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ORIGINAL REPORT

Association of *SLCO2B1* Genotypes With Time to Progression and Overall Survival in Patients Receiving Androgen-Deprivation Therapy for Prostate Cancer

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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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ABSTRACT

Purpose

To validate the association of three previously demonstrated *SLCO2B1* germline variants with time to progression (TTP) in patients receiving androgen-deprivation therapy (ADT), and to evaluate if the *SLCO2B1* genetic variants impacted overall survival (OS) for prostate cancer (PC).

Patients and Methods

Three single nucleotide polymorphisms (SNPs), exonic SNP rs12422149 and intronic SNPs rs1789693 and rs1077858, were genotyped in an independent validation cohort of 616 patients with PC who were treated with ADT at the Dana-Farber Cancer Institute from 1996 to 2013. Multivariable Cox proportional hazards regression adjusting for known prognostic factors estimated the association of these genetic variants with TTP and OS in patients receiving ADT. The expression of SLCO2B1 was examined in prostatectomy samples, and the impact of SLCO2B1 expression level on DHEAS (dehydroepiandrosterone sulfate) uptake was evaluated in cell lines.

Results

The association between exonic SNP rs12422149 and TTP in patients treated with ADT was confirmed in univariable ($P = .019$) and multivariable analyses (adjusted hazard ratio, 1.31; 95% CI, 1.00 to 1.72 for GG *v* AA/AG; $P = .049$). Because OS had not been previously evaluated, we examined the association in the combined initial and validation cohorts (N = 1,094). The intronic SNP rs1077858 was associated with OS in both univariable ($P = .009$; Bonferroni's method adjusted $P = .027$) and multivariable analyses (adjusted hazard ratio, 1.35; 95% CI, 1.07 to 1.71 for GG *v* AA/AG; $P = .012$). SLCO2B1 expression in normal prostate tissue and in 22RV1 cells carrying the major allele of SNP rs1077858 was significantly lower than in cells carrying the risk allele. We show in vitro that SLCO2B1 expression levels correlated with DHEAS uptake by PC cells.

Conclusion

The association of SNP rs1077858 with OS may be a result of differential SLCO2B1 expression and the consequent increased uptake of DHEAS and subsequent resistance to ADT, which, in turn, may contribute to decreased OS.

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INTRODUCTION

Androgen-deprivation therapy (ADT) is the primary treatment of newly diagnosed metastatic prostate cancer (PC) and works by suppressing testicular androgen production and diminishing androgen receptor (AR) activation.[1,2] Most patients will eventually progress to castration-resistant PC (CRPC), which is generally lethal.[3-6] The variability of time to CRPC may be partly a result of inherited factors that increase uptake or use of available circulating androgens.

Despite treatment with ADT, persistent AR activation, stimulated by residual androgen, remains an integral factor driving progression to castration resistance.[7,8] These residual androgens are derived either from increased de novo androgen synthesis or from uptake and conversion of circulating adrenal androgens.[6,9] One such adrenal androgen, dehydroepiandrosterone (DHEA), and its sulfated form, DHEAS (dehydroepiandrosterone sulfate), are secreted in large amounts by the adrenal cortex. In the tumor microenvironment, DHEAS is converted to testosterone and, subsequently, to dihydrotestosterone, the primary

androgen used by PC cells.[10-12] Even after ADT, DHEAS persists at a high concentration in the blood at a range of 1 μM to 20 μM.[13-15]

How patients use available DHEAS may impact their response to ADT. The super family of organic anion-transporting polypeptides, encoded by *SLCO* genes, mediates the sodium-independent uptake into cells of a wide variety of endogenous compounds and drugs.[16,17] SLCO2B1, of the *SLCO* gene family, mediates the transport of endogenous sex steroid conjugates, such as DHEAS,[17-20] and is expressed in a broad range of tissues, including the prostate. CRPC tumors exhibit higher SLCO2B1 expression than localized PC primary tumors.[21] Epidemiologic studies have demonstrated that sequence variations of *SLCO2B1* can have a significant impact on the time to progression (TTP) to CRPC or on PC survival.[21-23] In our previous study, we genotyped 18 single nucleotide polymorphisms (SNPs) of *SLCO2B1* in a cohort of 538 patients with PC who were treated with ADT.[22] Three SNPs were associated with TTP on ADT ($P < .05$): an exonic SNP, rs12422149G>A, and two intronic SNPs, rs1789693A>T and rs1077858A>G. Differences in median TTPs between the three identified SNPs were approximately 10 months (10 months for rs12422149, 7 months for rs1789693, and 12 months for rs1077858).

In this report, we attempt to validate whether the three *SLCO2B1* SNPs are associated with TTP in patients receiving ADT in an independent validation cohort. In addition, we examine the association of *SLCO2B1* SNPs with TTP and overall survival (OS) in the combined initial and validation cohorts, as well as assess the impact of metastatic disease status at ADT initiation.

PATIENTS AND METHODS

All cohorts were identified from our established, institutional Prostate Cancer Clinical Research Information System (CRIS) database.[24] We identified patients with biochemical recurrence (M0) or radiologically evident metastatic disease (M1) who had received ADT for hormone-sensitive PC. We began registering patients in CRIS in 2001, and all patients provided consent to an institutional review board–approved protocol that collects clinical and genomic data. We used a validation cohort of 616 patients who had not previously been analyzed. For additional analyses, we used a combined cohort (N = 1,094) composed of the 616-patient validation cohort and 478 patients from the initial cohort of our previous study.[22] The clinical data collection as well as detailed patient characteristics are described in the Appendix (online only) and have been previously described.[22]

Statistical methods are described in the Appendix. Details of SNP genotyping, plasmid preparation, cell cultures, transfections, and DHEAS uptake assays were described in our previous study.[22]

RESULTS

Patient Characteristics

Clinicodemographic, disease, and past treatment characteristics of the initial cohort (n = 478) and validation ADT cohort (n = 616) are presented in Appendix Table A1 (online only). Both cohorts had similar disease characteristics at diagnosis and at ADT initiation. In both cohorts, approximately 70% of patients had received a local therapy (radical prostatectomy or radiation therapy) and nearly 60% had metastases at the time of ADT initiation. The validation cohort had a lower prostate-specific antigen (PSA) at diagnosis than did the initial cohort (median, 9.9 ng/ml *v* 14 ng/ml, respectively), most likely a result of increased frequency of PSA screening that led to an earlier diagnosis of PC in the validation cohort. The validation cohort also more frequently received intermittent ADT than did the initial cohort (35.9% *v* 18.8%, respectively).

Validation of the Association of SLCO2B1 SNPs With TTP During ADT in the Validation Cohort

At the time of data retrieval, 66% (n = 408) of patients in the validation cohort had progressed on ADT. The median TTP on ADT was 20.9 months (95% CI, 18.0 to 24.0) and the median follow-up time was 4.2 years (range, 0.1 to 16.3 years).

The exonic SNP, rs12422149, in *SLCO2B1* was associated with TTP in patients receiving ADT as determined by univariable analysis (median TTP for AA/AG, 27.2 months; 95% CI, 18.9 to 48.9; and median TTP for GG, 20.0 months; 95% CI, 16.5 to 23.0; $P = .019$; Table 1; Appendix Fig A1A, online only). In multivariable analyses adjusting for clinical factors, the association between rs12422149 and TTP remained significant (hazard ratio [HR], 1.31; 95% CI, 1.00 to 1.72; $P = .049$). Thus, the association of rs12422149 with TTP in patients treated with ADT observed in the initial cohort was validated such that patients with PC who carry the major GG genotype exhibited a shorter TTP while being treated with ADT. Although there was a trend toward an association between rs1077858 and TTP in patients receiving ADT, the two intronic SNPs were not validated (Table 1; Appendix Figs A1B and A1C).

Association of SLCO2B1 SNPs With TTP During ADT in the Combined Cohort on the Basis of Prior ADT Use and Metastases Present at ADT Initiation

Radiographically evident metastatic PC (M1) carries a worse prognosis than does biochemically recurrent disease (PSA only or M0). Thus, we explored the correlation between the *SLCO2B1* SNPs and TTP in the combined cohort of initial and validation patients and when stratified by metastatic disease status at time of ADT initiation, both of which had not been previously evaluated. In the combined cohort (N = 1,094), 74% of patients (n = 811) had progressed on ADT (median TTP on ADT, 18.9 months; 95% CI, 16.5 to 21.1).

For the exonic rs12422149, we again found a significant association with TTP in patients receiving ADT in the combined cohort (adjusted HR, 1.33; 95% CI, 1.10 to 1.60; $P = .003$). When stratified by metastatic disease status, the association remained in the M1 population (adjusted HR, 1.65; 95%, 1.28 to 2.12), but was not seen in the M0 population (adjusted HR, 0.96; 95% CI, 0.72 to 1.27; P interaction = .006; Table 2; Appendix Fig A2, online only). The association between the intronic SNP rs1077858 and TTP was of borderline significance ($P = .075$; $P = .062$ if AA and AG were combined). No association was found for rs1789693 and TTP in patients receiving ADT in the combined cohort. Results were also negative in the M0 and M1 populations (P interaction > .5; Table 2; Appendix Fig A2).

The associations with TTP in patients treated with ADT were similar among patients with and without prior hormone treatments for the exonic SNP rs12422149. However, for the intronic SNP rs1077858, a significant association was observed only in

Table 1. *SLCO2B1* Genotype Distributions and Their Association With TTP in Patients Receiving ADT in the Validation Cohort

Genotype	No. (%)	Univariable Model			Multivariable Model*	
		Median (95% CI) TTP (months)	Log-Rank *P*	HR (95% CI)	Adjusted HR (95% CI)	*P*
rs12422149						
AA/AG	110 (18)	27.2 (18.9 to 48.9)	.019	1.00 (reference)	1.00 (reference)	.049
GG	506 (82)	20.0 (16.5 to 23.0)		1.39 (1.05 to 1.80)	1.31 (1.00 to 1.72)	
rs1789693						
AA	238 (39)	21.6 (17.5 to 34.1)	.176	1.00 (reference)	1.00 (reference)	.409
AT	281 (46)	19.4 (15.0 to 23.4)		1.22 (0.99 to 1.51)	1.15 (0.93 to 1.43)	
TT	90 (15)	25.2 (18.1 to 38.1)		1.07 (0.79 to 1.45)	1.02 (0.75 to 1.39)	
rs1077858						
AA	288 (47)	20.9 (17.0 to 25.1)	.383	1.00 (reference)	1.00 (reference)	.880
AG	242 (40)	23.0 (16.6 to 30.0)	.185†	0.96 (0.77 to 1.18)	0.98 (0.79 to 1.22)	.634†
GG	81 (13)	14.1 (9.7 to 23.7)		1.19 (0.88 to 1.59)	1.06 (0.79 to 1.44)	

Abbreviations: ADT, androgen-deprivation therapy; HR, hazard ratio; TTP, time to progression.
*Adjusting for biopsy Gleason tumor grading system score, type of primary therapy, prior treatment with ADT in conjunction with local therapy, metastatic status, and prostate-specific antigen at initiation of ADT.
†If AA and AG were combined.

patients without prior hormone treatment (adjusted HR, 1.32; 95% CI, 1.06 to 1.65), but not in patients with prior hormone treatment (adjusted HR, 0.87; 95% CI, 0.55 to 1.37; Appendix Table A2, online only).

Association of SLCO2B1 *SNPs With OS in the Combined ADT Cohort*

Because correlations with OS were not explored in our previous study, we therefore evaluated this end point in the combined cohort. Nearly half (49%; n = 537) of patients had died at the time of data collection. Median OS from ADT initiation was 6.5 years (95% CI, 6.0 to 7.0) and median follow-up time was 6.5 years (range, 0.1 to 16.3 years).

There was no statistically significant association with OS for the exonic SNP rs12422149 in either the univariable or multivariable analyses (Table 3; Appendix Fig A3A, online only). In the univariable analysis, there was no association between rs1789693 and OS from ADT initiation (P = .184; Bonferroni's method adjusted P = .552), but patients who carried the minor allele (AT or TT) had longer OS upon multivariable analysis than did those patients without the minor allele (HR, 0.81 and 0.78, respectively; P = .044; Table 3; Appendix Fig A3B). Of note, we found that patients who carried the minor genotype GG in the intronic SNP rs1077858 had a shorter OS from ADT initiation in both the univariable (P = .009; Bonferroni's method adjusted P = .027) and multivariable analyses (adjusted HR, 1.35; 95% CI, 1.07 to 1.71; P = .012; Table 3; Appendix Fig A3C). Median OS decreased from 6.7 years (95% CI, 6.2 to 7.2) to 5.2 years (95% CI, 4.3 to 6.8) in the AA/AG versus GG rs1077858 genotypes, respectively.

When stratified by metastatic disease status (M0 *v* M1), patients who carried the exonic SNP rs12422149 GG genotype expressed a trend for shorter OS only in the M1 population and at a

Table 2. Association of *SLCO2B1* Genotype With TTP in Patients Receiving ADT in the Combined Cohort (initial plus validation) and Stratified by Metastatic Disease Status at ADT Initiation

Genotype	Combined Cohort			Metastatic Disease Status*				
				M0		M1		
	No. (%)	Adjusted HR (95% CI)†	*P*	No.	Adjusted HR (95% CI)	No.	Adjusted HR (95% CI)	*P* for Interaction
rs12422149								
AA/AG	197 (18)	1.00 (reference)	.003	93	1.00 (reference)	104	1.00 (reference)	.006
GG	894 (82)	1.33 (1.10 to 1.60)		366	0.96 (0.72 to 1.27)	528	1.65 (1.28 to 2.12)	
rs1789693								
AA	427 (39)	1.00 (reference)	.978	186	1.00 (reference)	241	1.00 (reference)	.551
AT	486 (45)	0.99 (0.85 to 1.16)		200	1.07 (0.83 to 1.37)	286	0.95 (0.78 to 1.15)	
TT	169 (16)	1.02 (0.83 to 1.25)		68	1.18 (0.83 to 1.67)	101	0.94 (0.72 to 1.21)	
rs1077858								
AA	463 (43)	1.00 (reference)	.075	197	1.00 (reference)	266	1.00 (reference)	.610
AG	476 (44)	1.11 (0.95 to 1.29)	.062‡	200	1.21 (0.95 to 1.55)	276	1.05 (0.87 to 1.27)	
GG	147 (14)	1.27 (1.03 to 1.58)		60	1.27 (0.88 to 1.81)	87	1.27 (0.98 to 1.66)	

Abbreviations: ADT, androgen-deprivation therapy; HR, hazard ratio; TTP, time to progression.
*From multivariable Cox proportional hazards regression models, with the interaction of single nucleotide polymorphism (SNP) and metastatic disease, and simultaneously adjusted for other clinical variables. *P* for interaction is to test whether the association of SNP with TTP differs by metastatic disease status.
†Adjusting for biopsy Gleason tumor grading system score, type of primary therapy, prior treatment with ADT in conjunction with local therapy, metastatic status, and prostate-specific antigen at initiation of ADT.
‡If AA and AG were combined.

Table 3. Association of *SLCO2B1* Genotype With OS From the Initiation of ADT in the Combined Cohort (initial plus validation) and Stratified by Metastatic Disease Status at ADT Initiation

	Combined Cohort						Metastatic Disease Status*				
		Univariable Model			Multivariable Model†		M0		M1		
Genotype	No. (%)	Median (95% CI) OS (years)	Log-Rank *P* (Bonferroni's adjusted)	HR (95% CI)	Adjusted HR (95% CI)	*P*	No.	Adjusted HR (95% CI)	No.	Adjusted HR (95% CI)	*P* for Interaction
rs12422149											
AA/AG	197 (18)	7.1 (6.0 to 8.1)	.373 (.999)	1.00 (reference)	1.00 (reference)	.506	93	1.00 (reference)	104	1.00 (reference)	.038
GG	894 (82)	6.4 (5.9 to 6.9)		1.11 (0.89 to 1.39)	1.08 (0.86 to 1.36)		366	0.82 (0.58 to 1.14)	528	1.32 (0.97 to 1.80)	
rs1789693											
AA	427 (39)	6.1 (5.2 to 6.7)	.184 (.552)	1.00 (reference)	1.00 (reference)	.044	186	1.00 (reference)	241	1.00 (reference)	.999
AT	486 (45)	7.2 (6.1 to 7.7)		0.84 (0.70 to 1.01)	0.81 (0.67 to 0.98)		200	0.81 (0.60 to 1.11)	286	0.81 (0.64 to 1.03)	
TT	169 (16)	6.2 (5.0 to 7.8)		0.89 (0.69 to 1.14)	0.78 (0.60 to 1.00)		68	0.78 (0.50 to 1.21)	101	0.77 (0.57 to 1.06)	
rs1077858											
AA/AG‡	939 (86)	6.7 (6.2 to 7.2)	.009 (.027)	1.00 (reference)	1.00 (reference)	.012	397	1.00 (reference)	542	1.00 (reference)	.970
GG	147 (14)	5.2 (4.3 to 6.8)		1.36 (1.08 to 1.71)	1.35 (1.07 to 1.71)		60	1.36 (0.93 to 1.99)	87	1.35 (1.00 to 1.82)	

Abbreviations: ADT, androgen-deprivation therapy; HR, hazard ratio; OS, overall survival; TTP, time to progression.

*From multivariable Cox proportional hazards regression models, with the interaction of single nucleotide polymorphism (SNP) and metastatic disease, and simultaneously adjusted for other clinical variables. *P* for interaction is to test whether the association of SNP with TTP differs by metastatic disease status.

†Adjusting for biopsy Gleason tumor grading system score, type of primary therapy, prior treatment with ADT in conjunction with local therapy, metastatic status, prostate-specific antigen at initiation of ADT, and age at ADT initiation.

‡Distributions of OS were similar between AA and AG groups and were thus combined (median, 6.8 and 6.5 years, respectively).

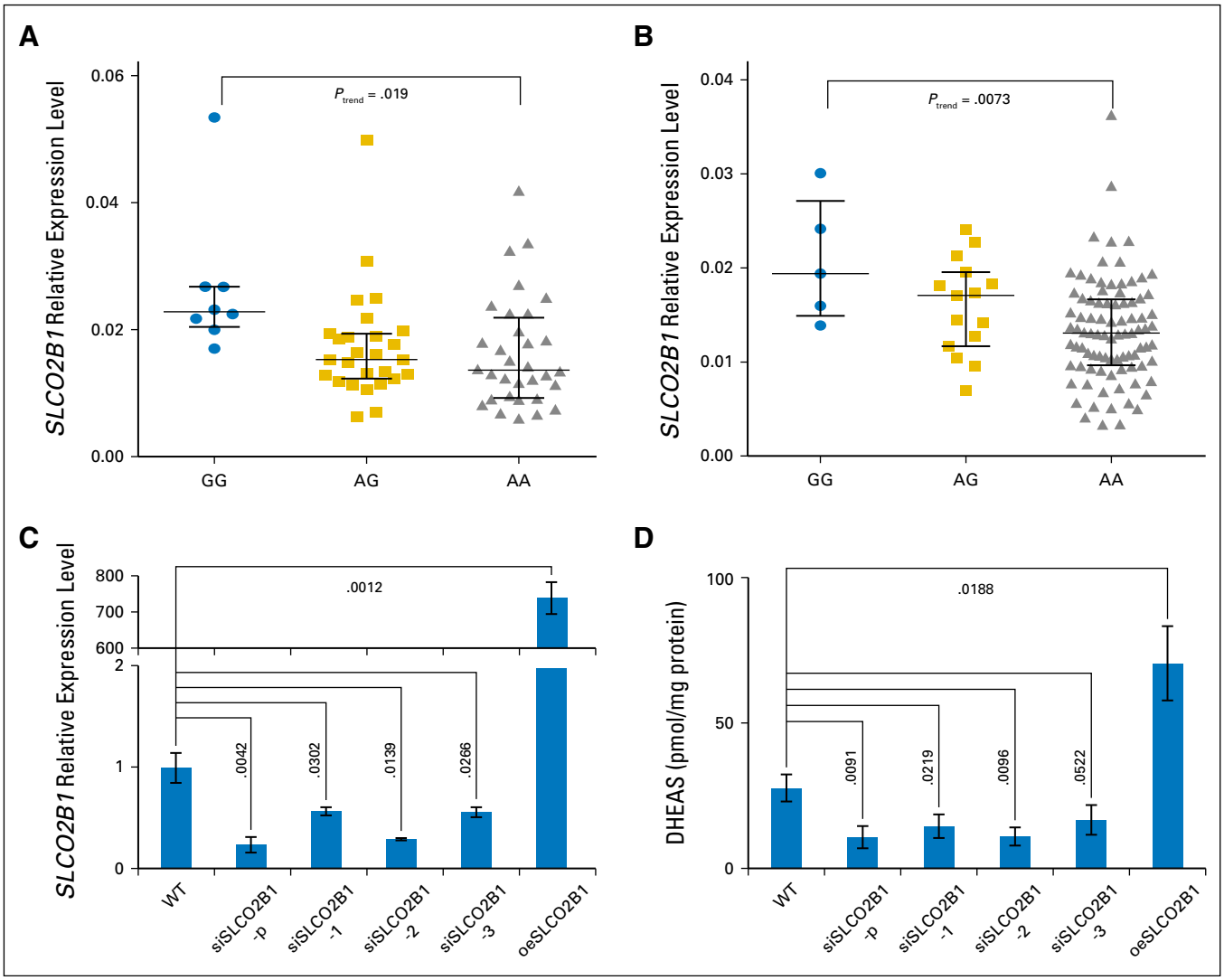

Fig 1. Impact of intronic single nucleotide polymorphism rs1077858 on the expression of SLCO2B1 and the effect on DHEAS (dehydroepiandrosterone sulfate) uptake. (A) Relative *SLCO2B1* mRNA levels compared with GAPDH (glyceraldehyde 3-phosphate dehydrogenase) in normal prostate tissue in patients with primary prostate cancer. Significant differences were observed among three groups ($P_{trend} < .05$). Lines represent the median with interquartile range. (B) Relative *SLCO2B1* mRNA levels compared with GAPDH in 22RV1 cells with different alleles after reconstitution using the Prostate Cancer Clinical Research Information System. Significant differences were observed among three groups ($P_{trend} < .01$). Lines represent the median with interquartile range. (C) *SLCO2B1* mRNA levels in mock-transfected LNCaP cells and in LNCaP cells transfected with different *SLCO2B1* variants. Values are fold differences relative to those in mock-transfected cells, which were set at 1.0. All experiments were repeated in triplex. Statistical analysis (unpaired *t* test) was performed by comparing each condition with the condition of LNCaP mock-transfected with either small interfering RNA (siRNA) control or pCMV6-XL4 vector (WT). All mRNA levels were analyzed by quantitative reverse transcriptase polymerase chain reaction and normalized by the expression level of GAPDH. (D) DHEAS uptake in LNCaP cells. Cells were incubated in DHEAS 2.5 μM for 10 minutes after cells had been transfected with siRNA or pCMV6-XL4-SLCO2B1 for 3 days. All experiments were repeated in triplex. Statistical analysis (unpaired *t* test) was performed by comparing each condition with the WT condition. oeSLCO2B1, LNCaP transiently transfected with pCMV6-XL4-SLCO2B1; siSLCO2B1, LNCaP transiently transfected with siRNA targeting SLCO2B1 (-p represents the SMARTpool siRNA from GE Healthcare, and -1, -2 and -3 represent three unique 27mer siRNAs from Origene; Appendix, online only).

borderline significance, but this difference was not observed in the M0 patients (for M0: adjusted HR for GG, 0.82; 95% CI, 0.58 to 1.14; for M1: adjusted HR for GG, 1.32; 95% CI, 0.97 to 1.80; *P* interaction = .038). The association of OS with rs1789693 and rs1077858 was similar in the M0 and M1 populations (*P* for interaction > .9; Table 3; Appendix Fig A4, online only). The minor rs1077858 GG genotype indicated shorter OS from ADT initiation in both groups (for M0: adjusted HR, 1.36; 95% CI, 0.93 to 1.99; for M1: adjusted HR, 1.35; 95% CI, 1.00 to 1.82; Table 3; Appendix Fig A4).

Impact of Intronic SNP rs1077858 on Expression of SLCO2B1

In our previous study, we showed that different *SLCO2B1* variants corresponding to the exonic SNP rs12422149G>A (Arg312Gln) influenced the efficiency of DHEAS transport into PC cells.[22] Given that intronic SNP rs1077858 was associated with OS, we studied the possible mechanism by which the SNP might affect OS from ADT initiation. Using 80 samples of normal prostate tissue from patients known to have PC that were available in our tissue repository, we analyzed SLCO2B1 expression (Fig 1A). Results

demonstrated that rs1077858 SNP variants were associated with significantly different SLCO2B1 expression levels (P_{trend} = .019), with the greatest SLCO2B1 expression in patients who carried the minor GG genotype. To prove that different rs1077858 SNPs impact the expression of SLCO2B1, we reconstituted the rs1077858 SNP variants using CRISPR in 22RV1 cells to convert the original A allele to the G allele (Appendix). The association between rs1077858 SNP variants and SLCO2B1 expression levels was significant (P_{trend} = .007) and, as before, the GG genotype in 22RV1 cells corresponded to the greatest SLCO2B1 expression level (Fig 1B). These results suggest that the association of rs1077858 with OS was possibly a result of changes in SLCO2B1 expression levels in these patients. Thus, alleles that correspond to greater SLCO2B1 expression may allow increased DHEAS uptake and, thereby, induce shorter OS in patients. To evaluate this hypothesis, we examined DHEAS uptake in an LNCaP PC cell line by altering the expression level of SLCO2B1 to mimic different rs1077858 allele conditions. After successfully knocking down or overexpressing SLCO2B1 (Fig 1C), we found that DHEAS uptake activity was dependent on the expression level of SLCO2B1 and that greater expression of SLCO2B1 resulted in increased DHEAS transport into cells (Fig 1D).

DISCUSSION

There is great variability in the durability of response to ADT, and multiple mechanisms are likely operative. Persistent androgens, particularly adrenal androgen precursors, such as DHEAS, play an important role in the evolution of CRPC by continuing to drive the AR signaling pathway.[15,25,26] SLCO2B1 is an active androgen transporter that delivers androgens and androgen precursors to prostate cells, potentially promoting resistance to treatment with ADT.[21,22,27] We found that *SLCO2B1* SNPs are significantly associated with either the efficiency of DHEAS transport or the expression level of SLCO2B1 on prostate cells, which, in part, may mechanistically determine the variability in outcomes in patients receiving ADT.

Our previous study revealed that three *SLCO2B1* SNPs significantly influenced TTP in patients with PC receiving ADT with approximately 10-month differences in median TTP.[22] In addition, an independent study of the *SLCO2B1* SNP in a Japanese patient population also found that rs12422149 is associated with TTP during treatment with ADT.[23] In the current study, we attempted to further validate those findings in an independent cohort of patients and to better elucidate the mechanism behind these effects. Only the exonic SNP rs12422149 was validated for its association with TTP in patients receiving ADT, which indicated that PC patients who carry the major GG genotype for rs12422149 may derive less benefit from ADT. Increased DHEAS uptake in the poorer-risk G allele carriers may be associated with greater intracellular androgen stores and, thus, is a plausible mechanism of resistance to ADT. In addition, in a subset analysis, the association of rs12422149 with TTP in patients receiving ADT was seen predominantly in M1 patients, suggesting additional unknown factors may participate in resistance in M1 patients compared with the M0 disease state.

The difference in median TTP between the AA and GG rs1077858 genotypes in the validation cohort was 6.8 months (AA: 20.9 months; 95% CI, 17.0 to 25.1 *v* GG: 14.1 months; 95% CI, 9.7 to 23.7), which has a similar trend to that observed in our initial study. This difference did not persist in the multivariable model after adjusting for other clinical factors. False discovery is certainly a possible reason for our inability to validate an association between the two intronic SNPs, rs1789693 and rs1077858, and TTP in patients receiving ADT in the current study.

To our knowledge, the current study is the first to demonstrate that an inherited variation in the *SLCO2B1* gene is significantly associated with OS after treatment with ADT. Whereas intronic SNP rs1077858 correlated with differences in TTP in the initial cohort, the current study should be viewed as a hypothesis-generating study. In the current study, it was found to be associated with OS in the larger combined cohort of patients. Of note, the phenotype of this functional SNP is independent of metastatic disease status at ADT initiation, indicating that the decreased OS cannot be attributed to lead time bias, that is, starting ADT later when patients have radiographically evident metastases compared with biochemical recurrence only. Further, we demonstrated that genetic variations in rs1077858 correlate with variable expression levels of SLCO2B1 in patient prostate tissue samples and in a cell line–based study. As such, it is plausible that the rs1077858 GG variant, with its greater SLCO2B1 expression level, results in enhanced DHEAS uptake and increased activation of the AR signaling pathway, with subsequent decreased OS in patients. It is unclear why we observed an OS benefit with no definite impact on TTP with rs1077858 in the validation cohort. However, there was a median increase in TTP of 6 months that was consistent with an improvement in outcomes with rs1077858 but this observation did not reach statistical significance in the univariable model (TTP in patients receiving ADT for AA and GG was 20.9 and 14.1 months, respectively; P = .383).

The question of why the only SNP validated to influence TTP in patients receiving ADT, exonic rs12422149, was not associated with OS must also be asked. It is worth mentioning that, though not associated with OS, the GG genotype of rs12422149 had decreased TTP and OS in M1 patients, suggesting that rs12422149, to some extent, could affect TTP and OS in a consistent fashion. We believe that mechanisms other than the expression level of SLCO2B1 may be involved in the association of rs1077858 with OS, because clinical evidence for a significant association of TTP in patients receiving ADT with OS has not been established, and because the functional effect of exonic SNP rs12422149 on DHEAS uptake efficiency has a significant association with TTP in patients treated with ADT, but not with OS in all patients. For example, variability in DHEAS uptake may be an important driver in earlier stages of metastatic disease but nonandrogen pathways may induce resistance in later stages. It is also possible that unidentified regulatory or functional SNPs cross-talk with known functional SNPs in different disease stages in this study. Hearn et al[28] have found that men inheriting the variant *HSD3B1* (1245C) allele that enhances dihydrotestosterone synthesis exhibit resistance to ADT, as manifested by worse clinical outcomes (decreased survival time). Therefore, those unidentified factors, such as an SNP in *HSD3B1* (A1245C), need to be considered in future analyses to further characterize the association of SNPs in *SLCO2B1* with TTP and OS. Finally and most plausibly, with respect to finding a difference in TTP but not OS, is heterogeneity in terms of access and administration of the five therapies currently approved for treatment of CRPC that improve survival, including enzalutamide, abiraterone, cabazitaxel, radium-223, and sipuleucel-T.[29-35]

Additional potential confounders of our results include differences in patient and disease characteristics at diagnosis and at ADT initiation between the initial and validation cohorts. Patients from the validation cohort were more likely to have a lower PSA at diagnosis and to have received hormonal therapy as part of local therapy (Appendix Table A1). Thus, the validation cohort could have been biased toward living longer, but this bias may be countered by increased prior treatment with ADT, which would indicate a decreased time on ADT.[3] When prior treatment with ADT was added to the multivariable model, it was found to have no impact on the result. Finally, differences in access and administration of subsequent survival-improving therapies, such as enzalutamide and abiraterone, may have also confounded the OS results. Whereas we found that the effect of exonic SNP rs12422149 on TTP was not dependent on prior treatment with ADT, the effect of the intronic SNP rs1077858 was shown to be dependent on prior treatment with ADT (Appendix Table A2). Thus, future analyses should be performed, stratifying by disease stage, use of ADT with local therapy, and use of subsequent therapies for metastatic disease, to discover the unknown factors that affect the SNP variant functions at different stages of disease and that result from prior ADT use.

In conclusion, germline variants within *SLCO2B1* seem to modulate function or expression of SLCO2B1 in vitro, which may affect the uptake of DHEAS and may impact TTP and OS in patients with PC. These findings suggest that *SLCO2B1* variants are prognostic markers of durability of response to ADT. Further, SLCO2B1 may serve as a target for novel agents and strategies. If prospectively validated, genotyping of *SLCO2B1* may help tailor more effective therapies to the individual patient.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at www.jco.org.

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GLOSSARY TERMS

androgen-deprivation therapy (ADT): treatment that suppresses or blocks the production or action of male hormones.

DHEAS (dehydroepiandrosterone sulfate): a metabolite of dehydroepiandrosterone (DHEA), an androgen produced by the adrenal gland.

overall survival: the duration between random assignment and death.

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

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

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Association of *SLCO2B1* Genotypes With Time to Progression and Overall Survival in Patients Receiving Androgen-Deprivation Therapy for Prostate Cancer

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Appendix

This appendix provides information that does not appear in the main article text.

Patients and Methods

In brief, the initial androgen-deprivation therapy (ADT) cohort (n = 595) was identified in June 2006 and the patient treatments and outcome data were updated in 2012. Twenty-seven patients were excluded from the initial cohort because of insufficient follow-up data after ADT initiation, and an additional 77 patients were excluded because they started ADT before the cutoff point of 1996. This year was used as the cutoff point because patients who had lived that long, that is, at least 6 years from time of ADT initiation, were excellent responders and inclusion would have created the potential for selection bias. Of the remaining 491 patients, 478 were genotyped for the three SLCO2B1 single nucleotide polymorphisms (SNPs). Seven hundred fifty-eight patients were identified for the validation cohort, and clinical data for analysis was retrieved from the Prostate Cancer Clinical Research Information System (CRIS) database in 2013. Of these 758 patients, 104 were excluded as a result of insufficient follow-up data after ADT initiation, and 11 patients were excluded because they started ADT before the 1996 cutoff point. Of the remaining 643 patients, 616 were genotyped for the three SLCO2B1 SNPs.

Time to progression (TTP) was defined as two increases in prostate-specific antigen (PSA), at least 1 week apart, while receiving ADT. The first increase was required to be greater than the nadir PSA value by greater than 0.02 ng/mL. Initiation of a secondary treatment for increasing PSA before fulfillment of the definition of progression was also considered a progression event; the start date of the secondary treatment was noted as the date of progression. TTP during ADT was defined as the duration of time from ADT initiation to the date of ADT progression or to the date of initiation of secondary therapy. Patients who did not experience progression were censored as of the date of their last known progression-free visit or the date of death for those patients who died without progression. Overall survival (OS) was defined as the period from ADT initiation to patient death or was censored at the date of the last follow-up.

Statistical Methods

SNPs were analyzed as three distinct genotype groups, with the exception of the exonic SNP rs12422149 wherein AA and AG genotypes were combined because only 1% of the population carries the AA genotype. AA and AG genotypes were also combined for intronic SNP rs1077858 during the analysis of their association with OS because the distributions of OS times were similar between the AA and AG groups.

Progression was defined as a minimum of two increases in PSA, with the date of first increase (nadir plus $>$ 0.02 ng/mL) used as the date of progression. TTP during ADT was defined as the duration of time from ADT initiation to the date of ADT progression or to the date of initiation of a secondary therapy for increasing PSA before fulfillment of the definition of progression, or was censored at the date of last follow-up visit or PSA value among patients who did not progress. OS was defined as the period from ADT initiation to patient death, or it was censored on the day of the last follow-up.

The distributions of TTP and OS were estimated using the Kaplan-Meier method and included 95% CIs. The association between SNPs and TTP was evaluated using the log-rank test or the Wald χ^2 test and by the multivariable Cox proportional hazards regression model adjusted for known prognostic factors, including biopsy Gleason score, type of primary therapy, prior treatment with ADT in conjunction with local therapy, metastatic status, and PSA at ADT initiation. OS was analyzed in a similar way, but age at ADT initiation was also included as a covariate in the multivariable models. To estimate whether the association of SNPs with OS and with TTP during ADT differed by metastatic disease status at ADT initiation, we used multivariable Cox proportional hazards regression models with the interaction term of SNP and metastatic disease status and simultaneously adjusted for the other clinical variables.

There was no correction for multiple comparisons for TTP analysis because validation aimed to reproduce the strength of association previously identified in our initial work in which we applied appropriate correction for multiple comparisons. Bonferroni's procedure was used to correct multiple comparisons to test OS.

For in vitro cell line studies, data were represented as mean $\pm$ standard deviation of at least three biologic repeats. Comparison between two independent groups was performed by an unpaired two-tailed t test for which $P < .05$ was considered statistically significant for all analyses.

Small Interfering RNA

We assayed the efficiency of knocking down SLCO2B1 expression after small interfering RNA transfection for 48 hours by reverse transcriptase polymerase chain reaction (forward: GTTTCGGCGAAAGGTCTTAGCAG; reverse: CCATCCTGCTTCTTCGTGGACT;

Origene). siSLCO2B1-p was a SMARTpool with four target sequences: CAUCCAUGGCUGCGGGCAU; GCCACCAGAUUGCGGGCAU; UCUCGGAGCCAUACCGCUA; and AUAAUGACCUGCUCCGAAA (GE Healthcare). siSLCO2B1-1, -2, and -3 were unique 25mer small interfering RNA duplexes: -1, AGUCGGGAAUUAUAGAUACAGCUTA; -2, CUACUACAAUAAUGACCUGCUCCGA; -3, GGAUAUGCCACAGGACUUCAAGGCT (Origene).

Point Mutation at Site of rs1077858 Using CRISPR

LNCaP cells (AG genotype for rs1077858) and 22RV1 cells (AA genotype for rs1077858) were chosen to reconstitute the rs1077858 GG genotype using CRISPR (Ran FA, et al: Cell 154:1380-1389, 2013; Sanjana NE, et al: Nat Methods 11:783-784, 2014). In brief, five different target oligos (oligo 1-forward, caccgGGCAGGGTCTCGCTCCATTC; oligo 1-reverse, aaacGAATGGAGCGAGACCCTGCCc; oligo 2-forward, caccgGCAGGGTCTCGCTCCATTCA; oligo 2-reverse, aaacTGAATGGAGCGAGACCCTGCc; oligo 3-forward, caccgGAGGTTACGTAGTCCCTGAA; oligo 3-reverse, aaacTTCAGGGACTACGTAACCTCc; oligo 4-forward, caccgTTGTATCTCTCACATTAGAC; oligo 4-reverse, aaacGTCTAATGTGAGAGATACAAc; oligo 5-forward, caccgCGAGACCCTGCCTTGTCTGA; oligo 5-reverse, aaacTCAGACAAGGCAGGGTCTCGc) were synthesized and cloned into the lentiCRISPRv2 one vector system. A single-strand DNA oligo of 101 nucleotides was synthesized as the donor template, which have 50 bp of homology arms on each side flanking the change site for rs1077858 A→G. The donor DNA sequence is AAAGGCCCCTGTCAGATGGGGGAGACAGTCTCTGAGCTGGGGGAAGCCCCTGTCTAATGTGAGAGATACAATCTCTCTGATGGAGGTTAC**G**TAGTCCCTGAATGGAGCGAGACCCTGCCTTGTCTGATGGAAGAGACCTGACCTCTGTGATGGAGAAGGCCCCTGTCTGATAAGGGAGACG. Two microliters of 10 μM donor oligo was used for each well of a 24-well plate to codeliver to the cells with five constructs. After puromycin selection, cells derived from a single colony were assayed for point mutations by using a Taqman SNP assay and for SLCO2B1 mRNA levels by using reverse transcriptase polymerase chain reaction.

We assayed 22 single colonies of LNCaP cells and 109 single colonies of 22RV1 cells. Unfortunately, we did not detect any A→G mutations for LNCaP cells. For 22RV1 cells, 89 single colonies remained the AA genotype, 15 single colonies were AG, and 5 were GG.

Table A1. Patient Characteristics and Outcomes in Initial, Validation, and Combined Cohorts

Characteristic	Initial Cohort (n = 478)		Validation Cohort (n = 616)		Combined Cohort (N = 1,094)	
	No.	%	No.	%	No.	%
At time of diagnosis						
PSA, ng/mL	412	14 (7.0-46.0)*	537	9.9 (5.7-24.8)*	949	11.6 (6.1-31.8)*
Age, years	458	62 (56-67)*	580	61 (56-67)*	1,038	61 (56-67)*
Clinical T stage						
Tx/Unknown	194	40.6	99	16.1	293	26.8
T1	122	25.5	379	61.5	501	45.8
T2	137	28.7	110	17.9	247	22.6
T3 to T4	25	5.2	28	4.5	53	4.8
Clinical N stage						
Nx/Unknown	278	58.2	349	56.6	627	57.3
N0	166	34.7	213	34.6	379	34.6
N1	34	7.1	54	8.8	88	8.0
Clinical M stage						
Mx/Unknown	217	45.4	316	51.3	533	48.7
M0	181	37.9	208	33.8	389	35.6
M1	80	16.7	92	14.9	172	15.7
Biopsy Gleason score						
≤ 6	77	16.1	81	13.1	158	14.4
7	153	32.0	198	32.1	351	32.1
≥ 8	171	35.8	274	44.5	445	40.7
Unknown	77	16.1	63	10.2	140	12.8
Type of local therapy						
RP ± RT	187	39.1	264	42.9	451	41.2
RT only/other	143	29.9	201	32.6	344	31.4
None	148	31.0	151	24.5	299	27.3
Use of ADT as part of local therapy	84	17.6	204	33.1	288	26.3
At time of ADT initiation						
PSA, ng/mL	380	14.9 (5.2-68.4)*	586	10.1 (4.1-36.5)*	966	11.9 (4.5-46.8)*
Presence of metastases	274	57.3	360	58.4	634	58.0
Received antiandrogen during ADT	333	69.7	414	67.2	747	68.3
Received intermittent ADT	90	18.8	221	35.9	311	28.4

Abbreviations: ADT, androgen-deprivation therapy; PSA, prostate-specific antigen; RP, radical prostatectomy; RT, radiation therapy.
*Data are given as median (interquartile rate).

Table A2. Association of *SLCO2B1* Genotype With TTP on ADT in Combined (initial plus validation) Cohort Stratified by Status of Prior Hormone Therapy

Genotype	No Prior Hormone Therapy		With Prior Hormone Therapy		*P* (for interaction)
	No.	Adjusted HR (95% CI)	No.	Adjusted HR (95% CI)	
rs12422149					
AA/AG	144	1 (reference)	53	1 (reference)	.642
GG	659	1.29 (1.04 to 1.60)	235	1.44 (0.97 to 2.13)	
rs1789693					
AA	318	1 (reference)	109	1 (reference)	.517
AT	358	0.95 (0.79 to 1.13)	128	1.17 (0.85 to 1.59)	
TT	123	0.99 (0.78 to 1.26)	46	1.09 (0.72 to 1.67)	
rs1077858					
AA/AG	684	1 (reference)	255	1 (reference)	.104
GG	116	1.32 (1.06 to 1.65)	31	0.87 (0.55 to 1.37)	

Abbreviations: ADT, androgen-deprivation therapy; HR, hazard ratio; TTP, time to progression.

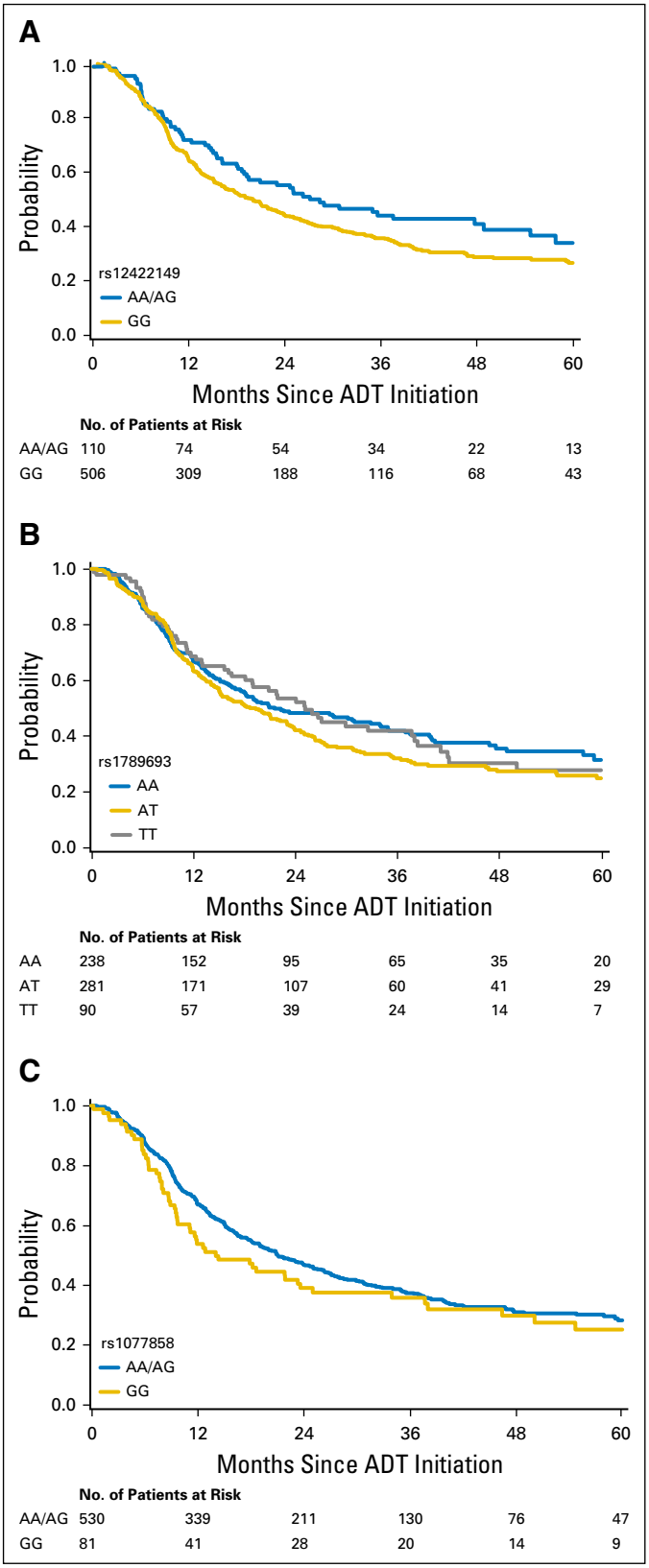

Fig A1. Kaplan-Meier curves of time to progression during treatment with androgen-deprivation therapy (ADT) in the validation cohort of *SLCO2B1* genotypes (A) rs12422149, (B) rs1789693, and (C) rs1077858.

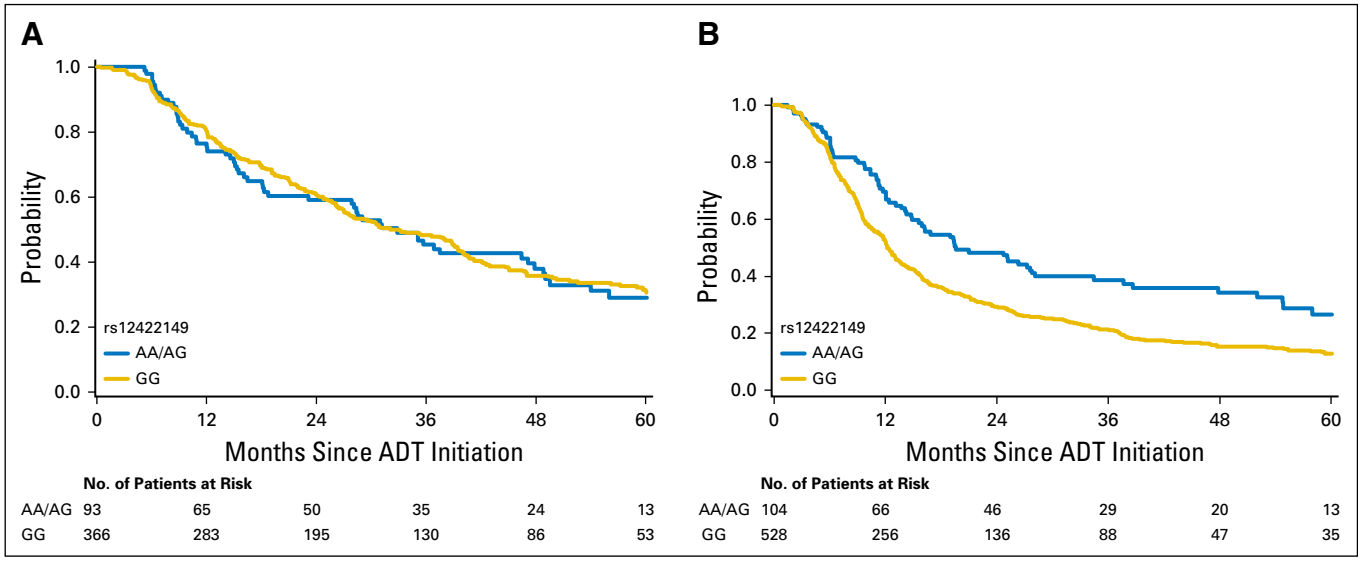

Fig A2. Kaplan-Meier curves of time to progression during treatment with androgen-deprivation therapy (ADT) in all cohorts (initial plus validation cohorts) by rs12422149 genotype as stratified by metastatic status (A) M0 versus (B) M1 at ADT initiation.

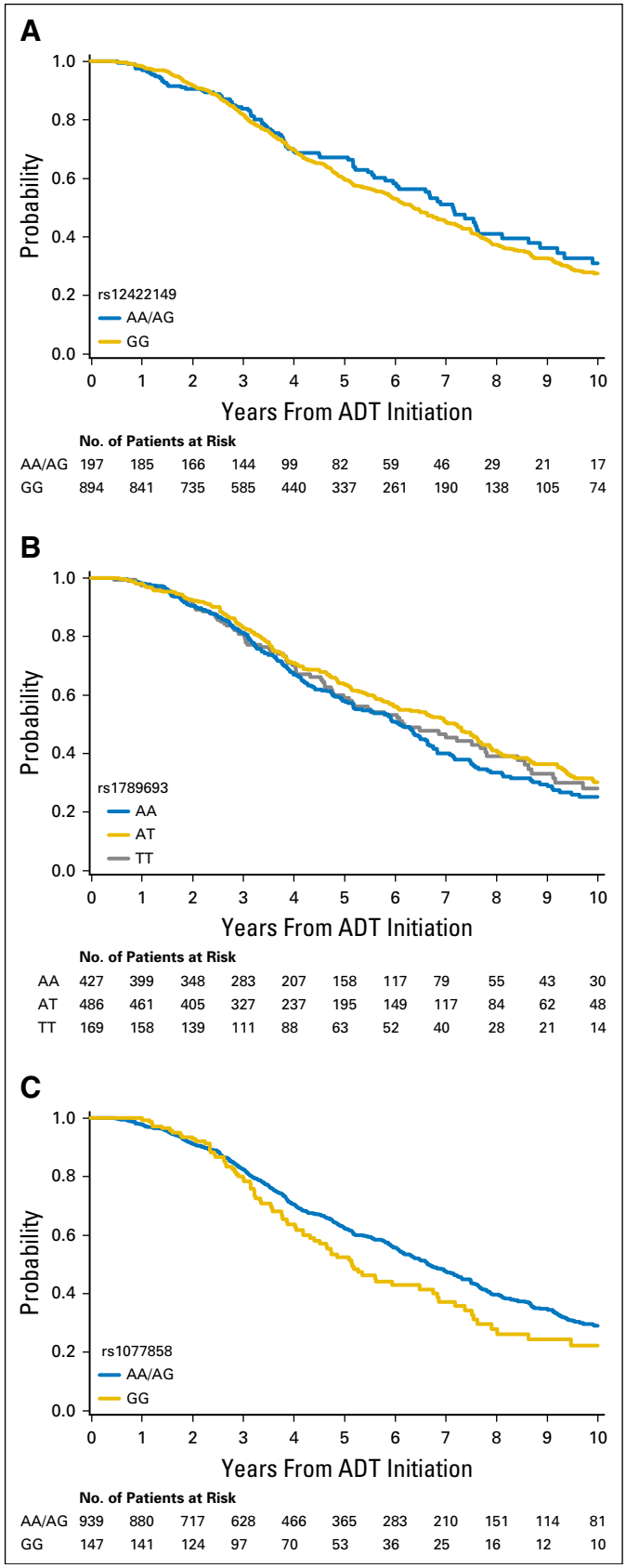

Fig A3. Kaplan-Meier curves of overall survival from androgen-deprivation therapy (ADT) initiation in all patients (initial plus validation cohorts) by *SLCO2B1* genotypes (A) rs12422149, (B) rs1789693, and (C) rs1077858.

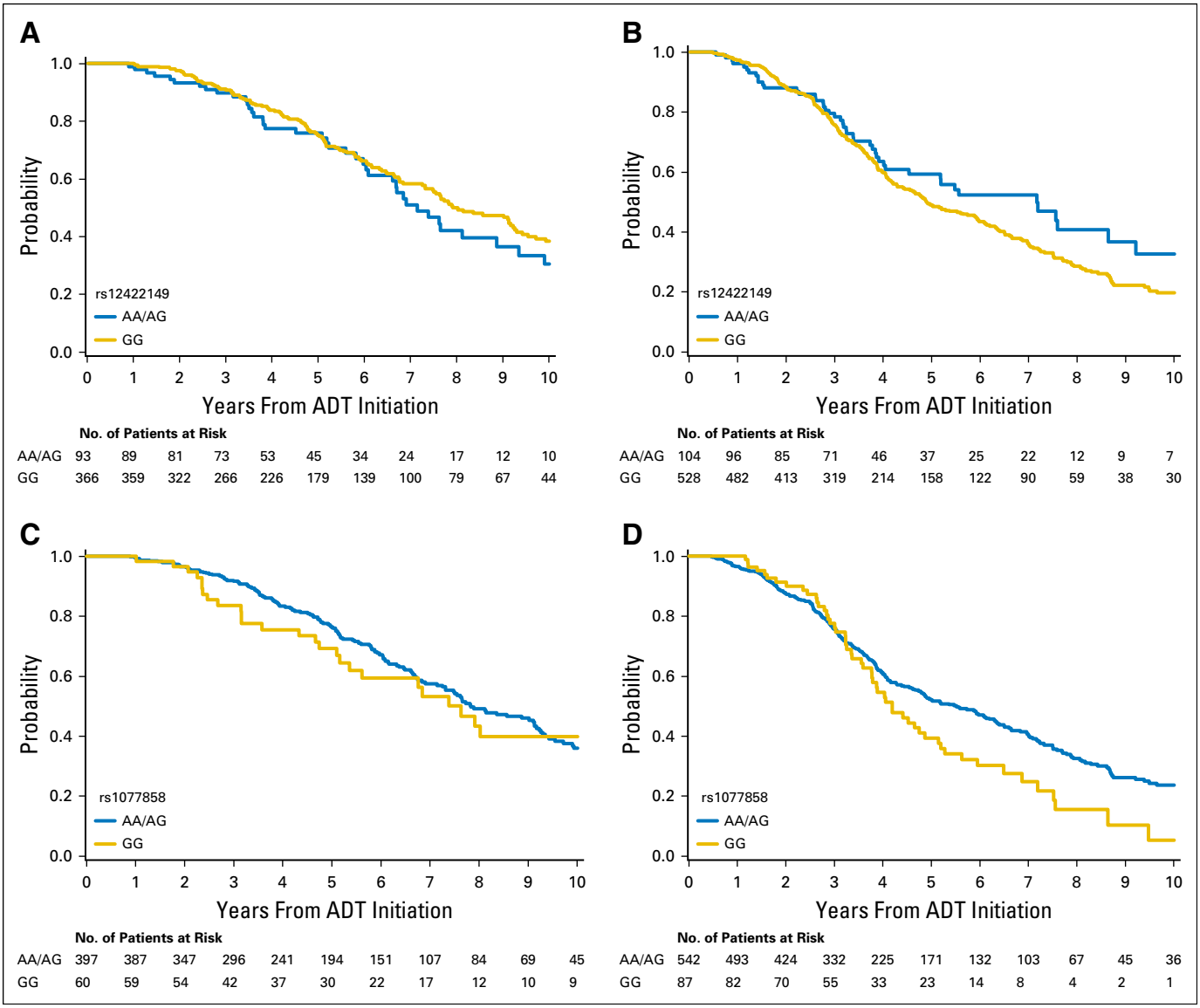

Fig A4. Kaplan-Meier curves of overall survival from androgen-deprivation therapy (ADT) initiation in all cohorts by *SLCO2B1* genotypes at (A, B) rs12422149 and (C, D) rs1077858, stratified by metastatic status at ADT initiation.