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ORIGINAL REPORT

SLCO2B1 and *SLCO1B3* May Determine Time to Progression for Patients Receiving Androgen Deprivation Therapy for Prostate Cancer

Ming Yang, Wanling Xie, Elahe Mostaghel, Mari Nakabayashi, Lillian Werner, Tong Sun, Mark Pomerantz, Matthew Freedman, Robert Ross, Meredith Regan, Nima Sharifi, William Douglas Figg, Steven Balk, Myles Brown, Mary-Ellen Taplin, William K. Oh, Gwo-Shu Mary Lee, and Philip W. Kantoff

Ming Yang, Wanling Xie, Mari Nakabayashi, Lillian Werner, Tong Sun, Mark Pomerantz, Matthew Freedman, Robert Ross, Meredith Regan, Myles Brown, Mary-Ellen Taplin, Gwo-Shu Mary Lee, Philip W. Kantoff, Dana-Farber Cancer Institute, Harvard Medical School; Steven Balk, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA; Elahe Mostaghe, University of Washington, Seattle, WA; Nima Sharifi, University of Texas Southwestern Medical Center, Dallas, TX; William Douglas Figg, National Cancer Institute, National Institutes of Health, Bethesda, MD; William K. Oh, Mount Sinai School of Medicine, New York, NY.

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Terms in blue are defined in the glossary, found at the end of this article and online at www.jco.org.

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Corresponding author: Gwo-Shu Mary Lee, PhD, Dana-Farber Cancer Institute, Dana 710B, 44 Binney St, Boston, MA 02115; e-mail: gwo-shu_lee@dfci.harvard.edu.

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ABSTRACT

Purpose
Androgen deprivation therapy (ADT), an important treatment for advanced prostate cancer, is highly variable in its effectiveness. We hypothesized that genetic variants of androgen transporter genes, *SLCO2B1* and *SLCO1B3*, may determine time to progression on ADT.

Patients and Methods
A cohort of 538 patients with prostate cancer treated with ADT was genotyped for *SLCO2B1* and *SLCO1B3* single nucleotide polymorphisms (SNP). The biologic function of a *SLCO2B1* coding SNP in transporting androgen was examined through biochemical assays.

Results
Three SNPs in *SLCO2B1* were associated with time to progression (TTP) on ADT ($P < .05$). The differences in median TTP for each of these polymorphisms were about 10 months. The *SLCO2B1* genotype, which allows more efficient import of androgen, enhances cell growth and is associated with a shorter TTP on ADT. Patients carrying both *SLCO2B1* and *SLCO1B3* genotypes, which import androgens more efficiently, exhibited a median 2-year shorter TTP on ADT, demonstrating a gene-gene interaction ($P_{interaction} = .041$).

Conclusion
Genetic variants of *SLCO2B1* and *SLCO1B3* may function as pharmacogenomic determinants of resistance to ADT in prostate cancer.

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INTRODUCTION

Androgens and androgen receptor (AR) participate in the development of prostate cancer (CaP). Androgen deprivation therapy (ADT), which suppresses testicular androgen production, is the most effective therapy for patients with hormone-sensitive CaP (HSPC).[1,2] Unfortunately, most HSPCs eventually become resistant to ADT and progress to castration-resistant CaP (CRPC), which is usually fatal.[1,3-5] Although mechanisms by which a CaP cell survives after ADT are not entirely understood, multiple AR dependent and independent pathways have been hypothesized.[6,7] Notably, patients with CRPC maintain significant levels of intraprostatic androgen despite castrate serum androgen levels.[8-10] In soft tissue metastases of CRPC, persistent levels of testosterone (T) have been detected.[11] In CRPC bone metastases, expression levels of several genes mediating androgen metabolism were increased.[12] All these findings suggested that maintaining intraprostatic androgen, via increased de novo androgen synthesis and/or uptake of circulating adrenal androgens, may activate AR signaling and may be a key factor in sustaining CRPC growth.[11,12]

Dehydroepiandrosterone (DHEA) and its sulfated form, DHEAS, which can be converted into more potent androgens, are secreted in large amounts by the adrenal cortex.[13] In men, the DHEAS level is 100- to 500-fold higher than T in serum[14] and is high in prostate.[10,15] DHEA is an important substrate for the production of T and dihydrotestosterone (DHT) in tumor microenvironments.[12,16] Other than T and DHT, DHEA can also bind to AR (DHEA also binds to a mutated AR with codon 877 A>G Thr-Ala, found in patients with CRPC and LNCaP [androgen-sensitive human prostate adenocarcinoma cell line]), and activate AR signaling, leading to enhanced CaP cell growth.[17,18] Taken together, intraprostatic androgens, particularly DHEA and DHEAS, may be directly involved

in the development and/or maintenance of CRPC. Thus, we hypothesized that the efficiency of androgen transport may have a significant impact on the CRPC emergence and the ADT efficacy.

The superfamily of organic anion-transporting polypeptides (OATP), encoded by *SLCO* genes, mediates the sodium-independent uptake of various endogenous compounds and drugs into cells.[18,19] For many members of the SLCO transporter family, their substrate(s) are not well characterized. However, SLCO2B1 and SLCO1B3 have been shown to be involved in the steroid hormone uptake. SLCO2B1 is expressed in a broad range of tissues and mediates the transport of steroid conjugates, such as DHEAS and estrone-3-sulfate.[19,20] SLCO1B3, involved in the uptake of several hormones including T, is mainly expressed in liver and various types of cancer cells.[21-23] Both *SLCO2B1* and *SLCO1B3* genes are polymorphic. Two single nucleotide polymorphisms (SNPs) in *SLCO1B3*, rs4149117 (334T>G/Ser112Ala) and rs7311358 (669G>A/Met233Ile), are in complete linkage disequilibrium (LD) and exhibit different T transport efficiencies.[22-24] The common *SLCO1B3* GG/AA haplotype, associated with the impaired T transport, appeared to be associated with survival and a prolonged response duration to ADT in a small white CaP cohort.[24,25] In *SLCO2B1*, two coding SNPs are associated with the cellular uptake of estrone-3-sulfate (rs2306168C>T)[26] or the plasma level of montelukast in patients with asthma (rs12422149G>A).[27]

We chose to study SLCO2B1 and SLCO1B3 because their roles in transporting T or DHEAS may be potentially important in CaP progression. We present here a comprehensive genetic and biochemical study of the impact of genetic variants of *SLCO2B1* and *SLCO1B3* on the time to progression (TTP) of ADT, which provides new mechanistic insights into the pharmacogenomics of resistance to ADT.

PATIENTS AND METHODS

The ADT cohort of 95% white was generated from the Prostate Clinical Research Information System[28] at Dana-Farber Cancer Institute. Patients provided written informed consent. The clinical data collection and detailed patient characteristics are described in the Data Supplement and are as previously described.[1,2]

Haplotype-tagging SNPs with minor allele frequency (MAF) of ≥ 0.05 from the *SLCO2B1* gene locus (approximately 59 kb) were chosen from genotyped SNPs in a white population (CEU, Utah residents with Northern and Western European ancestry) in the HapMap Project, with the program Tagger on a pairwise mode ($r^2 \geq 0.8$).[29] All nonsynonymous SNPs with MAFs greater than 0.05 or reported in literatures were included. Totally, 23 SNPs of *SLCO2B1* and two previously reported *SLCO1B3* functional SNPs[21] were selected for genotyping. Five SNPs with MAFs of fewer than 5% were excluded from analysis. Thus, 18 SNPs in *SLCO2B1* (for LD blocks see Data Supplement) and two SNPs in *SLCO1B3* were analyzed. Genomic DNA was prepared from peripheral blood and genotyping was performed using Sequenom technology (Carlsbad, CA). Sequences of primers and probes for each SNP are available on request.

Detailed SNP selections, genotype analyses, plasmid preparations, cell cultures, transfections, DHEAS uptake assays, and cell proliferation assays are described in the Appendix.

Table 1. *SLCO2B1* Genotype Distribution and Its Association With TTP on ADT

Gene and Genotype	No.	Median TTP (months)	95% CI	Univariate Model					Multivariate Model*		
				HR	95% CI	*P*	FDR	$P_{permutation}$	HR	95% CI	*P*
SLCO2B1 rs12422149											
AA/AG	98	31.5	18.1 to 42.6	1.00	Reference	.029	0.17	.33	1.00	Reference	.018
GG	434	20.9	17.5 to 25.2	1.35	1.03 to 1.77				1.40	1.06 to 1.84	
SLCO2B1 rs1789693											
AA	215	21.5	16.7 to 29.0	1.00	Reference	.007	0.08	.11	1.00	Reference	.051 (.018†)
AT	228	26.3	19.0 to 31.1	1.07	0.86 to 1.34				1.08	0.86 to 1.34	
TT	82	14.5	10.9 to 22.0	1.60	1.19 to 2.16				1.46	1.07 to 1.98	
SLCO2B1 rs1077858											
AA	188	28.1	22.7 to 36.6	1.00	Reference	.009	0.08	.13	1.00	Reference	.008
AG	231	19.3	15.7 to 25.5	1.25	1.00 to 1.58				1.33	1.05 to 1.68	
GG	73	16.4	12.0 to 27.5	1.59	1.17 to 2.17				1.57	1.15 to 2.14	
SLCO2B1, No. of risk genotype											
≤ 1	235	30.9	25.0 to 36.9	1.00	Reference	< .001			1.00	Reference	< .001
2	204	19.0	15.5 to 25.0	1.44	1.15 to 1.80				1.56	1.24 to 1.97	
3	50	12.8	9.0 to 22.0	2.01	1.42 to 2.85				1.91	1.34 to 2.71	
SLCO1B3 rs4149117											
GG	368	23.5	19.0 to 28.0	1.00	Reference	.717			1.00	Reference	.719
GT/TT	162	20.2	15.7 to 29.0	1.04	0.84 to 1.29				1.01	0.80 to 1.26	
SLCO1B3 rs7311358											
AA	372	23.5	19.0 to 28.0	1.00	Reference	.809			1.00	Reference	.810
AG/GG	166	20.2	15.5 to 32.0	1.03	0.83 to 1.27				0.98	0.78 to 1.22	

Abbreviations: TTP, time to progression; ADT, androgen-deprivation therapy; HR, hazard ratio; FDR, false discovery rate; $P_{permutation}$, *P* value of permutation tests; PSA, prostate-specific antigen.

*From separate Cox regression models, each adjusted for Biopsy Gleason score, type of primary therapy, whether hormone use occurred as part of local therapy, metastatic status and PSA at initiation of ADT.

†If AA and AT were combined.

The median TTP was estimated using the Kaplan-Meier method, with 95% CIs. To test the association between SNPs and TTP, the log-rank test was used. SNPs with *P* values lower than .05 from the log-rank test were further analyzed by multivariable Cox regression model to adjust for clinical variables and their associations with TTP. The Westfall and Young step-down permutation procedure was used for multiple testing.[30] Ten thousand permutation data sets were generated by randomly shuffling samples' outcomes. The permutation adjusted *P* value is defined as the proportion of resampled data sets where the minimum pseudo-*P* value is lower than or equal to the original *P* value.[30] Since we conducted hypothesis-generating experiments, the stringent control on family-wise type I error (as the permutation test does) may not have been necessary. We also reported the false discovery rate (FDR) of Benjamini and Hochberg.[31] The FDR represents the expected proportion of false positive results when declaring a test significant. Permutation tests were conducted using R 2.8.0 (R project; http://www.r-project.org/); all other statistical analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC). All *P* values are two sided.

RESULTS

Patient Characteristics

The ADT cohort (n = 538) is derived from a previously described cohort of patients with CaP treated with ADT[1,2] and its clinical characteristics are presented in Data Supplement. The efficacy of ADT was evaluated using TTP of prostate-specific antigen (PSA), defined as the duration of time from ADT initiation to the date of PSA progression (for details see Data Supplement).

Association of SLCO2B1 SNPs With TTP During ADT

A total of 18 SNPs in *SLCO2B1* were analyzed (Data Supplement). Three polymorphisms of *SLCO2B1*, rs12422149A>G (Arg312Gln) in exon 7, rs1789693A>T in intron 7 and rs1077858A>G in intron 8, were significantly associated with TTP during ADT in univariate analysis ($P = .029$, .007, and .009, respectively). The permutation adjusted *P* values for rs12422149, rs1789693, and rs1077858 were .33, .11, and .13, respectively, and their corresponding FDRs were .17, .08, and .08, respectively (Table 1 and Fig 1A, 1B, 1C). The differences in median TTP during ADT for the genotypes of these three SNPs were approximately 10 months (rs12422149), 7 months (rs1789693), and 12 months (rs1077858). In multivariate analyses adjusting for clinical factors associated with TTP, the association between each of the three SNPs and TTP remained significant ($P \leq .05$).

The LD among these three SNPs, with pairwise correlation (*r*) is from 0.11 to 0.21. An additive effect across the three *SLCO2B1* polymorphisms was observed (Table 1 and Fig 1D). The population was stratified by the number of risk genotypes that an individual carried. A

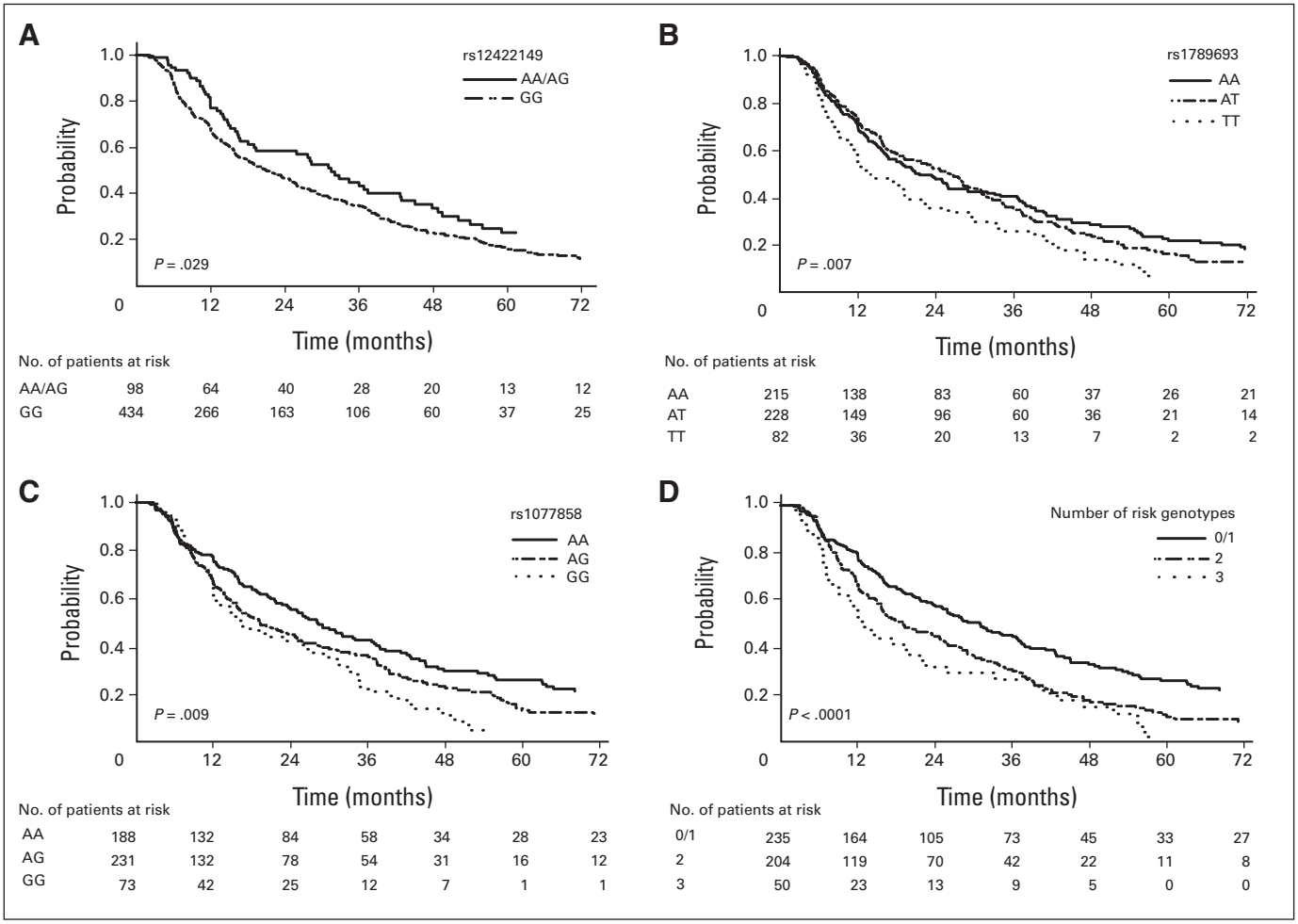

Fig 1. Kaplan-Meier curves of time to progression during androgen deprivation therapy, stratified by (A) genotypes at rs12422149, (B) genotypes at rs1789693, (C) genotypes at rs1077858, and (D) ≤ 1, 2, or 3 unfavorable genotypes of the three *SLCO2B1* single nucleotide polymorphisms.

risk genotype was defined as the genotype with a shorter TTP during ADT (GG at rs12422149, TT at rs1789693, and AG/GG at rs1077858). Increasing the number of risk genotypes reduced the TTP ($P < .001$). Approximately an 18-month difference in median TTP was observed between patients with three risk genotypes and those with zero or one risk genotype. Individuals with two risk genotypes showed a 12-month shorter TTP compared with those with zero or one risk genotype. No significant association was observed between genotypes of *SLCO2B1* and patient clinical characteristics.

In short, our data demonstrated that genetic variations in *SLCO2B1* may affect TTP on ADT. We hypothesized that different variants of *SLCO2B1* may have different efficiencies in transporting external androgens into CaP cells, resulting in varying durations responding to ADT.

Differential DHEAS Uptake of SLCO2B1 Variants

To address the biologic function of different *SLCO2B1* variants responding to ADT, we investigated the possibility that the *SLCO2B1* rs12422149A>G (Arg312Gln) SNP may influence the efficiency of DHEAS transport, thus affecting intracellular levels of androgen and cellular responses to ADT. We chose the *SLCO2B1* rs12422149A>G (Arg312Gln) SNP for functional analysis, because it is an exonic SNP, resulting in an amino acid change.

LNCaP cells that were transiently transfected with plasmids expressing SLCO2B1-312Gln or SLCO2B1-312Arg respectively expressed either variant at similar levels; the highest expression level was observed at 48 hours post-transfection (Fig 2A). DHEAS uptake increased with increasing incubation time, and reached maximum levels at 1 hour (Fig 2B). Compared with mock-transfected cells, the SLCO2B1-312Gln and -312Arg overexpressors exhibited significantly higher levels of DHEAS uptake. The SLCO2B1-312Gln expressor exhibited a maximal uptake of 223.38 ± 8.55 pmol/mg protein, the SLCO2B1-312Arg expressor exhibited a maximal uptake of 162.76 ± 25.73 pmol/mg protein, and the pCMV6-XL4 vector mock-transfected cells exhibited an uptake of 51.66 ± 7.44 pmol/mg protein.

Further analyses of the uptake kinetics of different *SLCO2B1* variants demonstrated that DHEAS uptake can be saturated at 60 to 100 μmol/L (Fig 2C). The best-fit K_m values for the SLCO2B1-312Gln and SLCO2B1-312Arg variants were 17.65 μmol/L (95% CI, 9.303 to 25.99 μmol/L) and 13.68 μmol/L (95% CI, 4.174 to 23.20 μmol/L), respectively. The maximum velocity values for the 312Gln and 312Arg variants were 281.4 pmol/mg protein/min (95% CI, 236.1 to 326.7 pmol/mg protein/min) and 189.5 pmol/mg protein/min (95% CI, 147.8 to 231.2 pmol/mg protein/min). These results indicate that both *SLCO2B1* variants can import DHEAS, although the SLCO2B1-312Gln variant exhibits a greater efficiency. We hypothesized that the higher efficiency of the SLCO2B1-312Gln variant in transporting DHEAS into CaP cells leads to a greater ability to activate AR, which may in turn sustain cell growth, thereby explaining why patients with the SLCO2B1-312Gln variant exhibited a shorter TTP during ADT.

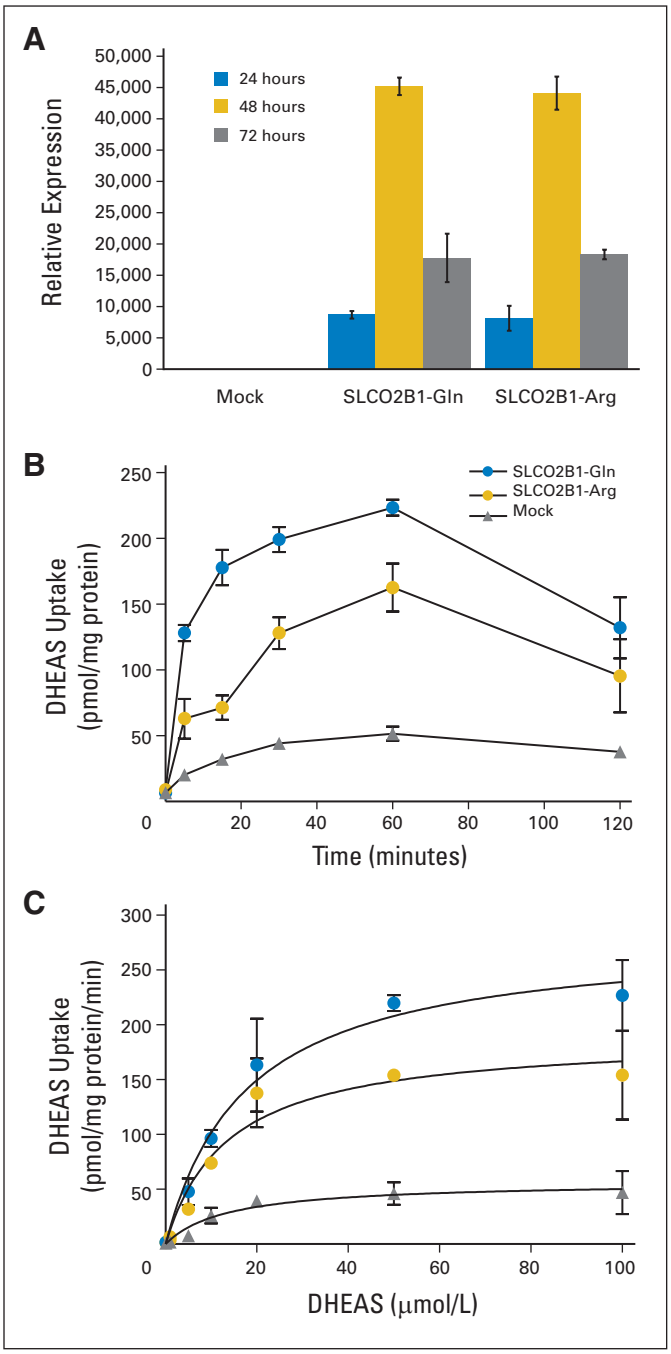

Fig 2. Sulfated dehydroepiandrosterone (DHEAS) uptake by *SLCO2B1* variants. (A) *SLCO2B1* mRNA levels in mock transfected LNCaP cells and LNCaP cells transfected with different SLCO2B1 variants. All mRNA levels were analyzed by quantitative reverse transcriptase polymerase chain reaction and normalized by the expression level of glyceraldehyde 3-phosphate dehydrogenase. Values represent the fold differences relative to those in mock transfected cells, which were set as 1.0. (B) Time course of DHEAS uptake. (C) Kinetics of DHEAS uptake (significant differences were observed among three groups, $P < .05$). Mock, LNCaP transfected with pCMV6-XL4 vector; SLCO2B1-Gln, LNCaP transiently transfected with pCMV-SLCO-312Gln; SLCO2B1-Arg, LNCaP transiently transfected with pCMV-SLCO-312Arg. All experiments were repeated in triplex.

Impact of DHEAS Import on AR Signaling and Cell Growth

To determine the biologic influence of DHEAS uptake in AR activation, we compared the AR-mediated PSA expression in LNCaP, LAPC-4, and cells that overexpress different *SLCO2B1* variants. Increasing the DHEAS level upregulated the PSA expression in both wild-type cells and cells transfected with *SLCO2B1* (Figs 3A and 3B). While both LNCaP and LAPC-4 transfected with the SLCO2B1-312Gln variant showed consistently higher PSA expression levels than those of cells transfected with the SLCO2B1-312Arg variant (Figs 3A

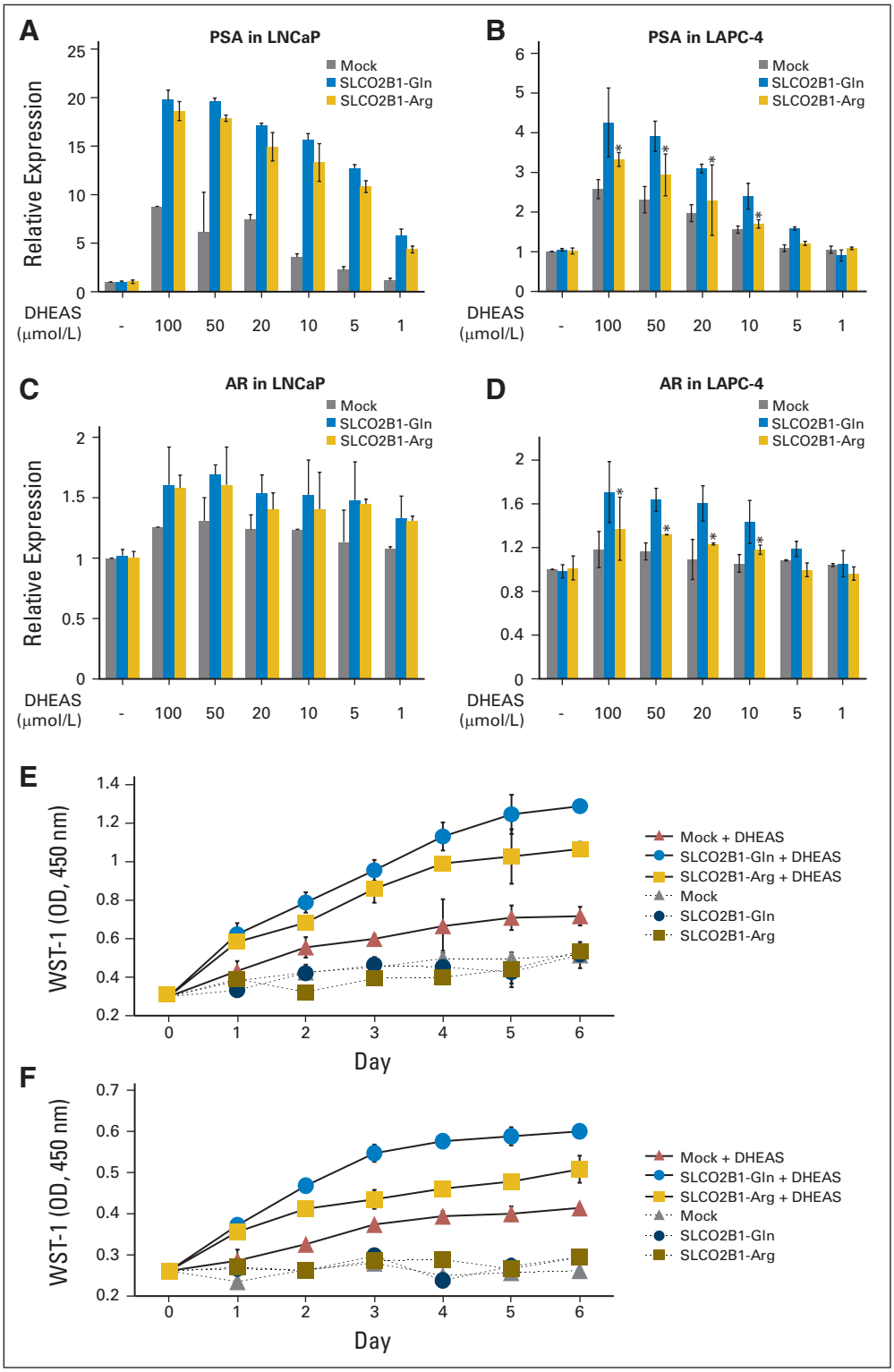

Fig 3. The impact of *SLCO2B1* variants on androgen receptor (AR) –mediated expression and cell growth. (A-D) Quantitative reverse transcriptase polymerase chain reaction analysis of expression levels of prostate-specific antigen (PSA) and AR in various LNCaP and LAPC-4 cell lines with or without sulfated dehydroepiandrosterone (DHEAS). In all experiments, the relative expression levels of PSA and AR in each sample were normalized by the expression level of glyceraldehyde 3-phosphate dehydrogenase. Values represent the fold differences relative to those in mock transfected cells without any drug treatment, which were set as 1.0. (E,F) Cell growth. WST-1 assay was used to determine cell growth. Solid lines and broken lines represent cell cultured in the presence or absence, respectively, of DHEAS. Triplicate experiments were performed for each set. Points, mean (n = 3); bars, standard deviation. Mock, cells transfected with pCMV6-XL4 vector; OD, optical density; SLCO2B1-Gln, cells transfected with the pCMV-SLCO-312Gln; SLCO2B1-Arg, cells transfected with the pCMV-SLCO-312Arg plasmid.

and 3B); this correlation was only statistically significant in LAPC-4 (Fig 3B). Specifically, treatment with 100 μmol/L DHEAS upregulated the PSA expression in LAPC-4 harboring the SLCO2B1-312Gln variant by 1.48-fold, compared to that in cells with the SLCO2B1-312Arg variant. Interestingly, exposure to DHEAS also increased AR mRNA levels in both LNCaP and LAPC4 (Figs 3C and 3D). Cells carrying the SLCO2B1-312Gln variant and treated with 100 μmol/L DHEAS exhibited a 1.24-fold higher level of AR expression than that in cells with the SLCO2B1-312Arg variant. Thus, upregulation of the PSA expression on treatment with DHEAS could be, in part, due to the increased levels of intracellular androgen and partly due to the increased expression of AR.

To corroborate the hypothesis that increasing intracellular androgen levels are involved in the emergence of CRPC, we further determined the impact of DHEAS uptake on cell growth. We compared the growth efficiency of wild-type LNCaP and LAPC4 and cell lines overexpressing different *SLCO2B1* variants in the presence or absence of 100 μmol/L DHEAS. Figure 3E shows that DHEAS increases the growth rate of LNCaP by approximately 1.4-fold. In the presence of DHEAS, the growth efficiency of LNCaP transfected with SLCO2B1-312Gln increased 2.5-fold, while the growth efficiency of LNCaP cells transfected with SLCO2B1-312Arg only increased 2.0 fold ($P < .05$). Similar results were also found in LAPC-4 (Fig 3F). In sum, our data suggested that the uptake of DHEAS activates AR signaling and promotes cell growth.

Gene-Gene Interaction Between SLCO2B1 and SLCO1B3 Polymorphisms

While patients carrying previously studied *SLCO1B3* risk genotypes (rs4149117-T or rs7311358-G) had a 3-month shorter median TTP compared to patients with favorable genotypes (rs4149117-GG or rs7311358-AA), these two SNPs alone did not have a statistically significant association with TTP on ADT (Table 1). Since both SLCO2B1 and SLCO1B3 are involved in transporting androgens (DHEAS and T), it is possible that the impact of *SLCO1B3* variants on TTP in ADT may be revealed in patients with different *SLCO2B1* genotypes. Thus, as an exploratory analysis, we investigated if *SLCO1B3* variants interact with the three *SLCO2B1* SNPs. As presented in Table 2 and Figure 4, the association of TTP on ADT with *SLCO2B1* polymorphisms was significantly modified by the genotype of *SLCO1B3*-rs4149117 ($P_{interaction} = .24$, .02, and .09, respectively; number of patients at risk is detailed in Data Supplement). Men with both *SLCO1B3*-rs4149117-GT/TT and *SLCO2B1*-rs12422149-GG, or -rs1789693-TT or -rs1077858-GG genotypes progressed more rapidly than men with either rs4149117-GT/TT genotype coupled with the favorable genotypes *SLCO2B1* (hazard ratio, 1.72, 95% CI, 1.04 to 2.85; hazard ratio, 2.90, 95% CI, 1.75 to 4.79; hazard ratio, 2.95, 95% CI, 1.65 to 5.28, respectively). The interaction remained significant in multivariable models adjusted for clinical factors affecting TTP. The additive effect of combinations of *SLCO2B1* three SNPs was also further enhanced by *SLCO1B3* SNPs ($P_{interaction} = .041$). As presented in Table 2 and Figures 4G and 4H, compared with men with one or zero risk genotype, the hazard ratio for three risk genotypes of *SLCO2B1* were 3.57 (95% CI, 2.03 to 6.26) in patients with the *SLCO1B3*-rs4149117-GT/TT genotypes and only 1.60 (95% CI, 0.99 to 2.58) in patients carrying the rs4149117-GG genotype. A similar trend was observed for the interaction between *SLCO2B1* genotypes and *SLCO1B3* rs7311358, which is in a strong LD with the rs4149117. The clinical implications of this interaction are significant. In some cases, the combinations of *SLCO1B3* and *SLCO2B1* genotypes resulted in a longer than 2-year difference in median TTP. These results suggested that *SLCO1B3* genotypes could significantly modify rates of disease progression, specifically for patients who carry the *SLCO2B1* risk genotypes.

DISCUSSION

The maintenance of AR signaling by sustaining intracellular androgen levels appears to be essential for the survival of CaP.[6,7] Intracellular androgens could be imported from the surrounding microenvironment or derived from de novo synthesis. In this study, we investigated the importance of genetic polymorphisms of *SLCO2B1* and *SLCO1B3* genes in affecting TTP on ADT in patients with HSPC. Three *SLCO2B1* SNPs, rs12422149A>G, rs1789693A>T and rs1077858A>G were associated with TTP on ADT. The differences in median TTP for each of these polymorphisms were approximately 10 months. We showed that the rs12422149G>A, an exonic SNP, can determine uptake of DHEAS. As such, the rs12422149G variant causes augmented DHEAS uptake, AR activation and increased cell proliferation

Table 2. Association Between *SLCO2B1* Genotypes and TTP During ADT, Listed Separately by *SLCO1B3*-rs4149117 Genotypes

SLCO2B1 Genotype	*SLCO1B3* rs4149117						
	GG			GT/TT			
	No.	HR	95% CI	No.	HR	95% CI	*P** (interaction)
rs12422149							
AA/AG	64	1.00	Reference	34	1.00	Reference	.237
GG	304	1.21	0.88 to 1.67	128	1.72	1.04 to 2.85	
rs1789693							
AA	153	1.00	Reference	59	1.00	Reference	.022
AT	158	0.91	0.70 to 1.17	67	1.68	1.07 to 2.65	
TT	47	1.20	0.80 to 1.81	33	2.90	1.75 to 4.79	
rs1077858							
AA	138	1.00	Reference	49	1.00	Reference	.092
AG	146	1.17	0.89 to 1.55	79	1.64	1.06 to 2.55	
GG	51	1.29	0.89 to 1.87	22	2.95	1.65 to 5.28	
No. of risk genotype							
≤ 1	167	1.00	Reference	67	1.00	Reference	.041
2	139	1.22	0.93 to 1.59	64	2.38	1.53 to 3.70	
3	29	1.60	0.99 to 2.58	20	3.57	2.03 to 6.26	

Abbreviations: TTP, time to progression; ADT, androgen-deprivation therapy; HR, hazard ratio.
*Wald χ^2 test for interaction from Cox regression.

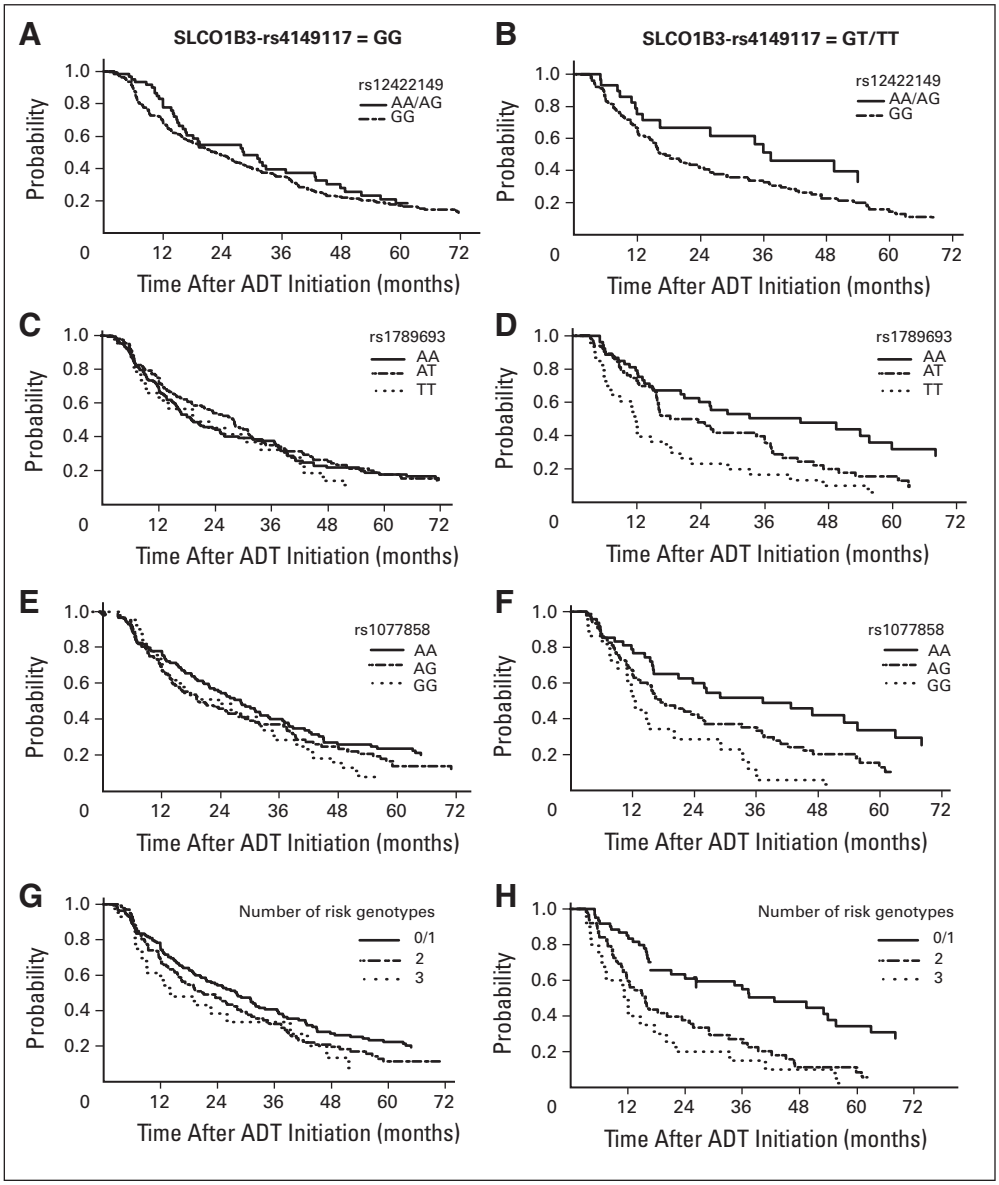

Fig 4. Kaplan-Meier curves of time to progression during androgen deprivation therapy, stratified by (A,B) genotypes at rs12422149 (*SLCO2B1*) and rs4149117 (*SLCO1B3*); (C,D) genotypes at rs1789693 (*SLCO2B1*) and rs4149117 (*SLCO1B3*); (E,F) genotypes at rs1077858 (*SLCO2B1*) and rs4149117 (*SLCO1B3*); and (G,H) ≤ 1, 2, or 3 unfavorable genotypes of the three *SLCO2B1* SNPs and rs4149117 (*SLCO1B3*).

in CaP cells, explaining its association with resistance to ADT. In addition, greater numbers of *SLCO2B1* risk genotypes are correlated with shorter TTPs on ADT. The additive effect of the three *SLCO2B1* risk genotypes suggested that these SNPs might independently affect *SLCO2B1* function. The biologic importance of rs1789693A>T and rs1077858A>G which are of nominal *P* values remains to be determined. Interestingly, although *SLCO1B3* SNPs alone did not have a significant association with TTP on ADT in our cohort, the importance of *SLCO1B3* responding to ADT was revealed via the analysis of the gene-gene interaction between the *SLCO2B1* and *SLCO1B3* variants. The TTP differences resulting from these gene-gene interactions were found to be potentially clinically important, with as much as 2-year differences in TTP. This finding needs further validation. These results strongly support our hypothesis that genetic variants of *SLCO2B1* and *SLCO1B3* loci can result in differences in progression after ADT, supporting the intracrine model for resistance to ADT.

The degree of significance of the association of *SLCO2B1* and *SLCO1B3* variants with patients' response to ADT is presumably driven by the expression level of the genes and the amount of substrate (androgens or androgen precursors) in the cancer microenvironment. The expression of *SLCO2B1* transcripts was found to be significantly increased in metastatic CRPC tissues compared to primary CaP tissues (5 fold, $P = .0012$; Mostaghel et al unpublished data). In addition, Titus et al[10] showed that adrenal derived DHEA levels in recurrent CaP tissues were 50% of the levels in benign prostate tissues but were sufficient for local androgen synthesis. The imported DHEAS can be converted to T and increase the intracellular androgen level, which may activate AR, thus sustaining tumor growth. The excess level of intracellular T may possibly be secreted, subsequently leading to an increased androgen level in the CaP microenvironment and this secreted T may be imported into tumor cells via SLCO1B3. This hypothesis may explain that *SLCO1B3* polymorphisms modify the association between TTP on ADT and *SLCO2B1* polymorphisms.

A small cohort study of 68 white patients with CaP showed that individuals with T genotype at the *SLCO1B3* rs4149117 had a shorter TTP than those with two copies of the G genotype ($P = .048$).[25]

However, we did not find any statistically significant association between this SNP and TTP on ADT. These inconsistent results may be due to the differences in patient populations and patient characteristics or more likely differences in sample sizes (68 *v* 538 patients).

We found that when treated with DHEAS, cells transfected with SLCO2B1-312Gln consistently exhibited higher PSA levels than those transfected with SLCO2B1-312Arg, although the significant differences was only observed in LAPC-4 cells. The failure to see this effect in LNCaP may be due to the expression of a mutated AR in LNCaP, which could be activated by T or DHT and many other weak androgens including DHEA,[32] while the wild-type AR in LAPC-4 may only be activated by T or DHT, metabolized from the imported DHEAS. It may also be due to different rates of DHEA metabolism in LNCaP and LAPC4. We also observed an increased expression of AR in both LNCaP and LAPC-4 on treatment with DHEAS. The exact mechanism leading to the upregulated AR by DHEAS is unclear. However, it has been noted that AR expression could be stimulated by androgens.[33]

In conclusion, to our knowledge, this report is the first study to demonstrate that inherited variations in the *SLCO2B1* gene, separately and in combination with the *SLCO1B3* polymorphisms, were significantly associated with response durations to ADT. Validation in an independent ADT cohort is underway. Our findings may potentially have several clinical implications. The *SLCO2B1* and *SLCO1B3* genotypes may act as prognostic tools for responses to ADT in CaP. Drugs may be developed to inhibit transporter activity to improve responses to ADT.[34] Importantly, our observations strongly suggest the essential role of adrenal androgens, such as DHEAS and DHEA, during CRPC development and the importance of considering antiadrenal androgen therapy including ketoconazole and abiraterone.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

Conception and design: Ming Yang, Elahe Mostaghel, Tong Sun, Nima Sharifi, William Douglas Figg, Gwo-Shu Mary Lee, Philip W. Kantoff
Financial support: Philip W. Kantoff
Administrative support: Gwo-Shu Mary Lee, Philip W. Kantoff
Provision of study materials or patients: Matthew Freedman, William K. Oh, Gwo-Shu Mary Lee, Philip W. Kantoff
Collection and assembly of data: Ming Yang, Wanling Xie, Gwo-Shu Mary Lee
Data analysis and interpretation: Ming Yang, Wanling Xie, Elahe Mostaghel, Mari Nakabayashi, Lillian Werner, Tong Sun, Mark Pomerantz, Matthew Freedman, Robert Ross, Meredith Regan, Nima Sharifi, William Douglas Figg, Steven Balk, Myles Brown, Mary-Ellen Taplin, William K. Oh, Gwo-Shu Mary Lee, Philip W. Kantoff
Manuscript writing: All authors
Final approval of manuscript: All authors

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Glossary Terms

OATP (organic anion-transporting polypeptide family): represents a family of the organic anion transporter which facilitates the Na ion independent transport of a variety of compounds including bile acids, bilirubin, steroid hormone conjugates, thyroid hormones, prostaglandins, clinically used drugs, and toxins. OATPs are transmembrane glycoproteins, expressed in various cells and tissues.

Pharmacogenomics: The study of how a person's genome can affect their reaction to medications.

PSA (prostate-specific antigen): A protein produced by cells of the prostate gland, the blood level of PSA is used as a tumor marker for men who may be suspected of having prostate cancer. Most physicians consider 0 to 4.0 ng/mL as the normal range. Levels of 4 to 10 and 10 to 20 ng/mL are considered slightly and moderately elevated, respectively. PSA levels have to be complemented with other tests to make a firm diagnosis of prostate cancer.

SLCO2B1: a member of the organic anion-transporting polypeptide family (OATP) and mediates the transport of montelukast and various sulfated steroids.

SNP (single nucleotide polymorphism): Genetic polymorphisms are natural variations in the genomic DNA sequence present in greater than 1% of the population, with SNP representing DNA variations in a single nucleotide. SNPs are being widely used to better understand disease processes, thereby paving the way for genetic-based diagnostics and therapeutics.