autopsy and are grouped by patient of origin. The heatmap depicts the mean centered expression of each gene across all samples. The scale is from bright green (lowest expression) to black (equivalent expression) to bright red (highest expression). Gray squares denote samples for which no transcript was detectable. Patients with prevalent versus rare expression of SLCO genes are as denoted.

Table 3. Risk of prostate cancer recurrence/progression by *SLCO2B1* and *SLCO1B3* SNP genotypes in a population-based prostate cancer cohort[a]

	SNP	Genotype	Recurrence/progression		HR (95% CI)[b]
			No (*n* = 326)	Yes (*n* = 143)	
SLCO2B1	rs12422149	GG	254 (69.2)	113 (30.8)	1.00 (referent)
		GA + AA	47 (66.2)	24 (33.8)	1.09 (0.68–1.75)
SLCO2B1	rs949069	GG	216 (69.5)	95 (30.6)	1.00 (referent)
		GA + AA	87 (66.4)	44 (33.6)	1.19 (0.82–1.73)
SLCO2B1	rs2712819	AA	194 (70.6)	81 (29.5)	1.00 (referent)
		AG + GG	109 (65.7)	57 (34.3)	1.35 (0.95–1.91)
SLCO2B1	rs2851069	CC	116 (70.3)	49 (29.7)	1.00 (referent)
		CT + TT	184 (66.9)	91 (33.1)	1.31 (0.92–1.89)
SLCO2B1	rs7947726	GG	259 (69.1)	116 (30.9)	1.00 (referent)
		AG + AA	44 (67.7)	21 (32.3)	1.21 (0.75–1.95)
SLCO1B3	rs4149117	GG	221 (68.0)	104 (32.0)	1.00 (referent)
		GT + TT	84 (70.0)	36 (30.0)	0.85 (0.57–1.26)
SLCO1B3	rs3829311	GG	206 (68.0)	97 (32.0)	1.00 (referent)
		GA + AA	98 (69.5)	43 (30.5)	0.83 (0.57–1.21)
SLCO1B3	rs4762803	CC	239 (68.3)	111 (31.7)	1.00 (referent)
		CG + GG	66 (70.2)	28 (29.8)	0.86 (0.56–1.32)

[a]Recurrence/progression data available for 469 cases, variable number of cases due to failed genotyping.
[b]Adjusted for age, PSA at diagnosis, Gleason score, tumor stage, primary treatment, BMI, and smoking status.

Table 4. Risk of PCSM by *SLCO2B1* and *SLCO1B3* SNP genotypes in a population-based prostate cancer cohort[a]

	SNP	Genotype	Prostate cancer death		HR (95% CI)[c]
			No[b]	Yes	
SLCO2B1	rs12422149	GG	993 (95.6)	46 (4.4)	1.00 (referent)
		GA + AA	210 (92.5)	17 (7.5)	1.99 (1.11–3.55)
SLCO2B1	rs949069	GG	811 (95.5)	38 (4.5)	1.00 (referent)
		GA + AA	397 (93.9)	26 (6.2)	1.39 (0.82–2.34)
SLCO2B1	rs2712819	AA	777 (95.0)	41 (5.0)	1.00 (referent)
		AG + GG	431 (95.1)	22 (4.9)	1.02 (0.60–1.77)
SLCO2B1	rs2851069	CC	452 (95.2)	23 (4.8)	1.00 (referent)
		CT + TT	752 (94.7)	42 (5.3)	1.21 (0.72–2.05)
SLCO2B1	rs7947726	GG	999 (95.1)	51 (4.9)	1.00 (referent)
		AG + AA	204 (94.0)	13 (6.0)	0.75 (0.37–1.53)
SLCO1B3	rs4149117	GG	885 (95.4)	43 (4.6)	1.00 (referent)
		GT + TT	326 (93.7)	22 (6.3)	1.76 (1.00–3.08)
SLCO1B3	rs3829311	GG	812 (95.0)	43 (5.0)	1.00 (referent)
		GA + AA	396 (94.7)	22 (5.3)	1.28 (0.74–2.23)
SLCO1B3	rs4762803	CC	958 (94.8)	53 (5.2)	1.00 (referent)
		CG + GG	251 (95.4)	12 (4.6)	1.16 (0.59–2.30)

[a]Variable number of cases due to failed genotyping.
[b]Includes 115 men who died of nonprostate cancer causes and who were censored at time of death.
[c]Adjusted for age, PSA at diagnosis, Gleason score, stage, primary treatment, BMI, and smoking status.

important limitation of gene expression data is that transcript level does not necessarily equate with protein expression; however, our findings are consistent with a hypothesis that in certain individuals, steroid transport proteins may play a role in the tumor androgen levels observed in CRPC metastases. In our analysis of data from a population-based cohort of prostate cancer patients, we found that 2 SNPs (*SLCO2B1* SNP rs12422149 and *SLCO1B3* SNP rs4149117) were associated with an increased risk of PCSM.

The increased risk of PCSM in carriers of the *T* versus *G* allele in *SLCO1B3* SNP rs4149117 (334T>G; adjusted HR: 1.76; 95% CI: 1.00–3.08), although of borderline significance, is consistent with the study of Hamada and colleagues (13). In their study of 180 men with CRPC, the HR for overall mortality was 1.57 (95% CI: 1.11–2.24) for men carrying the *T* allele in rs4149117. Notably, they showed that SLCO1B3-transfected cells actively transported testosterone, that testosterone uptake was higher for carriers of the *T* versus *G* allele, and that men homozygous for the *334GG* genotype (associated with impaired testosterone uptake) had a longer median survival (8.5 vs. 6.4 years; *P* = 0.02) and improved 10-year survival (42% vs. 23%; *P* = 0.023). In a separate study of 68 men treated with ADT for advanced prostate cancer, those with 2 copies of the *G* allele in rs4149117 had a longer time to androgen independence (1.6 vs. 1.2 years; *P* < 0.05; ref. 12). Of potential importance is that in the Caucasian population comprising these reports (and ours), SNP rs4149117 (334T>G) is in perfect linkage disequilibrium with SNP rs7311358 (699G>A). The study of Hamada and colleagues found that only the double-mutant haplotype showed impaired testosterone transport *in vivo*. Thus, different outcomes might be observed in non-Caucasian populations in which the SNPs may vary independently.

We also found an increased risk of PCSM in carriers of the *A* versus *G* allele in *SLCO2B1* SNP rs12422149 (935A>G; adjusted HR: 1.99; 95% CI: 1.11–3.55). This coding, nonsynonymous SNP has been reported in *in vitro* studies to have altered cellular uptake of certain medications (i.e., montelukast) and DHEA-S (dehydroepiandrosterone sulfate; ref. 32, 33). In a study of 538 castrate men with advanced prostate cancer receiving ADT (a different setting than the population-based cohort of incident prostate cancer cases in our study), the *A* allele in *SLCO2B1* SNP rs12422149 was associated with a longer time to progression (HR for progression: 1.40; 95% CI: 1.06–1.84 for the *G* vs. *A* allele; Phil Kantoff, personal communication). Interestingly, allelic variation in DHEA-S uptake by SLCO2B1 (and accordingly, any impact of cellular DHEA-S uptake on prostate cancer outcome) might be anticipated to differ in the eugonadal and castrate settings, as testosterone has been shown to inhibit SLCO2B1-mediated transport of DHEA-S (23). Thus, the association of *SLCO2B1* with prostate cancer outcome may reflect the transport of DHEA-S in advanced disease but that of an alternative substrate in incident prostate cancer cases.

We did not find any associations between *SLCO1B3* or *SLCO2B1* genotypes and prostate cancer recurrence/progression. This may be due to several reasons. First, recurrence/progression data were only available for one third of the cohort, which limited our power to detect a difference. Second, there can be significant variation (up to 35%) in reported PSA progression rates depending on how it is defined (34). Furthermore, considering the natural history of prostate cancer, many men who experience recurrence/progression events will not die of their disease (35). Finally, consistent with our findings of increased expression of *SLCO* gene members in metastatic tumors compared with primary tumors, it may be that alteration in *SLCO* activity is important in men with advanced, end-stage tumors but not in men with early recurrence, which may be due to other mechanisms.

Our data from a population-based cohort of prostate cancer patients suggest that genetic variation in the *SLCO* transport gene family is associated with prostate cancer mortality. These findings are consistent with results from studies of men with advanced prostate cancer, suggesting that genetic variation in *SLCO* genes may allow stratification of patients at higher risk for PCSM or poor response to ADT for consideration of either more aggressive hormonal therapies or incorporation of nonhormonal-based treatment strategies. In addition, we observe significantly increased expression of *SLCO* gene members in CRPC metastases, suggesting steroid transport proteins may contribute to the elevated tumoral androgen levels observed during CRPC progression. Future studies include immunohistochemical assessment of OATP expression in primary and metastatic prostate cancer tissues, and using *in vivo* models to evaluate the ability of SLCO transporters to measurably alter intratumoral androgen levels. The family of OATP/SLCO steroid transport proteins may serve as novel biomarkers of response to ADT, and common genetic variants in these genes may be associated with enhanced risk of prostate cancer mortality. Thus, their role in prostate cancer warrants further investigation.

Disclosure of Potential Conflicts of Interest

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References

1. Nelson PS, Clegg N, Arnold H, Ferguson C, Bonham M, White J, et al. The program of androgen-responsive genes in neoplastic prostate epithelium. Proc Natl Acad Sci U S A 2002;99:11890–5.
2. Pritchard CC, Nelson PS. Gene expression profiling in the developing prostate. Differentiation 2008;76:624–40.
3. Visakorpi T, Hyytinen E, Koivisto P, Tanner M, Keinänen R, Palmberg C, et al. *In vivo* amplification of the androgen receptor gene and progression of human prostate cancer. Nat Genet 1995;9:401–6.
4. Chen CD, Welsbie DS, Tran C, Baek SH, Chen R, Vessella R, et al. Molecular determinants of resistance to antiandrogen therapy. Nat Med 2004;10:33–9.
5. Feldman BJ, Feldman D. The development of androgen-independent prostate cancer. Nat Rev Cancer 2001;1:34–45.
6. Geller J, Albert J, Nachtsheim D, Loza D, Lippman S. Steroid levels in cancer of the prostate—markers of tumor differentiation and adequacy of anti-androgen therapy. Prog Clin Biol Res 1979;33:103–11.
7. Mohler JL, Gregory CW, Ford OH III, Kim D, Weaver CM, Petrusz P, et al. The androgen axis in recurrent prostate cancer. Clin Cancer Res 2004;10:440–8.
8. Page ST, Lin DW, Mostaghel EA, Hess DL, True LD, Amory JK, et al. Persistent intraprostatic androgen concentrations after medical castration in healthy men. J Clin Endocrinol Metab 2006;91:3850–6.
9. Mostaghel EA, Page ST, Lin DW, Fazli L, Coleman IM, True LD, et al. Intraprostatic androgens and androgen-regulated gene expression persist after testosterone suppression: Therapeutic implications for castration-resistant prostate cancer. Cancer Res 2007;67:5033–41.
10. Montgomery RB, Mostaghel EA, Vessella R, Hess DL, Kalhorn TF, Higano CS, et al. Maintenance of intratumoral androgens in metastatic prostate cancer: a mechanism for castration-resistant tumor growth. Cancer Res 2008;68:4447–54.
11. Stanbrough M, Bubley GJ, Ross K, Golub TR, Rubin MA, Penning TM, et al. Increased expression of genes converting adrenal androgens to testosterone in androgen-independent prostate cancer. Cancer Res 2006;66:2815–25.
12. Sharifi N, Hamada A, Sissung T, Danesi R, Venzon D, Baum C, et al. A polymorphism in a transporter of testosterone is a determinant of androgen independence in prostate cancer. BJU Int 2008;102:617–21.
13. Hamada A, Sissung T, Price DK, Danesi R, Chau CH, Sharifi N, et al. Effect of SLCO1B3 haplotype on testosterone transport and clinical outcome in Caucasian patients with androgen-independent prostatic cancer. Clin Cancer Res 2008;14:3312–8.
14. Hagenbuch B, Meier PJ. The superfamily of organic anion transporting polypeptides. Biochim Biophys Acta 2003;1609:1–18.
15. Pizzagalli F, Varga Z, Huber RD, Folkers G, Meier PJ, St-Pierre MV. Identification of steroid sulfate transport processes in the human mammary gland. J Clin Endocrinol Metab 2003;88:3902–12.
16. Tamai I, Nezu J, Uchino H, Sai Y, Oku A, Shimane M, et al. Molecular identification and characterization of novel members of the human organic anion transporter (OATP) family. Biochem Biophys Res Commun 2000;273:251–60.
17. Alcorn J, Lu X, Moscow JA, McNamara PJ. Transporter gene expression in lactating and nonlactating human mammary epithelial cells using real-time reverse transcription-polymerase chain reaction. J Pharmacol Exp Ther 2002;303:487–96.
18. Ugele B, St-Pierre MV, Pihusch M, Bahn A, Hantschmann P. Characterization and identification of steroid sulfate transporters of human placenta. Am J Physiol Endocrinol Metab 2003;284:E390–8.
19. Valle LD, Toffolo V, Nardi A, Fiore C, Bernante P, Di Liddo R, et al. Tissue-specific transcriptional initiation and activity of steroid sulfatase complementing dehydroepiandrosterone sulfate uptake and intracrine steroid activations in human adipose tissue. J Endocrinol 2006;190:129–39.
20. Miki Y, Suzuki T, Kitada K, Yabuki N, Shibuya R, Moriya T, et al. Expression of the steroid and xenobiotic receptor and its possible target gene, organic anion transporting polypeptide-A, in human breast carcinoma. Cancer Res 2006;66:535–42.
21. Al Sarakbi W, Mokbel R, Salhab M, Jiang WG, Reed MJ, Mokbel K. The role of STS and OATP-B mRNA expression in predicting the clinical outcome in human breast cancer. Anticancer Res 2006;26:4985–90.
22. Nozawa T, Suzuki M, Yabuuchi H, Irokawa M, Tsuji A, Tamai I. Suppression of cell proliferation by inhibition of estrone-3-sulfate transporter in estrogen-dependent breast cancer cells. Pharm Res 2005;22:1634–41.
23. Grube M, Köck K, Karner S, Reuther S, Ritter CA, Jedlitschky G, et al. Modification of OATP2B1-mediated transport by steroid hormones. Mol Pharmacol 2006;70:1735–41.
24. Roudier MP, True LD, Higano CS, Vesselle H, Ellis W, Lange P, et al. Phenotypic heterogeneity of end-stage prostate carcinoma metastatic to bone. Hum Pathol 2003;34:646–53.
25. Rozen S, Skaletsky HJ. Primer3 on the WWW for general users and for biologist programmers. In:Krawetz S, Misener S, editors. Bioinformatics Methods and Protocols: Methods in Molecular Biology. Totowa, NJ: Humana Press; 2000. p. 365–86. Available from:http://fokker.wi.mit.edu/primer3/.
26. Genome Variation Server [Internet]. SeattleSNPs Program for Genomic Applications(Version 5.11) [cited 2009 Sep 10]. Seattle, WA: Genome Variation Server. Available from:http://gvs.gs.washington.edu/GVS/.
27. Applied Biosystems [Internet][cited 2009 Sep 10]. Carlsbad,CA: Life Technologies Corporation.Available from:www.appliedbiosystems.com.
28. Wright JL, Kwon EM, Lin DW, Kolb S, Koopmeiners JS, Feng Z, et al. CYP17 polymorphisms and prostate cancer outcomes. Prostate2010;70:1094–101.
29. Harrell F. Regression Modeling Strategies. New York: Springer; 2001.
30. Roach M III, Hanks G, Thames H Jr, Schellhammer P, Shipley WU, Sokol GH, et al. Defining biochemical failure following radiotherapy with or without hormonal therapy in men with clinically localized prostate cancer: recommendations of the RTOG-ASTRO Phoenix Consensus Conference. Int J Radiat Oncol Biol Phys 2006;65:965–74.
31. Shah RB, Mehra R, Chinnaiyan AM, Shen R, Ghosh D, Zhou M, et al. Androgen-independent prostate cancer is a heterogeneous group of diseases: lessons from a rapid autopsy program. Cancer Res 2004;64:9209–16.
32. Mougey EB, Feng H, Castro M, Irvin CG, Lima JJ. Absorption of montelukast is transporter mediated: a common variant of OATP2B1 is associated with reduced plasma concentrations and poor response. Pharmacogenet Genomics 2009;19:129–38.
33. Yang M, Oh WK, Xie W, Mostaghel EA, Sun T, Sharifi N, et al. Genetic variations in SLCO2B1 and SLCO1B3 and the efficacy of androgen-deprivation therapy in prostate cancer patients. In:ASCO Genitourinary Cancers Symposium 2010 (San Francisco, CA, March 5–7).
34. Stephenson AJ, Kattan MW, Eastham JA, Dotan ZA, Bianco FJ Jr, Lilja H, et al. Defining biochemical recurrence of prostate cancer after radical prostatectomy: a proposal for a standardized definition. J Clin Oncol 2006;24:3973–8.
35. Collette L. Prostate-specific antigen (PSA) as a surrogate end point for survival in prostate cancer clinical trials. Eur Urol 2008;53:6–9.

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Expression of *SLCO* Transport Genes in Castration-Resistant Prostate Cancer and Impact of Genetic Variation in *SLCO1B3* and *SLCO2B1* on Prostate Cancer Outcomes

Jonathan L. Wright, Erika M. Kwon, Elaine A. Ostrander, et al.

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