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Association of Prostate Cancer *SLCO* Gene Expression with Gleason Grade and Alterations Following Androgen Deprivation Therapy

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Abstract

Background.—*SLCO*-encoded transporters have been associated with progression to castration resistant prostate cancer (CRPC) after initiation of androgen deprivation therapy (ADT). Although expressed at lower levels than in CRPC tissues, *SLCO*-encoded transporters may also play a role in response of primary prostate cancer (PCa) to ADT and biochemical recurrence.

Methods.—We systematically explored expression of the 11 human *SLCO* genes in a large sample of untreated and ADT-treated normal prostate (NP) and primary PCa tissues, including tumors treated with neoadjuvant abiraterone.

Results.—Transporters with the most recognized role in steroid uptake in PCa, including *SLCO2B1* (DHEAS) and *1B3* (testosterone), were consistently detected in primary PCa.

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SLCO1B3 was nearly 5-fold higher in PCa vs NP with no difference in Gleason 3 vs 4 and no change with ADT. *SLCO2B1* was detected at 3-fold lower levels in PCa than NP but was nearly 7-fold higher in Gleason 4 vs Gleason 3 and increased 3-fold following ADT ($p<0.05$ for all).

Conclusions.—We observed clear differences in *SLCO* expression in PCa vs NP samples, in Gleason 4 vs Gleason 3 tumors, and in ADT-treated vs untreated tissues. These findings are hypothesis generating due to small sample size, but suggest that baseline and ADT-induced changes in PCa OATP expression may influence steroid uptake and response to ADT, as well as uptake and response to drugs such as abiraterone and docetaxel which are also subject to OATP-mediated transport and are now being routinely combined with ADT in the metastatic castration sensitive setting.

Keywords

OATP; SLCO; androgen transport; biochemical recurrence; genetic variation; primary prostate cancer

INTRODUCTION

Androgen deprivation therapy (ADT) is standard of care for men with advanced prostate cancer (PCa) but is inevitably followed by castration-resistant prostate cancer (CRPC). Despite suppression of circulating androgens, prostatic androgens following castration remain well above levels capable of engaging AR, and CRPC metastases contain testosterone levels four times higher than prostate tissue of eugonadal men.[1–3]

The organic anion transporting polypeptides (OATP) are *SLCO*-encoded proteins that transport bile acids, xenobiotics, steroid conjugates, and important drugs including taxanes.[4–6] Several OATPs (e.g. OATP1A2, 1B1, 1B3, and 2B1) mediate uptake of steroids into PCa cells *in vitro* and *in vivo*,[7–11] and single nucleotide polymorphisms (SNPs) of *SLCO1B3* and *SLCO2B1* that demonstrate enhanced androgen uptake are associated with more rapid disease progression in men with metastatic disease treated with ADT.[8,12,13] CRPC metastases express transcripts encoding *SLCO* transporters at significantly higher levels than in primary PCa.[14] These data strongly support a role for OATP-mediated steroid transport in moderating the tissue response to ADT and promoting disease progression in men with advanced disease.

In contrast, in primary PCa the extent to which *SLCO* genes influence disease or become altered in response to hormonal therapy is not well-explored. Pressler et al examined *SLCO1B1*, *SLCO1B3* and *SLCO2B1* in a five normal prostate and 21 PCa samples and observed higher *SLCO1B3* in primary PCa vs normal prostate, and an association of *SLCO1B3* with Gleason score.[15] Wright et al evaluated *SLCO1B1, SLCO1B3, SLCO2A1, SLCO2B1, SLCO3A1,* and *SLCO4A1* levels in eight benign and eight PCa samples without significant differences in tumor vs normal tissue, likely due to limited number of samples.[14] They also found no association of genetic differences in *SLCO1B3* or *SLCO2B1* with PCa recurrence in a case control sample of 469 men with localized PCa (no hormone therapy). However, an impact of *SLCO* expression on steroid transport in primary PCa may be most relevant in the setting of ADT (e.g. ADT combined with definitive radiation therapy). In

particular, the extent to which expression of *SLCO* genes in primary PCa is altered by ADT is unknown but may provide insight into induction of *SLCO* transporters as a mechanism of resistance to ADT.

We evaluated the eleven human *SLCO* genes in microdissected benign (n=20) and cancer (n=35) tissues from untreated men with localized PCa, including separately microdissected foci of Gleason grade 3 vs grade 4 tumors. We profiled *SLCO* expression in a cohort of matched benign (n=20) and cancer (n=18) prostate samples from men enrolled in a trial of neoadjuvant ADT prior to radical prostatectomy (RP), and in primary PCa samples (n=13) from men enrolled in a trial of neoadjuvant ADT plus abiraterone acetate (ABI) prior to RP.[16,17] Finally, we evaluated the impact of 12 SNPS in 7 *SLCO* genes on risk of biochemical recurrence in a cohort of 147 men with localized PCa treated with radical prostatectomy (RP).

MATERIALS AND METHODS

Patient Samples

All procedures were approved by Institutional Review Boards of University of Washington and Fred Hutchinson Cancer Research Center. Frozen prostate tissue was collected from men with Gleason grade 3 and/or grade 4 PCa under an approved protocol for use of excess tissue after RP, and from men with intermediate to high risk PCa (Gleason score ≥7) treated on a previously published trial of ADT prior to RP (NCT00298155).[17] (Greater than 95% of men for whom data was available self-identified as Caucasian). Hormonal regimens included 1) goserelin with bicalutamide, 2) goserelin with dutasteride (3.5 mg), 3) goserelin with bicalutamide and dutasteride, and 4) goserelin with bicalutamide, dutasteride and ketoconazole (200 mg three times daily; with prednisone 5 mg daily). Formalin fixed prostate tissue (FFPE) was obtained from men with intermediate to high risk PCa (Gleason score ≥7) treated on a previously published trial of lupron plus ABI prior to RP (NCT00924469).[16] Genomic DNA was isolated via macrodissection of benign prostate tissue from hematoxylin and eosin (H&E) stained FFPE tissue sections from 147 patients who underwent RP between 1995 and 2010 at the University of Washington for whom recurrence data was available.

Laser Capture Microdissection and RNA Isolation

Microdissection and RNA isolation was performed from frozen prostate tissue as epreviously described.[3] In untreated tissues, foci of Gleason 3 and Gleason 4 cancer were separately microdissected. All areas to be micro-dissected were selected reviewed by a pathologist (X.Z., L.T. or H.Y). Microdissection and RNA isolation of FFPE samples from ABI treated samples was performed as described.[18]

SLCO Transcript Profiling

Quantitative reverse transcription (qPCR) was performed as described.[14] The mean Ct for each gene was normalized to the housekeeping gene *RPL13A* (delta C_t or dCt). No consistent differences in RPL13A expression itself were observed in the normal vs prostate samples (Supplementary Fig S1). Samples with undetectable expression of a given gene

were assigned d*Ct* value of 33 for purposes of calculation. Fold change was calculated from the difference in mean d*Ct* between sample groups (dd*Ct* method; fold = 2^dd*Ct*). Primer sequences are as published.[14]

SLCO Genotyping Analysis

Genomic DNA was purified using the QIAamp® DNA FFPE Tissue Kit. Twelve single nucleotide polymorphisms (SNPs) with minor allelic frequency (MAF) > 10% in 6 *SLCO* genes (*SLCO1B1, 1B3, 2B1, 2A1, 3A1,* and *5A1*) were genotyped using TaqMan SNP assays as described.[13] The SNP assay numbers, minor allele frequency and potential role in PCa are shown in Supplementary Table 1.

Statistical Analysis

Comparisons of *SLCO* gene expression in PCa vs NP, Gleason grade 3 vs 4 samples, and treated vs untreated PCa were performed with un-paired two-tailed t tests. The variance between groups was compared using an F test and if significantly different ($p<0.05$) Welch's correction was applied. Time to recurrence was number of months from RP to the first measurement of biochemical relapse (BCR; PSA ≥ 0.2), death, loss to follow-up or 10 years. Binary recurrence within 10 years was compared across different genotypes using Fisher's Exact Test. Cox proportional hazards models were used to compare time to recurrence by genotype, adjusted for tumor volume, TNM staging, pre-prostatectomy PSA, Gleason grade, and margin status. Heterozygous and rare homozygous variants were combined if total number of cases in any genotype was fewer than 10.

RESULTS

Expression of SLCO genes in untreated prostate tissue

We evaluated *SLCO* expression in normal prostate (NP; n=20) and separately microdissected foci of Gleason grade 3 and grade 4 PCa (n=35) from men with localized PCa undergoing RP. In untreated tissues (cancer or benign), the percentage of samples that were undetectable for a given gene varied and did not represent a unique population (0–3% samples undetectable for *SLCO2B1*, *3A1*, and *5A1;* 10–20% for *1B3* and *4A1*; 40% for *1C1 and 4C1*; 60–80% for *1A2* and *1B1*; and 93% for *6A1).* Transcript data in NP vs PCa (comprising both Gleason grade 3 and 4 tumors) are shown in Fig 1 and summarized in Table 1.

In NP, levels of *SLCO2A1, 3A1* and *5A1* (Fig 1A-C) were most abundant (average d*Ct* vs *RPL13A* of −4 to −6), while *SLCO2B1*, *4A1* and *4C1* (Fig 1D–F) were several orders of magnitude lower (d*Ct* −14 to −17), but still easily detectable. Average levels of *SLCO1B3*, *1C1* and *1B1* were very low in NP (d*Ct* of −19–22), and largely undetectable for *SLCO1A2* (Fig 1G-J). *SLCO6A1* was only detected in a few samples (not shown) and was not analyzed further.

Multiple *SLCO* genes showed differential expression in PCa vs NP. PCa expressed higher *SLCO1B3* (4.8 fold, p=0.045; Fig 1G), *SLCO1C1* (21 fold, p=0.002; Fig 1H), and *1B1* (2.7 fold, p=0.016; Fig 1I), and lower *SLCO4C1* (−37 fold, p<0.0001; Fig 1E), 3A1 (−10 fold,

p=<0.0001; Fig 1B) and 2B1 (−2.8 fold, p=0.011; Fig 1D). Thus, *SLCO2A1, 3A1* and *5A1* remain the most highly expressed genes in tumor (d*C*t −4 to −8), while *SLCO1B3* and *1C1* move up to join *SLCO2B1* and *4A1* as the next most abundant (dCt −15 to −16), and *SLCO4C1* moves down with *SLCO1A2* and *1B1* as the least abundant (dCt-21 to −22). Consistent with a prior report, we detected minimal to no expression of the truncated *SLCO1B3* splice variant in primary PCa (not shown).[11]

Association of Gleason grade with SLCO gene expression

Differences in tumor androgen levels have been reported in high vs low grade PCa.[19] Therefore, we sought to determine whether differential expression of *SLCO* genes (and thereby differences in androgen uptake) might plausibly contribute to this difference. Gleason 4 tumors (n=18) had significantly higher *SLCO1C1* (24-fold, p= 0.007, Fig 2A), 2B1 (6.7-fold, p=0.0001; Fig 2B) and 3A1 (3.5-fold, p=0.014; Fig 2C) than Gleason 3 tumors (n=17; Table 1 and Fig 2). No differences were observed in the remaining *SLCO* genes (Supplementary Fig S2).

Impact of androgen deprivation on SLCO gene expression

To determine whether expression of *SLCO* genes is altered by hormonal therapy, we microdissected PCa and NP in frozen prostate tissue from 20 men with high risk localized PCa treated with various combinations of goserelin (Zoladex, Z), bicalutamide (Casodex, C), dutasteride (D) and ketoconazole (K) in a previously completed trial of neoadjuvant ADT for three months prior to surgery.[17] Due to morphologic changes caused by ADT, Gleason grade was not assessed. Specimen numbers in each subset do not enable a statistically rigorous assessment Specimen numbers in each subset do not enable a statistically rigorous assessment among the different regimens (ZC, n=2; ZD n=4; ZCD n=6; and ZCDK n=8; Supplementary Fig S3), and samples were grouped into treated NP or treated PCa sets for subsequent analysis.

Compared to untreated tissues, ADT induced differential expression of *SLCO* genes in both NP and PCa (Fig 1 and Table 2). ADT-treated tumor samples had higher *SLCO3A1* (4.4 fold, p=0.0007; Fig 1B), 2B1 (3.6 fold, p=0.032; Fig 1D), and *1C1* (9.8 fold, p=0.03; Fig 1H), but lower *2A1* (−2.3 fold, p=0004; Fig 1A) and *5A1* (−4.2 fold, p=0.0004; Fig 1C). The direction and magnitude of change was generally similar for PCa and NP except for *SLCO5A1,* in which NP showed a significantly larger decrease in expression (−15 fold, p<0.0001; Fig 1C) than that in tumor; *SLCO1B3,* in which NP showed a decrease after ADT (−5 fold, p=014; Fig 1G) while tumor did not; and *SLCO3A1* and *2B1,* in which NP did not show the increase in expression observed in tumor (Fig 1B and 1D).

To assess the impact of more potent androgen suppression we microdissected FFPE PCa samples from a trial of Lupron plus 3 to 6 months of ABI prior to RP.[16] While absolute transcript levels were lower than those in frozen tissue, the four *SLCO* genes most highly expressed in PCa in the studies above (*SLCO2A1, 3A1, 5A1 and 2B1)* were also detected in the FFPE samples (Fig 3). Moreover, there was a near statistically significant decrease in *SLCO2A1* (p=0.07; Fig 3A) and increase in *SLCO2B1* (p=0.09; Fig 3D) in the ABI plus ADT-treated samples, similar to our findings in the ADT-treated tumor samples. While the

changes in *SLCO3A1* and *SLCO5A1* were not statistically significant, the direction of changes in the tumor samples following treatment (increased for *SLCO3A1*, decreased for *SLCO5A1*) were also consistent with the findings above.

Association of SLCO genotype with prostate cancer recurrence after prostatectomy

Genomic DNA was obtained from 147 patients with PCa who underwent RP between 1995 and 2010. Longitudinal follow up (median 60 months) yielded 67 patients with and 80 without evidence of BCR. Demographics and clinical characteristics of patients are shown in Supplementary Table 2. PCa patients with BCR had more aggressive features on final prostate pathology; higher stage disease (30% had pT3 N0 vs. only 8.8% in non-BCR group), higher Gleason scores (80.6% Gleason 7 vs. 42.5 % in non-BCR), more positive margins and larger tumor volumes. Samples were genotyped for 12 SNPS with MAF>10%, selected based a published role in hormone transport/metabolism and/or significance in PCa.[13] Binary recurrence within 10 years showed no statistically significant difference across the different SNP genotypes except for *SLCO2A1* SNP rs34550074 (p= 0.025). However, after adjustment for clinical variables the resultant model was no longer significant (Supplementary Table 3).

DISCUSSION

Although expressed at lower levels than in CRPC, *SLCO*-encoded transporters may also play a role in response of primary PCa to neoadjuvant/adjuvant ADT and in driving BR following definitive therapy. We systematically explored expression of *SLCO* genes in a large sample of untreated and ADT-treated primary PCa tissues. Transporters with the most recognized role in steroid uptake in PCa, including *SLCO2B1* (DHEAS) and *1B3* (testosterone), were consistently detected in primary PCa tissue.[8–11] We observed clear differences in PCa vs NP samples, in Gleason 4 vs Gleason 3 tumors, and in ADT-treated vs untreated tissues.

The increased expression of *SLCO1B3* observed in PCa vs NP is consistent with prior reports showing higher *SLCO1B3* in PCa vs NP.[8,15] While Pressler et al noted an association of Gleason grade with *SLCO1B3,* we observed an association of Gleason grade with *SLCO2B1* (this may reflect differences in sample preparation as we report microdissected foci of Gleason 3 and 4 tumors whereas Pressler et al reported tumor grade as the Gleason sum). However, the increased expression of *SLCO2B1* in higher Gleason grade tumors is consistent with findings recently reported in primary PCa samples from The Cancer Genome Atlas (TCGA).[20]

SLCO-encoded genes may influence primary PCa in multiple ways. While the higher *SLCO1B3* expression in cancer, or the higher *SLCO2B1* levels in Gleason 4 vs Gleason 3 tumors might associate with higher tissue androgen levels and more aggressive cancer, the association of tissue androgens and PCa risk is not well understood. In one study of 196 patients, higher tissue testosterone levels were significantly related to higher Gleason scores, but no association was noted with DHT, whereas in a different study of 81 patients, DHT levels were lower in patients with Gleason 7–10 disease vs Gleason 6 (testosterone not

measured), actually suggesting an inverse association of androgen uptake with tumor aggressiveness.[19,21]

Importantly, testosterone interferes with OATP2B1-mediated transport of DHEAS, and thus OATP-mediated steroid uptake in the eugonadal setting may not follow the same paradigm as in ADT.[22] This is consistent with our data showing no association of *SLCO* genotype with PCa recurrence in 147 men not treated with ADT. Our recurrence data set clearly has limitations due to small cohort size, but is consistent with the larger study of Wright et al showing no association of *SLCO* genotype with recurrence in 489 patients.[14]

However, in a study of 494 primary PCa cases from TCGA, high *SLCO2B1* was associated with worse disease free survival (DFS) after RP (no association was noted for *SLCO1B3*). [20] Notably, this study reported higher *SLCO2B1* in higher Gleason grade tumors (consistent with our data), and found the association with DFS was only significant for Gleason score ≥8. This suggests that any impact of *SLCO2B1* expression (or genotype) on recurrence or progression is most important in high grade disease in which it is more highly expressed. In this regard, our study and that of Wright et al may have been negative because the populations were low risk (81% and 85% with Gleason score 6 or 3+4, respectively), whereas >40% of patients in the TCGA study had a Gleason score ≥8.

Alternatively, *SLCO*-encoded transporters may mediate uptake of other OATP substrates relevant to prostate carcinogenesis and/or progression, including prostaglandins (PGs), thyroid hormones, green tea catechins, taxanes, statins, cardiac glycosides, glitazones, and metformin.[23–33] In this regard, several of the transporters most abundantly expressed in the prostate do not have a recognized role in androgen transport. Of these, *SLCO3A1* (estrone-sulfate, PGs), and *4C1* (estrone-sulfate, cAMP, thyroid hormones) were expressed at significantly lower levels in CP vs NP, possibly suggesting transport of a substrate with anti-tumor activity. In contrast, increased expression of the thyroid hormone transporter *SLCO1C1* may represent a substrate with tumor promoting properties as thyroid hormones have been shown to promote PCa cell proliferation *in vitro*, and several studies have suggested an association between increased thyroid hormone levels and PCa risk.[24,29,34–41]

As in advanced PCa, OATP-mediated uptake of androgens in primary PCa may be most relevant in modifying response to ADT. The most substantive evidence for OATP-mediated androgen uptake in PCa is that of DHEAS by OATP2B1. DHEAS uptake is dependent on the expression of *SLCO2B1* and greater expression of *SLCO2B1* resulted in increased DHEAS transport into cells.[9] Thus, the increased *SLCO2B1* observed with standard and ABI containing neoadjuvant ADT may adversely influence response to therapy, such as in patients with localized PCa receiving neoadjuvant/adjuvant ADT in context of definitive radiation, or patients with newly diagnosed metastatic disease being treated with ADT and ABI. This may be particularly relevant for *SLCO2B1* genotypes associated with increased transport efficiency or higher protein expression.[9,12] Additionally, the 6.7 fold higher *SLCO2B1* in Gl4 vs Gl3 tumors may render Gl4 disease more resistant to ADT, which would be consistent with data from a small study of 28 patients which showed that the decrease in tissue DHT following 6 months of ADT was less in Gleason 7–10 disease than Gleason 6 disease.[42] As OATP2B1 transports both DHEAS and ABI, any net impact of the

increase in *SLCO2B1* expression induced by ADT and ABI remains to be empirically determined from clinical studies.[43]

OATP proteins are critical mediators of hepatic taxane uptake and primary determinants of systemic taxane exposure.[29,40] A pharmacokinetic study reported that docetaxel clearance increased by approximately 100% in castrate compared to non-castrate men with PCa, and rat studies found that castration increased docetaxel clearance and was associated with increased hepatic expression of rOat2 (*Slc22a7*), a mouse Oatp shown to transport docetaxel.[44] OATPs also modify intratumoral accumulation of docetaxel and cabazitaxel, strongly influencing response of PCa xenografts to treatment[28,45], and loss of tumor *SLCO1B3* expression is a mechanism of resistance in taxane-refractory prostate tumors.[41] Thus, an increase in tumor *SLCO* expression following ADT could potentially influence docetaxel transport and enhance treatment response.

Regulation of OATP expression and function occurs at both the transcriptional and post-transcriptional level and is, at least in part, tissue-specific.[46,47] While androgen regulation of rodent renal Oatp expression has been demonstrated in male and female rodents, androgen regulation of *SLCO* genes in prostate tissue has not been explored.[48,49]

In summary, clear differences in *SLCO* transcript expression are present in primary PCa compared to NP in Gleason 4 vs Gleason 3 tumors, and in ADT-treated vs untreated tissues. These findings are hypothesis generating due to small sample size, but suggest that baseline and ADT-induced changes in PCa OATP expression may influence steroid uptake and response to ADT, as well as uptake and response to critical PCa drugs such as ABI and docetaxel, which are subject to OATP-mediated transport and are now being routinely combined with ADT in the metastatic castration sensitive setting. Future work may identify how targeting the induction, repression or inhibition of these transporters might be exploited for therapeutic benefit.[50,51] Supplementary information is available at PCAN's website.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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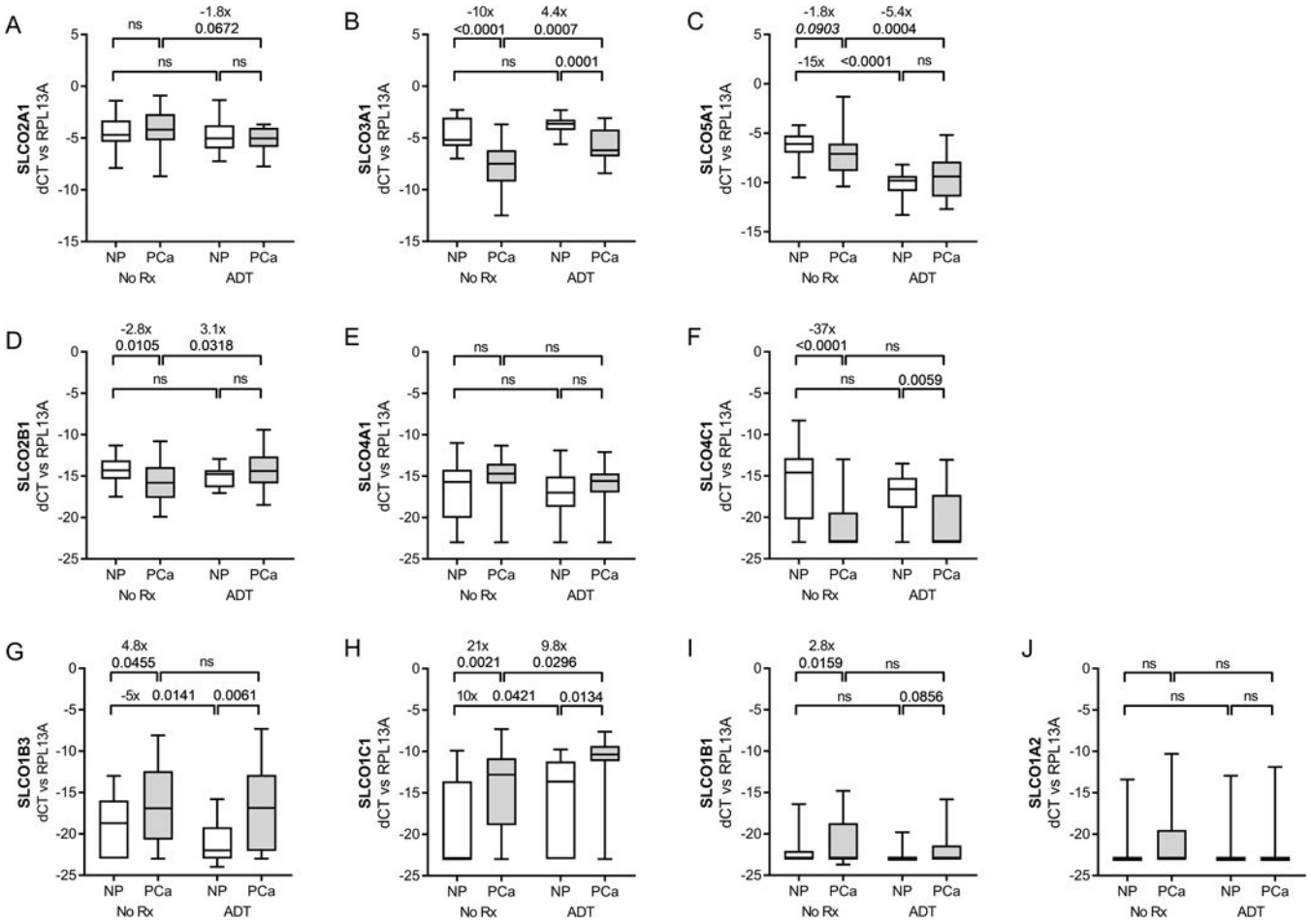

Figure 1. Expression of *SLCO* genes, ranked by abundance in primary prostate tissues.

Transcript levels of the indicated *SLCO* genes (normalized to the housekeeping gene *RPL13A*) were evaluated in microdissected normal prostate (NP) and prostate cancer (PCa) epithelium from untreated men (No Rx) or from men treated with neoadjuvant androgen deprivation therapy (ADT) for three months prior to prostatectomy. Data are shown as box-and-whisker plots, where horizontal lines indicate median values; white boxes denote the 75th (upper margin) and 25th percentiles (lower margin), and upper and lower bars indicate minimum and maximum values, respectively. Fold changes and p values for the indicated comparisons are given. P-values calculated using a two-sided t test (P values ≤0.05 were considered significant; and those ≤ 0.01 as trending toward significance - *italicized*).

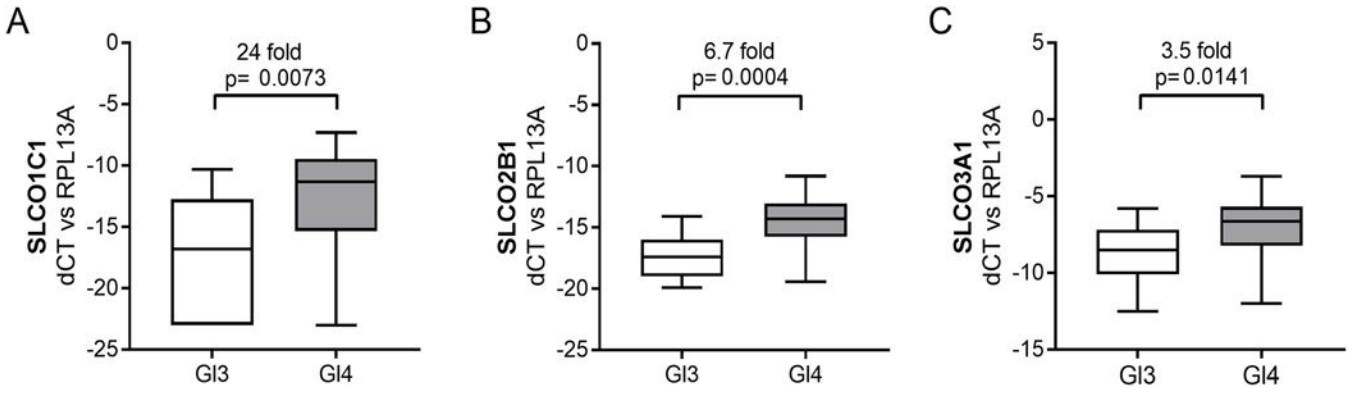

Figure 2. *SLCO* gene expression in Gleason 3 vs Gleason 4 prostate tumors.

Transcript levels of the indicated *SLCO* genes were evaluated in microdissected foci of Gleason 3 (Gl3, n=18) or Gleason 4 (Gl4, n=17) PCa from untreated men. Presentation of data as box and whisker plots and statistical analyses are as described in the legend for Figure 1.

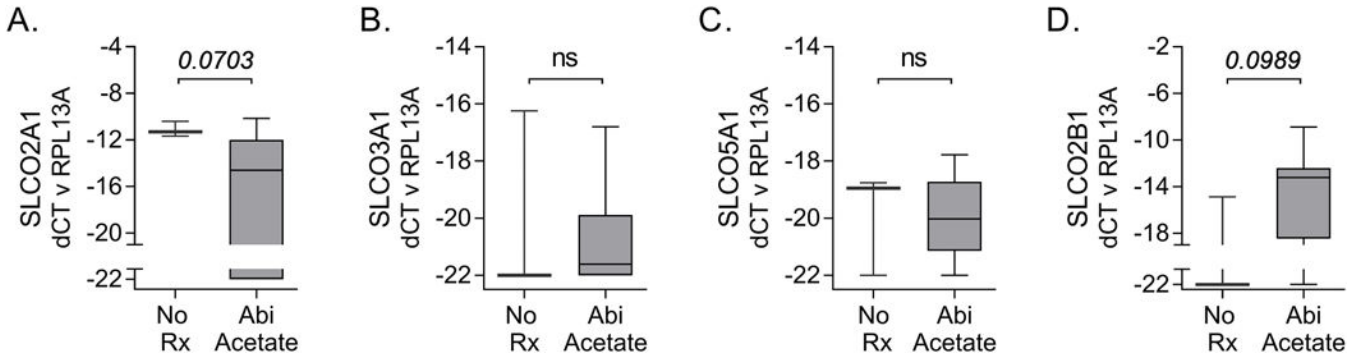

Figure 3. Expression of *SLCO* genes in primary prostate cancer after neoadjuvant treatment with abiraterone.

Transcript levels of the indicated *SLCO* genes (normalized to the housekeeping gene *RPL13A*) were evaluated in microdissected foci of cancer from untreated men or those treated with neoadjuvant abiraterone for 3–6 months prior to prostatectomy. Presentation of data as box and whisker plots and statistical analyses are as described in the legend for Figure 1.

Table 1.

Expression of *SLCO* Genes in Hormone Naive Primary Prostate Cancer

	Normal Prostate (NP)	Prostate Cancer (PCa)	PCa vs NP		Gleason 4 vs 3	
	avg dCt*	avg dCt*	fold	P value**	fold	P value**
SLCO1A2	−22	−21	2.0	ns	−1.5	ns
SLCO1B1	−22	−21	**2.7**	**0.016**	1.0	ns
SLCO1B3	−19	−16	**4.8**	**0.045**	−1.3	ns
SLCO1C1	−19	−15	**21**	**0.002**	**24**	**0.007**
SLCO2A1	−5	−4	1.7	*0.055*	1.8	ns
SLCO2B1	−14	−16	**−2.8**	**0.011**	**6.7**	**0.0001**
SLCO3A1	−4	−8	**−10**	**<0.0001**	**3.5**	**0.014**
SLCO4A1	−17	−15	2.8	ns	1.6	ns
SLCO4C1	−16	−21	**−37**	**<0.0001**	2.3	ns
SLCO5A1	−6	−7	−1.8	*0.09*	1.5	ns

* normalized to RPL13A, d*C*t of −23 considered negative

** p values from two-sided t tests, p≤0.05 significant, p≤0.10 (*italicized*) trending toward significance

Table 2.

Change in *SLCO* Gene Expression in Normal and Cancer Prostate Epithelium after Androgen Deprivation

	Normal Prostate (NP)		Prostate Cancer (PCa)	
	fold*	**p value****	**fold***	**p value****
SLCO1A2	−1.3	ns	−1.9	ns
SLCO1B1	−1.4	ns	−1.7	ns
SLCO1B3	**−4.9**	**0.014**	−1.2	ns
SLCO1C1	**10.2**	**0.042**	**9.8**	**0.030**
SLCO2A1	−1.3	ns	−1.8	*0.07*
SLCO2B1	−1.8	*0.07*	**3.1**	**0.032**
SLCO3A1	1.7	*0.06*	**4.4**	**0.0007**
SLCO4A1	−1.2	ns	−1.4	ns
SLCO4C1	−2.8	ns	1.5	ns
SLCO5A1	**−15.3**	**<0.0001**	**−5.4**	**0.0004**

* fold change relative to untreated tissue

** p values calculated as in Table 1