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Association of Tissue Abiraterone Levels and *SLCO* Genotype with Intraprostatic Steroids and Pathologic Response in Men with High-Risk Localized Prostate Cancer

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Abstract

Purpose: Germline variation in solute carrier organic anion (*SLCO*) genes influences cellular steroid uptake and is associated with prostate cancer outcomes. We hypothesized that, due to its steroidal structure, the CYP17A inhibitor abiraterone may undergo transport by *SLCO*-encoded transporters and that *SLCO* gene variation may influence intracellular abiraterone levels and outcomes.

Experimental Design: Steroid and abiraterone levels were measured in serum and tissue from 58 men with localized prostate cancer in a clinical trial of LHRH agonist plus abiraterone acetate plus prednisone for 24 weeks prior to prostatectomy. Germline DNA was genotyped for 13 SNPs in six *SLCO* genes.

Results: Abiraterone levels spanned a broad range (serum median 28 ng/mL, 108 nmol/L; tissue median 77 ng/mL, 271 nmol/L) and were correlated ($r = 0.355$, $P = 0.001$). Levels correlated positively with steroids upstream of CYP17A (pregnenolone, progesterone), and inversely with steroids downstream of CYP17A (DHEA, AED, testosterone). Serum PSA and tumor volumes were higher in men with undetectable versus detectable tissue abiraterone at prostatectomy (median 0.10 vs. 0.03 ng/dL, $P = 0.02$; 1.28 vs. 0.44 cc, $P = 0.09$, respectively). SNPs in *SLCO2B1* associated with significant differences in tissue abiraterone (rs1789693, $P = 0.0008$; rs12422149, $P = 0.03$) and higher rates of minimal residual disease (tumor volume < 0.5 cc; rs1789693, 67% vs. 27%, $P = 0.009$; rs1077858, 46% vs. 0%, $P = 0.03$). LNCaP cells expressing SLCO2B1 showed two- to fourfold higher abiraterone levels compared with vector controls ($P < 0.05$).

Conclusions: Intraprostatic abiraterone levels and genetic variation in *SLCO* genes are associated with pathologic responses in high-risk localized prostate cancer. Variation in *SLCO* genes may serve as predictors of response to abiraterone treatment. *Clin Cancer Res; 23(16); 4592–601. ©2017 AACR.*

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Introduction

Although clinical responses to abiraterone acetate plus prednisone in men with castration-resistant prostate cancer (CRPC) have been impressive, not all men respond, and the duration of response is variable (1, 2). Abiraterone acetate is a prodrug of abiraterone, a CYP17A inhibitor. Factors influencing the efficacy of abiraterone acetate plus prednisone in suppressing serum and tissue androgens and inhibiting tumor growth in prostate cancer are not well understood. Proposed associations with improved responses in men with CRPC include the presence of serum androgen levels above versus below the median at baseline (3, 4), the detection of intense nuclear androgen receptor (AR) expression combined with at least 10% CYP17A tumor staining in tumor-infiltrated bone marrow samples (5), the absence of the constitutively active AR splice variant ARV7 in circulating tumor cells prior to the start of therapy (6), and the absence of AR and *CYP17A* gene aberrations, such as copy-number increase and/or mutation (7–9).

Organic anion transport proteins (OATPs) transporters, encoded by the solute carrier organic anion (*SLCO*) gene family,

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Translational Relevance

Factors influencing the efficacy of abiraterone in suppressing androgens and inhibiting tumor growth in castration-resistant prostate cancer are not well understood. Germline variation in solute carrier organic anion (*SLCO*) genes influences the cellular uptake of steroids and is associated with prostate cancer outcomes. On the basis of its steroidal structure, we hypothesized abiraterone may undergo transport mediated by *SLCO*-encoded transporters and that variation in *SLCO* genes may associate with tissue abiraterone levels and treatment response. In a neoadjuvant trial of abiraterone acetate plus prednisone prior to prostatectomy in men with localized prostate cancer, we find that intraprostatic abiraterone levels are associated with pathologic responses and that genetic variation in *SLCO2B1* is associated with tissue abiraterone levels and rates of minimal residual disease. *In vitro*, we show higher abiraterone uptake in prostate cancer cell lines expressing SLCO2B1 compared with vector controls. These data suggest variation in *SLCO* genes should be evaluated as predictors of response to abiraterone acetate plus prednisone treatment in men with castration-resistant prostate cancer.

mediate the cellular uptake of numerous drugs and steroids and have been implicated in prostate cancer progression (10–13). We and others have shown that *SLCO* genes are expressed in prostate tumors, that germline variation in *SLCO1B3* and *SLCO2B1* can influence the uptake of steroids such as testosterone and DHEA sulfate (DHEAS) into prostate cancer cells *in vitro*, and that genetic variants in certain *SLCO* genes are associated with time to progression (TTP) and overall survival (OS) in response to androgen deprivation therapy (ADT; refs. 14–19). Recently, we have also demonstrated that expression of SLCO2B1 can measurably alter intratumoral DHEA levels in prostate tumors *in vivo* (20).

On the basis of its steroidal structure, we hypothesized that abiraterone may undergo transport mediated by *SLCO*-encoded transporters and that variation in *SLCO* transport genes may be associated with prostate tissue abiraterone levels and treatment response. In a neoadjuvant trial of abiraterone acetate plus prednisone prior to prostatectomy in men with intermediate or high-risk localized prostate cancer, we find that intraprostatic abiraterone levels are associated with pathologic responses and that genetic variation in *SLCO2B1* is associated with differences in mean tissue abiraterone levels at prostatectomy and with higher rates of achieving minimal residual disease (MRD).

Materials and Methods

Patients, study design, and treatment

The patients and study design have been reported previously (21). The study (COU-AA-201; NCT00924469) was a randomized, open-label phase II clinical trial of men with intermediate- or high-risk localized prostate cancer ($\geq$3 positive biopsies) and $\geq$1 high-risk feature: PSA > 10 ng/mL, PSA velocity > 2 ng/mL/y, or Gleason score $\geq$ 7. Sixty-nine percent of patients had Gleason score 8; the remaining patients had Gleason score 7 and PSA $\geq$ 10 ng/mL or elevated PSA velocity. Baseline patient characteristics as previously reported are provided in Supplementary Table S1 for reference (21). Patients were randomized to receive a luteinizing hormone-releasing hormone agonist (LHRHa) alone for 12 weeks followed by the combination of LHRHa plus abiraterone acetate (1,000 mg/day) and prednisone (5 mg/day) for an additional 12 weeks (LHRHa 24/abiraterone 12), versus LHRHa plus upfront abiraterone acetate and prednisone for 24 weeks (LHRHa 24/abiraterone 24). Patients took abiraterone acetate in a fasted state, and drug levels were drawn 2 to 4 hours after daily dose. Snap-frozen prostate tissue for analysis of hormone and abiraterone levels was obtained via a research biopsy at week 12 and at radical prostatectomy at week 24. All patients signed written informed consent, and participating Institutional Review Boards approved the study, which was conducted per the Declaration of Helsinki in accordance with the World Medicines Association and its amendments.

Pathology

Methods for determination of pathologic outcomes for this study were as previously reported (21). In brief, residual tumor volume was calculated using the largest cross-sectional dimension of tumor in reconstructed "whole cross-sectional slices" with MRD defined as $\leq$0.5 cc. Tumor volume was independently scored by five separate study pathologists involved in the original study, and the residual tumor volume was determined from the median of their estimates.

Steroid and abiraterone measurements

Methods for determination of steroids and abiraterone in serum and prostate tissue samples by mass spectrometry were as previously reported (21, 22). Briefly, frozen needle biopsy tissue cores were weighed, added to 60°C water containing deuterated internal standards, heated to 60°C for 10 minutes, and homogenized using a tissue homogenizer (Precellys; Bertin); supernatant was extracted twice with hexane [ethyl acetate (80:20 v/v)], and the organic layer was dried (SpeedVac; Thermo Fisher Scientific), derivatized with 0.025 mol/L hydroxylamine hydrochloride for 24 hours at room temperature to form oximes, and quantified using liquid chromatography/electrospray ionization tandem mass spectrometry. Lower limits of detection and quantitation (LLOD and LLOQ) for steroids in serum were 0.49 pg/sample (0.02 ng/mL) for progesterone, androstenedione (AED), and testosterone, 0.98 pg/sample (0.04 ng/mL) for pregnenolone and DHT, and 3.9 pg/sample (1.2 ng/mL) for DHEA. The LLOD for steroids in tissue was 0.49 pg/sample (0.02 pg/mg) for progesterone, AED, DHT, and testosterone, 0.98 pg/sample (0.04 pg/mg) for DHEA, and 1.96 pg/sample (0.08 pg/mg) for pregnenolone. The LLOD and LLOQ for abiraterone in serum was 0.06 ng/sample (0.005 ng/dL) and in tissue was 1.2 ng/sample (0.06 pg/gm). Additional information on assay methodology is provided in Supplementary Fig. S1.

Genotyping

Genomic DNA was extracted from buffy coat and snap-frozen prostate cores using commercial isolation kits (Qiagen Inc.). SNPs were analyzed using TaqMan SNP genotyping assays (Life Technologies) on the Applied Biosystems 7900HT Fast Real-Time PCR system (Applied Biosystems). The specific TaqMan probes used are presented in Supplementary Table S2.

Abiraterone and DHEAS uptake assay

LNCaP cells were engineered to overexpress SLCO2B1 and 1B3 as recently described (20). In brief, LNCaP cells were obtained

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from ATCC in 2010 and transduced at passage 25 to 30. Cell line authentication of stable lines was performed by STR profiling (DDC Medical). For abiraterone uptake assays, LNCaP-SLCO cells were plated in RPMI1640 supplemented with 10% FBS and 1% antibiotics at 37°C with 5% CO_2. When the cells grew to 80% to 85% confluency, complete culture medium was replaced with serum-free medium for 48 hours followed by 0.5 or 5 μmol/L abiraterone treatment for 4 hours. Cells were trypsinized, counted, washed three times with PBS, snap frozen in liquid nitrogen, and stored at −80°C until assay. Pierce BCA Protein Assay Kit (Thermo Fisher Scientific). Abiraterone levels were measured by mass spectrometry and adjusted to the protein concentration of the cell lysate.

For DHEAS uptake, cells were pretreated with 5 μmol/L abiraterone for 1 hour, then trypsinized, counted, washed twice, and resuspended in uptake buffer (135 mmol/L NaCl, 5 mmol/L KCl, 2.5 mmol/L $CaCl_2$, 0.6 mmol/L $MgSO_4$, 6 mmol/L D-glucose, and 10 mmol/L HEPES, pH 7.4) for 15 minutes at 37°C. Cells were then incubated for 1 hour in uptake buffer supplemented with 5 μmol/L of DHEAS (Sigma), washed three times in ice-cold PBS, and resuspended in DHEAS assay buffer followed by sonication. Intracellular DHEAS level was measured by DHEAS ELISA Kit (RCAN-DHS-480R, BioVendor) and adjusted to total protein concentration. The specificity of the antibody in this kit is given as 100% cross-reactivity with DHEAS, 16% with androsterone, 1.7% with androstenedione, and less than 1% with eight other tested steroids.

Statistical analyses

Statistical analysis was performed using GraphPad Prism (GraphPad Software Inc.). Correlations of serum and tissue abiraterone levels were computed by Spearman rank correlation. Steroid levels, serum PSA, and tumor volume in patients with detectable and undetectable tissue levels of abiraterone, and abiraterone and pregnenolone levels in SLCO-expressing versus vector control cell lines, were compared using the nonparametric Mann–Whitney test. Genotype frequencies were tested for Hardy–Weinberg equilibrium. Rare homozygotes were combined with the heterozygote if the frequency of the rare homozygote was below 10%. Comparisons of tissue abiraterone levels and tumor volume based on genotype were performed using the nonparametric Mann–Whitney test (or by ANOVA for nondichotomized groups). Categorical data (e.g., achieving MRD or not) were compared using χ^2 analysis. Correction for multiple testing was performed using the Bonferroni method. Unpaired t tests were used to compare difference in abiraterone uptake and impact of abiraterone on DHEAS uptake. P values ≤ 0.05 were considered significant.

Results

Serum and intraprostatic abiraterone levels

Steroid and abiraterone levels were measured by mass spectrometry in serum and tissue from 58 men with intermediate or high-risk localized prostate cancer enrolled in a previously reported clinical trial of castration plus abiraterone for 12 or 24 weeks prior to prostatectomy (21). Abiraterone levels in serum (median 28 ng/mL, 108 nmol/L) and tissue (median 77 ng/mL, 271 nmol/L) spanned a broad range (Fig. 1A; similar to prior reports for serum; refs. 23, 24) and were moderately correlated (Spearman $r = 0.355$, $P = 0.001$; Fig. 1B). Prostate tissue abiraterone levels were undetectable in eight men at the time of radical prostatectomy, of which six were also associated with undetectable abiraterone levels in serum (Supplementary Table S3). Chart review revealed that these six patients had discontinued abiraterone acetate ≥1 month prior to prostate surgery (due to liver abnormalities, noncompliance, or delay in surgery, two patients each). Serum abiraterone levels were easily detectable in one patient (54 ng/mL) and unavailable in the other patient with undetectable tissue abiraterone levels at prostatectomy, both of whom were reported to be taking drug until the day of surgery.

Steroid levels in patients with undetectable versus detectable tissue abiraterone levels

Abiraterone levels in both serum and prostate tissue were strongly correlated with steroids upstream of CYP17A (pregnenolone, progesterone) and inversely correlated with steroids downstream of CYP17A (DHEA, AED, testosterone; Table 1). In

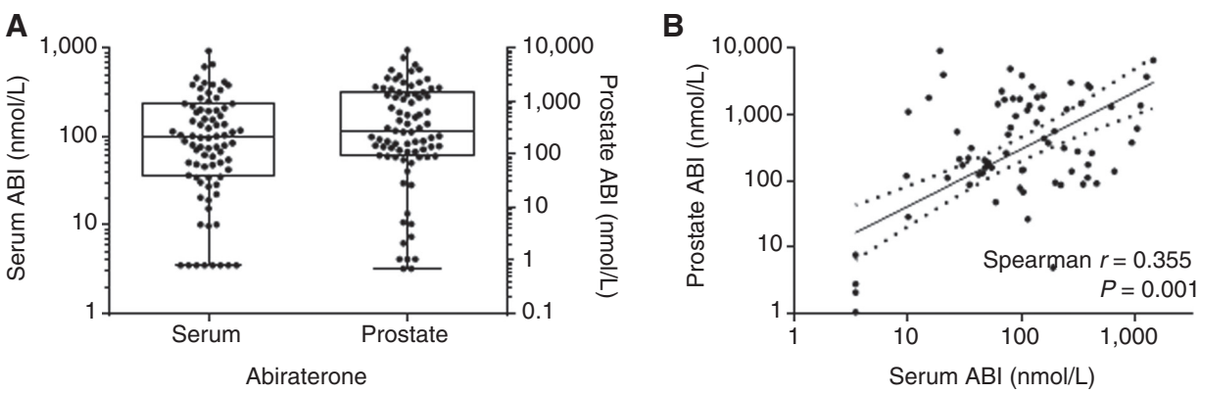

Figure 1.

Abiraterone levels in serum and prostate tissue of men treated with neoadjuvant abiraterone prior to prostatectomy. **A,** Levels of abiraterone measured by mass spectrometry in serum (left axis, median 108 nmol/L) and prostate tissue (right axis, median 271 nmol/L). Lines, median with interquartile range. Data include samples obtained at both 12- and 24-week time points of abiraterone treatment. **B,** Linear regression and Spearman rank correlation of serum versus prostate abiraterone levels.

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Table 1. Correlation of serum and prostate abiraterone with serum and tissue steroids[a]

		Serum abiraterone		Tissue abiraterone	
		r	*P*	*r*	*P*
Serum	ABI			0.355	0.001
	Pregnenolone	0.490	<0.0001	0.341	0.002
	Progesterone	0.601	<0.0001	0.392	0.000
	DHEAS	−0.323	0.003	−0.353	0.001
	DHEA	−0.23	0.035	−0.248	0.027
	AED	−0.391	0.0002	−0.288	0.01
	Testosterone	−0.297	0.006	−0.346	0.002
Prostate	Pregnenolone	0.311	0.005	0.489	<0.0001
	Progesterone	0.272	0.014	0.33	0.002
	DHEA	−0.280	0.012	−0.226	0.041
	AED	−0.169	0.135	−0.16	0.151

Abbreviation: ABI, abiraterone.
[a]Spearman rank correlation.

prostate tissue samples from patients with undetectable tissue abiraterone levels (including the two men who were taking abiraterone acetate plus prednisone at surgery), the characteristic increase in steroids upstream of CYP17A (Fig. 2A) and decrease in downstream steroids (Fig. 2B) was significantly muted compared with patients with detectable abiraterone levels. The same was observed in serum androgens from patients with detectable versus undetectable abiraterone levels (Supplementary Fig. S2). Previously reported serum steroid levels in men with CRPC were consistent with our data (3, 4), as were previously reported levels of DHEA, androstenedione, and testosterone in abiraterone acetate plus prednisone-treated patients (23, 25).

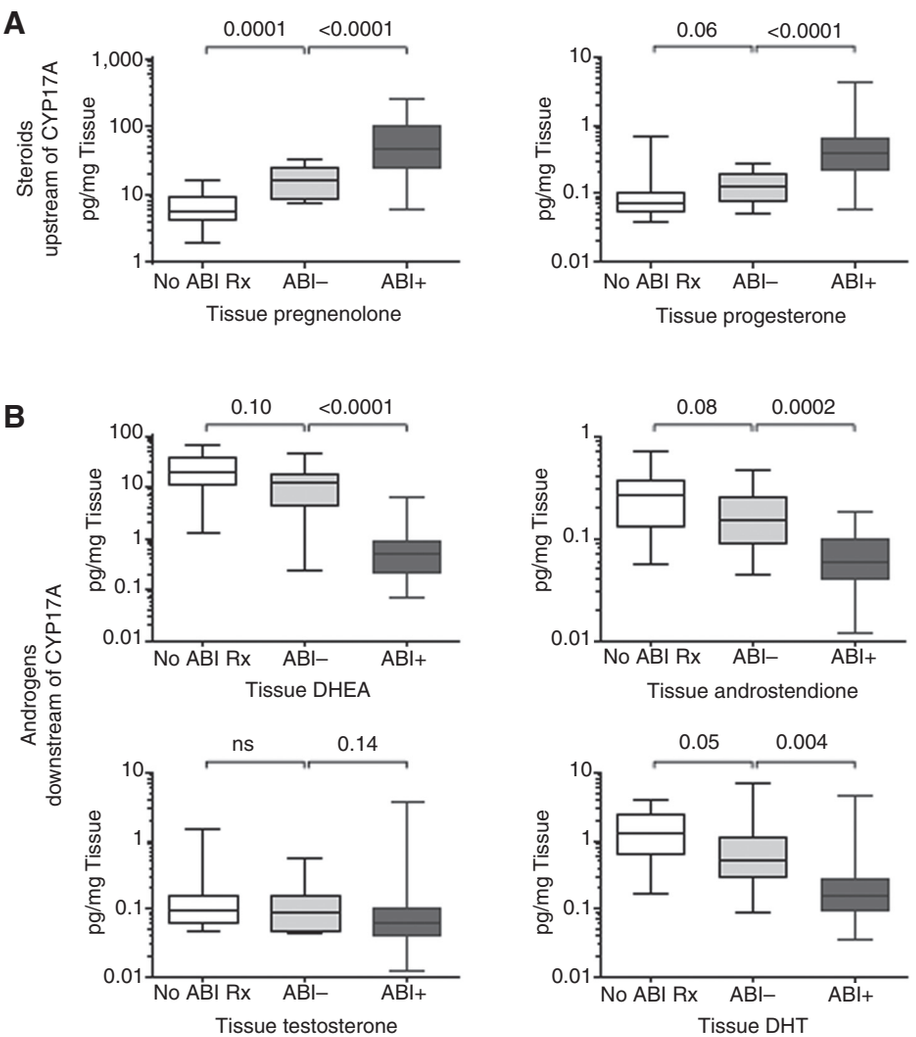

Figure 2.
Prostate tissue steroid levels in samples with undetectable (ABI−) versus detectable (ABI+) prostate abiraterone levels. **A,** Levels of steroids upstream of CYP17A. **B,** Levels of steroids downstream of CYP17A. Steroid levels measured by mass spectrometry and compared with steroid levels in tissues obtained at 12 weeks from patients treated with LHRHa therapy who had not yet started abiraterone treatment (no ABI Rx). Lines, median with interquartile range. *P* values from nonparametric Mann–Whitney *t* tests.

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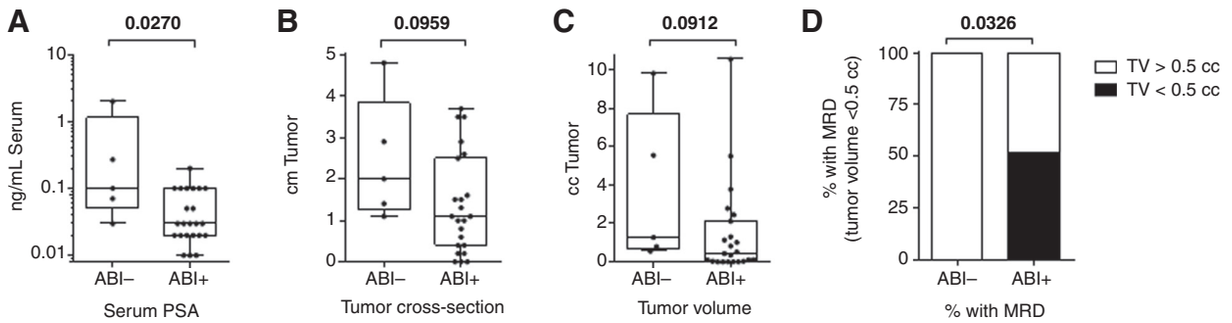

Figure 3.

PSA and pathologic responses in samples with undetectable (ABI−) versus detectable (ABI+) prostate abiraterone levels. **A,** Serum PSA levels at 24 weeks. **B–D,** Largest tumor cross-sectional dimension (**B**), tumor volume (TV; **C**), and percent of samples meeting criteria for MRD (**D**; defined as tumor volume 0.5 cc) in prostate samples obtained at prostatectomy. Lines, median with interquartile range. *P* values from nonparametric Mann–Whitney *t* tests (**A–C**) and the χ^2 test (**D**).

Serum PSA and pathologic response in patients with undetectable versus detectable tissue abiraterone levels

Among patients randomized to receive 24 weeks of abiraterone acetate plus prednisone treatment, end-of-study PSA levels and pathologic measures of disease revealed a consistent trend toward higher values in patients with undetectable versus detectable abiraterone levels. PSA levels (median 0.10 vs. 0.03 ng/dL, $P = 0.027$; Fig. 3A), largest cross-sectional tumor dimension (2.0 vs. 1.1 cm, $P = 0.09$; Fig. 3B), and tumor volume (1.28 vs. 0.44 cc, $P = 0.09$; Fig. 3C) were all higher in patients with undetectable abiraterone levels. Consistent with these observations, the proportion of patients achieving MRD (tumor volume < 0.5 cc) was also lower in the patients with undetectable versus detectable tissue abiraterone levels (0% vs. 50%, $P = 0.03$; Fig. 3D). Both the increase in serum and tissue steroids upstream of CYP17A and the decrease in those downstream of CYP17A, as well as the median PSA nadir achieved in patients with detectable tissue abiraterone levels, were consistent with observations in a neoadjuvant study of abiraterone acetate plus prednisone given for 12 weeks prior to radiotherapy. In that study, all patients had PSA nadirs $\leq$0.3 ng/mL, except one patient who was not compliant with abiraterone acetate treatment (PSA, 0.81 ng/mL; ref. 22).

Association of *SLCO* genotype with prostate tissue abiraterone levels and pathologic response

Thirteen SNPs in six human *SLCO* genes were selected for genotyping based on having a previously published role in steroid hormone transport or metabolism, a previously published significance in prostate cancer, and/or minor allelic frequency $\geq$10% (11). Characteristics of the specific SNPs in each gene and published associations with prostate cancer are summarized in Supplementary Table S4. All SNPs were found to be in Hardy–Weinberg equilibrium (Supplementary Table S2).

SNPs in *SLCO1B3* (rs4149117), *SLCO2B1* (rs1789693, rs12422149, rs1077858, and rs949069), and *SLCO2A1* (rs34550074) showed one or more associations with tissue abiraterone levels, estimated tumor volume, or the presence of MRD (summarized in Supplementary Table S5). *SLCO2B1* (rs1789693 and rs12422149) demonstrated the most significant associations with tissue abiraterone levels. The TT/AT genotype of the intronic *SLCO2b1* SNP rs1789693 (associated with longer survival after initiation of ADT; ref. 16) was associated with lower mean tissue abiraterone levels than the dominant AA allele [Fig. 4A, 59 pg/mg (TT/AT) vs. 172 pg/mg (AA), $P = 0.0008$]. The AA/AG genotype of the exonic *SLCO2B1* SNP rs12422149 (associated with lower DHEAS uptake and longer TTP on ADT; refs. 16, 19) was associated with higher mean tissue abiraterone levels than the dominant GG allele [258 pg/mg (AA/AG) vs. 99 pg/mg (GG), $P = 0.03$]. The AA/AG genotype of intronic *SLCO2B1* SNP rs949069 (unknown function) trended toward higher tissue abiraterone levels than the dominant GG allele [178 pg/mg (AA/AG) vs. 57 pg/mg (GG), $P = 0.12$], whereas the TT/GT genotype of the exonic *SLCO1B3* SNP rs4149177 (associated with enhanced testosterone uptake and shorter TTP on ADT; refs. 14, 15) trended toward lower tissue abiraterone levels than the common GG allele [45 pg/mg (TT/GT) vs. 123 pg/mg (GG), $P = 0.17$]. After correction for multiple testing, the difference for rs1789693 remained statistically significant (adjusted Bonferroni significance level $P = 0.004$). SNPs associated with increased tissue abiraterone levels were not associated with increased serum abiraterone levels (Fig. 4B). These data suggest that the potential association of *SLCO* genotype with tissue abiraterone levels does not necessarily track with the predicted direction of steroid transport and that the influence of a particular SNP on response to abiraterone may be different than its impact on outcomes in the setting of ADT.

Contingency analysis showed that the alleles of rs1789693 in *SLCO2B1* and of rs4149177 in *SLCO1B3* that were associated with higher tissue abiraterone levels were also associated with higher rates of MRD [67% (AA) vs. 27% (TT/AT), $P = 0.009$; 50% (GG) vs. 24% (TT/GT), $P = 0.07$, respectively, Fig. 5]. Although not associated with tissue abiraterone levels, the AG/AA genotype of the intronic SLCO2B1 SNP rs1077858 (associated with lower expression and longer TTP after initiation of ADT; ref. 19) associated with a higher rate of MRD than the minor GG allele [46% (AG/AA) vs. 0% (GG), $P = 0.03$], whereas the TT/CT genotype of *SLCO2A1* SNP rs34550074 (unknown function) associated with a lower rate of MRD compared with the minor CC allele [19% (TT/CT) vs. 52% (CC), $P = 0.03$]. After correction for multiple testing, the difference for rs1789693 remained near statistically significant (adjusted significance level $P = 0.004$).

We found relatively few significant associations of *SLCO* genotypes with serum and tissue androgens. However, among these were several of the *SLCO2B1* SNPs associated with tissue abiraterone levels and/or MRD. The AA/AG genotype of *SLCO2B1* SNP rs12422149 (associated with lower DHEAS uptake, longer

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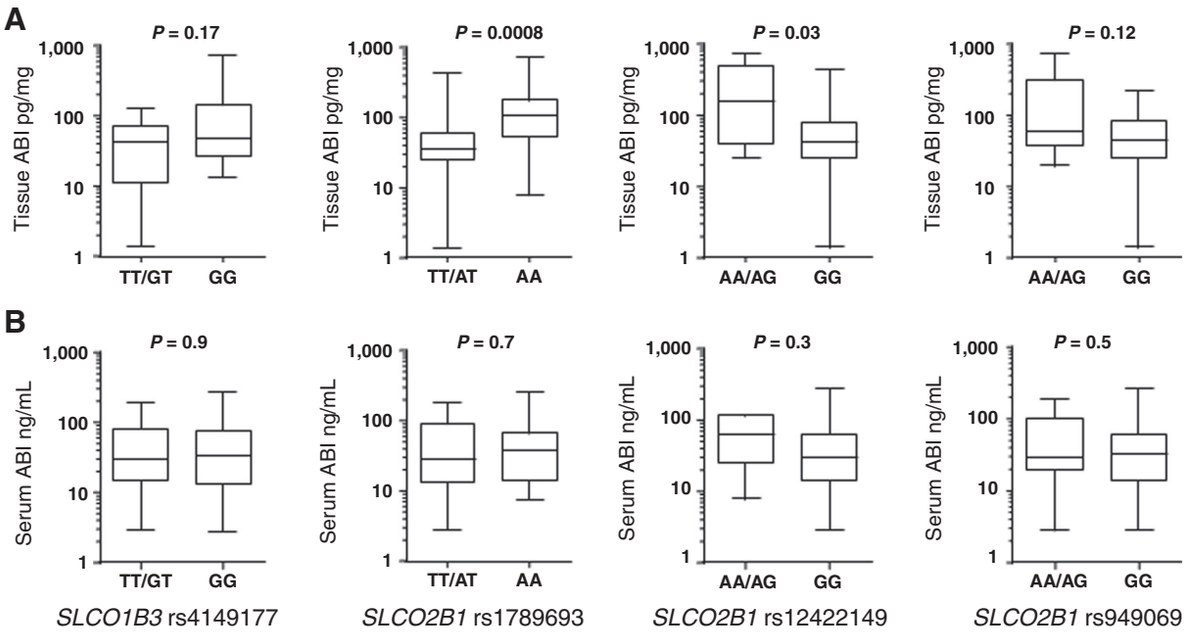

Figure 4.

Association of *SLCO* SNPs with prostate and serum abiraterone levels and the presence of MRD (defined as tumor volume (TV) <0.5 cc). **A,** Differences in tissue abiraterone levels based on the given *SLCO* genotypes. **B,** Differences in serum abiraterone levels based on the same *SLCO* genotypes as shown in **A**. Lines, median with interquartile range. *P* values from nonparametric Mann–Whitney *t* tests.

TTP on ADT, and with increased tissue abiraterone levels; Fig. 4A) was weakly associated with higher serum progesterone and pregnenolone levels (Supplementary Fig. S3; $P = 0.09$ for both). The AA/AG genotype of *SLCO2B1* SNP rs949069 (unknown function, associated with higher tissue abiraterone; Fig. 4A) was similarly associated with higher serum pregnenolone levels ($P = 0.02$). As well, the AG/AA genotype of *SLCO2B1* rs1077858 (associated with lower expression, longer TTP after initiation of ADT, and with a higher rate of MRD; Fig. 5) was associated with lower levels of serum DHEAS ($P = 0.002$). Otherwise, we found associations of *SLCO1B3* SNP rs60140950 (unknown function) with serum progesterone and tissue pregnenolone ($P = 0.033$ and 0.029, respectively).

Abiraterone levels in prostate cancer cells expressing SLCO genes and impact on DHEAS uptake

To demonstrate the impact of SLCO expression on abiraterone uptake, we generated LNCaP prostate cancer cells stably transfected to express SLCO1B3 and SLCO2B1 (as recently described; ref. 20). Treatment with 0.5 or 5 μmol/L abiraterone (concentrations that are in the range observed in the treated tissues in Fig. 1) resulted in two- to fourfold higher levels of intracellular abiraterone in SLCO1B3 or SLCO2B1-overexpressing cell lines ($P < 0.05$; including two independently cloned SLCO2B1 lines) compared with the vector control (Fig. 6A). Although the absolute levels achieved were higher at the 5 μmol/L dose (shown in Supplementary Fig. S4), the fold increase was higher at the 0.5 μmol/L

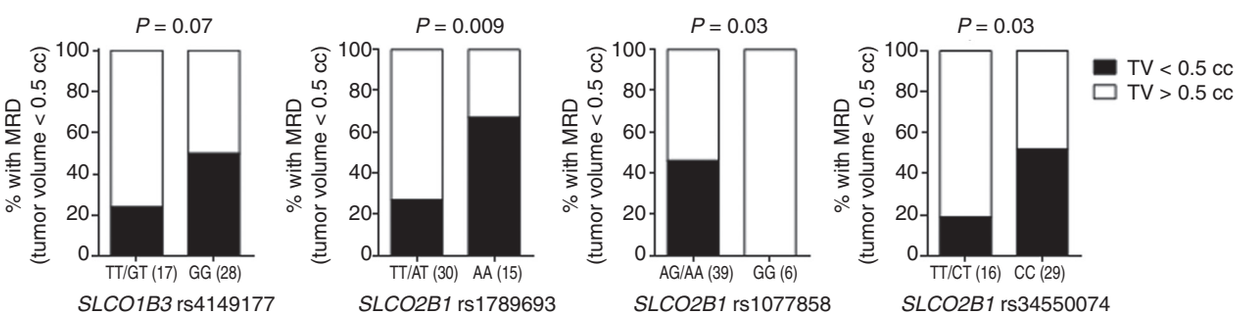

Figure 5.

Association of *SLCO* SNPs with achieving MRD. The percent of pathologic samples achieving criteria for MRD (defined as tumor volume (TV) <0.5 cc) based on the given *SLCO* genotype. Lines, median with interquartile range. *P* values from χ^2 test.

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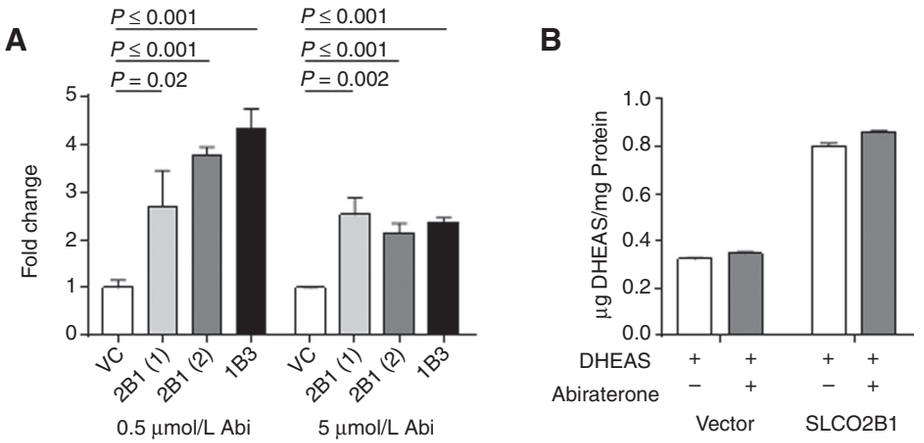

Figure 6.

Uptake of abiraterone and impact on DHEA uptake by SLCO-expressing LNCaP cells *in vitro*. **A,** Abiraterone levels (mean and SD) measured in LNCaP cells stably expressing vector control (VC) or the indicated SLCO construct after incubation with abiraterone at 0.5 or 5 μmol/L for 4 hours [cell lines denoted 2B (1) and 2B (2) indicate two independently cloned cell lines]. Abiraterone levels adjusted to the protein concentration of the cell lysate. *P* values from unpaired two-sample tests between each SLCO-expressing cell line and the vector control (VC). **B,** DHEAS levels in vector control and SLCO2B1-expressing lines preincubated without or with abiraterone (5 μmol/L) for 1 hour prior to treatment with DHEAS (5 μmol/L) for 1 hour. DHEAS levels adjusted to the protein concentration of the cell lysate.

dose, suggesting SLCO-mediated transport may be relatively more important in mediating tumor exposure at low drug levels. We then sought to determine whether abiraterone could decrease tissue androgen levels by interfering with uptake of DHEAS in addition to its effects on inhibiting androgen synthesis. However, incubation of vector control and SLCO2B1-expressing cell lines with 5 μmol/L abiraterone showed no impact on DHEAS uptake in either cell line (Fig. 6B).

Discussion

In this study, we investigated the association of abiraterone levels and *SLCO* genotype with intraprostatic steroids and treatment response in men with localized prostate cancer. Abiraterone levels in serum and prostate tissue were highly variable and were strongly correlated with changes in serum and tissue androgens. Consistent with this observation, serum PSA levels and measures of pathologic response, including tumor volume and the proportion of patients achieving MRD, were higher in patients with undetectable versus detectable tissue abiraterone levels.

Although serum and tissue abiraterone levels were statistically significantly correlated, there was still marked scatter in the data, suggesting a contribution of tissue-specific factors. Our hypothesis that abiraterone may undergo SLCO-mediated transport was supported by our *in vitro* studies showing increased intracellular abiraterone levels in prostate cancer cells overexpressing SLCO2B1 or SLCO1B3. We found two SNPs in *SLCO2B1* (rs1789693 and rs12422149) to be associated with approximately 2.5-fold statistically significant differences in tissue abiraterone levels, while a third SNP in *SLCO2B1* (rs949069) and one SNP in *SLCO1B3* (rs4149177) showed trends toward a significant difference. Notably, the alleles of rs1789693 in *SLCO2B1* and rs4149177 in *SLCO1B3* that associated with higher tissue abiraterone levels were also associated with approximately 2-fold higher rates of achieving MRD. As well, although not associated with tissue abiraterone levels, additional SNPs in *SLCO2B1* (rs1077858) and *SLCO2A1* (rs34550074) were also associated with significant differences in MRD.

As increased expression of CYP17A is noted in CRPC rather than primary prostate cancer (26), the primary target for abiraterone in patients with localized prostate cancer is likely adrenal expression of CYP17A. Accordingly, the observed correlations of prostate abiraterone levels with tissue steroid levels are likely to reflect the changes in serum steroid levels associated with adrenal CYP17A inhibition rather than a direct impact of abiraterone on prostate tissue CYP17A.

Abiraterone inhibits the type I isoform of HSD3B expressed in the prostate (27, 28), potentially mediating a direct effect of abiraterone on prostate tissue androgens downstream of HSD3B1 (i.e., AED, T, DHT) and pathologic outcomes. This may underlie the observation that the AA allele of *SLCO2B1* rs1789693 and the GG allele of *SLCO1B3* rs4149177 (associated with higher tissue abiraterone levels) were associated with approximately 2-fold higher rates of MRD (i.e., via increased inhibition of HSD3B1-dependent production of downstream androgens). However, we did not observe a direct association between either rates of MRD or of these transporter genotypes with tissue levels of AED, T, or DHT (not shown), suggesting the association of these transporters with MRD may reflect the ability of abiraterone (and its downstream metabolite, D4A) to directly inhibit AR in addition to its impact on suppressing tissue androgens (27, 29).

The association of *SLCO* genotype with MRD observed in this study may reflect a contribution of SLCO-mediated steroid transport in addition to (or independently of) an effect on abiraterone transport. Thus, the association of the GG allele of *SLCO1B3* rs4149177 with higher MRD may reflect the higher tissue abiraterone levels observed with this allele, but may also reflect the impaired uptake of testosterone imparted by this allele (and previously associated with a decreased risk of prostate cancer–specific mortality in men with CRPC and decreased risk of progression in men on ADT; refs. 14, 15). Similarly, association of the AA allele of *SLCO2B1* rs1789693 with a higher rate of

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achieving MRD may reflect an effect of higher tissue abiraterone levels, but this allele has been associated with improved prostate cancer outcomes in non-abiraterone–treated patients as well (16). Notably, the GG allele of *SLCO2B1* rs1077858, which was associated with a lower rate of achieving MRD (but not a difference in abiraterone levels), has been associated with higher SLCO2B1 expression and with shorter TTP and OS in men with CRPC on ADT, an effect proposed to occur via an increase in SLCO2B1-mediated import of DHEAS (19). Although tissue levels of DHEAS were not evaluated in this study, these observations suggest the effect of this SNP on achieving MRD in abiraterone acetate plus prednisone-treated patients could also be related to DHEAS uptake due to increased transporter expression (19).

Statins (themselves a well-known SLCO substrate; ref. 30) have recently been demonstrated to inhibit SLCO2B1-mediated DHEAS uptake, an effect that may contribute to the beneficial effect of statin use on TTP to CRPC (31). Whether statins inhibit SLCO-mediated uptake of abiraterone remains to be established. However, given the depot of residual DHEAS present in abiraterone-treated patients (32), any decrease in DHEAS uptake, whether due to the presence of low import SNPs in *SLCO* genes or pharmacologic modulation of DHEAS uptake by statins, may result in enhanced treatment efficacy.

Factors other than *SLCO* genes may also influence the pathologic response of prostate tumors to abiraterone. The D4A metabolite of abiraterone, generated by the activity of 3BHSD, is present in the serum of abiraterone acetate plus prednisone-treated CRPC patients at levels ranging from approximately 1.7% to 17% of total abiraterone levels and has been demonstrated to inhibit the activity of CYP17A, 3BHSD, and SRD5A enzymes as well as AR chromatin binding more strongly than abiraterone (27). Conversely, the 5-α reduced metabolite of D4A, generated by the activity of SRD5A, has been demonstrated to stimulate AR activity (33). Although not evaluated in this study, the relative levels of D4A and 5α-D4A in prostate tumor tissue could influence treatment efficacy. An SNP variant of HSD3B1 (367T) has been associated with increased protein stability and enhanced activity, both in generating AED and testosterone from upstream precursors, and in generating D4A from abiraterone (34). In this small cohort, we observed only three patients that were homozygous for this SNP, and we did not otherwise detect significant associations of this SNP with tissue steroid levels or MRD (data not shown). However, this SNP may have discordant effects, with its role in directly promoting androgen synthesis countered by an indirect role in suppressing androgen synthesis via enhanced production of D4A.

SLCO genes may also influence response to abiraterone via an impact on systemic abiraterone exposure (13, 35, 36). Although >50% of abiraterone is excreted unchanged in the feces (suggesting intestinal transport of abiraterone is not clinically significant; refs. 37, 38), hepatic expression of SLCO1B1, SLCO1B3, and SLCO2B1 genes is robust (39, 40). As hepatic activity of CYP3A4 is a primary route of abiraterone metabolism (37), variation in hepatic abiraterone uptake could plausibly influence the half-life of circulating abiraterone levels. Mixed effects, due to both increased tumor uptake and increased hepatic clearance, are possible, in which case, hepatic effects on abiraterone uptake might be expected to predominate, as they would ultimately influence both adrenal and tumor exposure to abiraterone.

To the extent that CRPC tumors are more likely to demonstrate increased expression of CYP17A than localized tumors, a potential association of tissue abiraterone levels and pathologic response may be stronger in CRPC tissues than in our study. Progesterone is increased in the setting of abiraterone-mediated CYP17A inhibition (21) and enhances uptake of DHEAS by SLCO2B1 in non–prostate cancer models (41, 42). This suggests abiraterone acetate plus prednisone-treated patients may be particularly susceptible to the SLCO-mediated transport of key steroid and treatment-related substrates. Abiraterone has also been demonstrated to inhibit SLCO1B1 transport of certain substrates (37, 43). As many SLCO substrates are also SLCO inhibitors (13, 36), this is consistent with abiraterone being an SLCO substrate. Of note, however, we do not find evidence that abiraterone itself inhibits SLCO-mediated tumoral DHEAS uptake.

This study has several important limitations, including the relatively small sample size and exploratory nature of the analyses, which clearly influence the stability of the observed *SLCO* genotype associations. Furthermore, although hypothesis generating, the potential clinical utility of our findings must be evaluated in a larger dataset of men with CRPC undergoing treatment with abiraterone acetate plus prednisone. In summary, we find that intraprostatic abiraterone levels are associated with pathologic responses in men with intermediate or high-risk localized prostate cancer and that genetic variation in *SLCO* genes associate with differences in tissue abiraterone levels and rates of MRD. Our findings suggest that heterogeneity in the response to abiraterone acetate plus prednisone may reflect differences in intratumoral abiraterone and residual tumor androgens achieved in each patient and that variation in *SLCO* genes may serve as predictors of response to treatment with abiraterone acetate plus prednisone.

Disclosure of Potential Conflicts of Interest

L.D. True reports receiving commercial research grants from Ventana/Roche. T.N. Penning holds ownership interest (including patents) in Penzymes and is a consultant/advisory board member for Sage Pharmaceuticals and Tokai Pharmaceuticals. M. Taplin reports receiving other commercial research support from and is a consultant/advisory board member for Janssen. B. Montgomery reports receiving commercial research grants from Janssen. No potential conflicts of interest were disclosed by the other authors.

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