Differential Associations of SLCO Transporters with Prostate Cancer Aggressiveness between African Americans and European Americans

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ABSTRACT

Background: Androgen receptor signaling is crucial to prostate cancer aggressiveness. Members of the solute carrier family of the organic anion transporting peptides (SLCO) are potential regulators of androgen availability in prostate tissue. It remains unknown whether genetic variations in SLCOs contribute to the differences in prostate cancer aggressiveness in African Americans (AA) and European Americans (EA).

Methods: SNPs in 11 SLCO members were selected, with addition of 139 potentially functional SNPs and 128 ancestry informative markers. A total of 1,045 SNPs were genotyped and analyzed in 993 AAs and 1,057 EAs from the North Carolina–Louisiana Prostate Cancer Project. Expression and cellular localization of SLCOs were examined using qRT-PCR, IHC, and *in situ* RNA hybridization in independent sets of prostate cancer cases.

Results: Significant associations with prostate cancer characteristics were found for SNPs in *SLCO2A1* and *SLCO5A1*. The associations differed by race ($P_{interaction} < 0.05$). SNPs in *SLCO2A1* were associated with reduced tumor aggressiveness and low Gleason score in AAs; whereas, SNPs in *SLCO5A1* were associated with high clinical stage in EAs. In prostate tissue, SLCO2A1 and SLCO5A1 were the most expressed SLCOs at the mRNA level and were expressed predominantly in prostate endothelial and epithelial cells at the protein level, respectively.

Conclusions: SLCO2A1 and SLCO5A1 play important but different roles in prostate cancer aggressiveness in AAs versus EAs.

Impact: The finding calls for consideration of racial differences in biomarker studies of prostate cancer and for investigations on functions of SLCO2A1 and SLCO5A1 in prostate cancer.

Introduction

Compared with European Americans (EA), African Americans (AA) suffer higher incidence of, and greater mortality from, prostate cancer (1). The racial difference in prostate cancer survival between AAs and EAs could be reduced by improving access to care (2). However, the evidence for racial difference in the biology of prostate cancer is substantial (3). Androgen-stimulated androgen receptor (AR) signaling is crucial to progression and survival of prostate cancer cells throughout all stages of prostate cancer (4–6). Expressions of a cell proliferation marker Ki-67 and AR were found higher in prostate biopsy specimens from AAs than EAs after controlling age and Gleason score (7). Furthermore, percentage of AR-positive cells and AR expression were higher in benign and malignant prostate tissue from AAs when compared with those from EAs (8). These findings suggest that AR activity, and subsequently, AR-driven cell progression may be upregulated in prostate cancer in AAs relative to EAs. Although serum androgen concentrations are similar between adult AAs and EAs, tissue androgen concentrations vary among individuals (9). We reported that the ability to accumulate androgens differed among prostate cancer cell lines, and these differences were mediated by a selective, active transport mechanism (10, 11).

The solute carrier family of organic anion transporting peptides (SLCO) family is comprised of 11 transmembrane transporters that mediate cellular uptake of a wide range of chemicals that include androgens (12–16). Expression of SLCO transporters was altered in malignant tissues compared with benign tissues (14), and was associated with prostate cancer outcomes (12, 13, 17). Existing studies that examined genetic variations of SLCO transporter genes in prostate cancer primarily focused on clinical outcomes and included only one or two SLCO family members (12, 13, 17–20). To our knowledge, there has not been a systematic investigation of genetic variants of SLCO transporters in prostate cancer, nor an investigation of their roles in racial disparity in prostate cancer aggressiveness. Here, we reported a comprehensive examination of SNPs of all 11 SLCO transporter genes in relation to prostate cancer characteristics in AA and EA participants in the North Carolina-Louisiana Prostate Cancer Project (PCaP), a population-based, case-only study of racial/ethnic differences in prostate cancer (21). Expression profiles and cellular localization of SLCO transporters in malignant and benign prostate tissues also were examined in independent sets of prostate cancer cases. We hypothesized that differences in genetic variations or expression of SLCO transporters may regulate the availability of androgens to prostate cancer cells, in that high or more efficient uptake of androgens may lead to development of more aggressive prostate cancer, thus contribute to the differences in prostate cancer aggressiveness between AAs and EAs.

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Materials and Methods

Study population of PCaP

The genotyping study utilized data and samples collected from PCaP participants. Eligible participants were residents of the NC or LA study areas who had a first diagnosis of histologically confirmed adenocarcinoma of the prostate, were 40–79 years of age at diagnosis, and self-identified as AA/Black or as Caucasian/Caucasian-American (EA)/White (21). PCaP enrolled 2,246 men from 2003 to 2009. A subset of participants ($N = 2,115$) with germline DNA samples and relevant clinical and epidemiologic data were included in the study. Informed written consent was obtained from each participant for the release and use of their medical records and biological samples for research. The research was conducted in accordance with recognized ethical guidelines and was approval by Roswell Park Comprehensive Cancer Center (Buffalo, NY) and other relevant Institutional Review Boards. The genotyping data generated in this study can be accessed upon request to PCaP.

PCaP epidemiologic and clinical data

Epidemiologic data were collected by a series of structured questionnaires administered at time of enrollment by a registered nurse during an in-person interview, which occurred averagely within 3–4 months after prostate cancer diagnosis (21). Epidemiologic information included demographics, race/ethnicity, personal/family history of cancer, and prostate cancer screening history. Because the study focused on racial disparity in prostate cancer aggressiveness, only the data from self-reported race were considered in the analysis along with ancestry data from genotyping.

Clinical data were extracted from medical records and tumor information was derived independently based on a standardized protocol. Clinical data included tumor stage, Gleason grade, history of prostate cancer screening examinations, and laboratory assays at or near diagnosis. Combinations of the Gleason score, clinical stage, and PSA at diagnosis were used to define prostate cancer aggressiveness in three categories: highly aggressive (Gleason score ≥ 8, or PSA > 20 ng/mL, or Gleason score = 7 and clinical stage T3-T4); nonaggressive (Gleason score < 7 and stage T1-T2 and PSA < 10 ng/mL); and intermediate aggressive (all other cases). The definition was developed by PCaP based on the literature reviews of risk factors and characteristics that are strongly associated with survival and aligned with other risk classification systems (21, 22).

SNP selection, genotyping, and quality control

Tag SNPs were selected for all 11 members of the SLCO family based on HapMap genotype and linkage disequilibrium (LD) data from the YRI (Yoruba in Ibadan, Nigeria) and CEU (Utah residents with Northern and Western European ancestry) populations to most closely resemble AA and EA populations, respectively, with LD threshold (r^2) ≥ 0.8 and minor allele frequency (MAF) ≥ 5%. The panel of SNPs included 139 potentially functional SNPs identified using HuGE Literature Finder on significant associations and/or functional impacts in human disease (23) and 128 ancestry informative markers (AIM; ref. 24) for population structure analysis. A customized 1152-plex chip was assembled using the Illumina GoldenGate platform. Genotyping was conducted using the Illumina Bead Station System in the Genomics Shared Resource at Roswell Park Comprehensive Cancer Center (Buffalo, NY). A total of 5% random duplicates and in-house trio samples were included in each run for quality control. The final analysis was performed on a total of 1,045 SNPs in 2,050 PCaP participants (993 AAs and 1,057 EAs), after removing DNA samples ($n = 65$) and SNPs ($n = 107$) with call rate <95%. None of the SNPs included in the final analysis violated the Hardy–Weinberg equilibrium at $P < 0.05$. The 2,050 DNA samples were: 1,916 (93.5%) from blood, 102 (5%) from mouth wash, and 32 (1.5%) from immortalized lymphocytes derived from participants. No significant difference in distribution of DNA sources were found between AAs and EAs. Sensitivity analysis on participants with DNA samples from blood only ($N = 1,916$, 912 AAs and 1,004 EAs) showed similar results, hence the results based on all 2,050 DNA samples were presented.

qRT-PCR analysis

Total RNA from 50 pairs of matched malignant and benign prostate tissues were requested from Roswell Park Comprehensive Cancer Center Pathology Network Shared Resource (PNSR) and subjected to qRT-PCR examination (25). The patients were self-reported non-Hispanic Whites (94%) primarily, 46% cases had primary Gleason grade ≥ 4, and 67% cases had tumor stage 3 and above. cDNA was generated using the SuperScript VILO cDNA Synthesis Kit (Thermo Fisher Scientific). Premade FAM-MGB real-time PCR probes for 11 SLCO transporters and *GUS* as a housekeeping gene for reference were obtained from Thermo Fisher Scientific. Real-time PCR reactions were set up using the Taqman Universal PCR Master Mix (Thermo Fisher Scientific) and run on an Applied Biosystems 7900HT Fast Real-Time PCR System.

In situ RNA hybridization

The RNAScope 2.5 HD Brown Assay kit and probes for human *SLCO2A1* and *SLCO5A1* were used, which is developed by Advanced Cell Diagnosis, Inc. by using specific probes to detect mRNA of genes of interest with high specificity. Images of stained sections were acquired using an Aperio ScanScope XT (Leica Biosystems), and processed using Aperio eSlide Manager (Leica Biosystems). *In situ* RNA hybridization was tested using a working tissue microarray (TMA) consisting of 10 prostate cancer cases randomly selected by the PNSR with race information unavailable. Serial sections of formalin-fixed paraffin-embedded (FFPE)-procured prostate cancer tissue specimen from two additional independent cases were requested from the PNSR to confirm *in situ* RNA hybridization results. Comparison of *in situ* expression by race was conducted using an IHC staining method as described in the next section.

IHC staining and quantification

A TMA set was constructed at PNSR using malignant and distant benign prostate tissue specimens from 92 AAs and 92 EAs. The TMA contained three cores of each type of tissue from each patient. Sections were requested for IHC staining for SLCO2A1 using a custom antibody developed (0.0035 mg/mL) by Pacific Immunology, SLCO5A1 using antibody LS-C179257 (0.0017 mg/mL) from Lifespan Biosciences, and CD31 (an endothelial cell–specific marker) using antibody Dako M0823 (1:50) from Agilent. Relevant nonimmune rabbit or mouse IgG were used as negative control. IHC staining was performed using a published method (25). Digital images of the stained sections were acquired using an Aperio ScanScope XT and captured using the Aperio Digital Image Analysis Software (Leica Biosystems Inc). Prostate epithelial cells in malignant and benign cores were circled manually under the supervision of a pathologist (G. Azabdaftari) and scored using algorithms optimized for SLCO2A1 and SLCO5A1 at PNSR.

Statistical analysis

Demographic and tumor characteristics were compared between AAs and EAs using χ^2 tests for categorical variables and Student t

tests for continuous variables if normally distributed, otherwise the Kruskal–Wallis tests were used. Genotype and allele frequency were compared between groups using χ^2 tests and adjusted for multiple comparisons using Bonferroni correction. A total of 122 AIMs passed quality control and were analyzed using Structure v2.3.4 to ascertain genetic ancestry (26). A two-population model was assumed since there was a minimal Asian ancestry component found in the study population. Proportions of African and European ancestry were estimated for each PCaP participant and African ancestry proportion was included as a continuous covariate in statistical models.

For genetic analysis, primary outcomes were prostate cancer aggressiveness (low/intermediate vs. high), Gleason score (summary of primary and secondary Gleason grades, ≥8 vs. <8), primary Gleason grade (≥4 vs. <4), and clinical stage (T1/T2 vs. T3/T4). Logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CI) separately in AAs and EAs with adjustment of age at diagnosis (continuous), study site (NC or LA), first-degree family history of prostate cancer (yes or no), and African ancestry proportion (continuous). A genotypic (codominant) model was assumed, after which a dominant model was included with the consideration of small number of homozygotes for most of SNPs in the analysis. The common genotype in the EA group was used as the reference category for analyzing both AAs and EAs to facilitate comparisons between the groups. A log-additive genetic model was used to test genetic dose response by coding genotypes as 0, 1, 2 according to the number of variant alleles with P_{trend} presented. False discovery rate (FDR) was applied for adjusting multiple comparisons and only SNPs with adjusted P values (P_{adj}) < 0.05 in either AAs or EAs were presented. Interaction between genotype and race was tested for significant SNPs in the models by including a relevant multiplicative term and $P_{interaction}$ was generated using the Wald test of the product term for the differences in associations between AAs and EAs. The joint effects of significant SNPs were modeled using the number of risk alleles or genotypes as ordinal variables and were tested separately in AAs and EAs.

For qRT-PCR data, relative expression level of each SLCO transporter was presented as a ratio to the housekeeping gene of *GUS* as well as a percentage of the transporter relative to the total expression of all SLCO transporters. The Wilcoxon signed-rank test was used for the comparisons between paired malignant and benign prostate tissues.

For IHC staining, H-scores were derived from the staining intensity (0, 1, 2, 3) multiplied by the percentage of positive cells for each intensity category. An average of H-scores was calculated from the three cores per patient for malignant tissue and for benign tissue, respectively. The Kruskal–Wallis test by rank was used to compare H-scores by cancer characteristics and between AAs and EAs. All analyses were conducted using the 64-bit build of R 3.6.2 and/or SAS 9.4.

Results

Characteristics of the study population of PCaP

Table 1 summarized the descriptive characteristics of the study population of PCaP by self-reported race information. Compared with EAs, AAs were diagnosed at younger ages (61.9 vs. 64.2, $P < 0.001$) and

Table 1. Demographic and tumor characteristics of PCaP research participants by self-reported race.

	African Americans ($N = 993$)		European Americans ($N = 1,057$)		P^a
	Mean	**SD**	**Mean**	**SD**	
Age at diagnosis, years	62	8	64	8	<0.001
European ancestry	0.098	0.160	0.975	0.086	<0.0001
African ancestry	0.902	0.160	0.025	0.086	<0.0001
	N	**%**	***N***	**%**	
Study site					0.416
North Carolina	436	43.9%	483	45.7%	
Louisiana	557	56.1%	574	54.3%	
Family history of prostate cancer					0.299
No	656	66.0%	732	69.2%	
Yes	253	25.5%	246	23.3%	
Unknown	84	8.5%	79	7.5%	
Pretreatment PSA level, ng/mL					<0.0001
<10	742	74.7%	890	84.2%	
10–20	140	14.1%	112	10.6%	
>20	111	11.2%	55	5.2%	
Primary Gleason grade					0.082
<4	770	80.0%	858	83.0%	
≥4	193	20.0%	176	17.0%	
Summary Gleason score					0.147
<8	861	86.9%	936	89.0%	
≥8	130	13.1%	116	11.0%	
Clinical stage					0.825
T1/T2	946	98.1%	1019	98.3%	
T3/T4	18	1.9%	18	1.7%	
Prostate cancer aggressiveness					0.0007
Low/intermediate	750	79.0%	872	84.9%	
High	199	21.0%	155	15.1%	

[a]χ^2 tests were used for categorical variables, and Student t tests were used for continuous variables if normally distributed; otherwise, Kolmogorov–Smirnov tests were used.

had a higher percentage of cancer with aggressive phenotypes as defined by Gleason score ≥ 8, or PSA > 20 ng/mL, or Gleason score = 7 and clinical stage T3-T4 (21% vs. 15%, $P < 0.001$). There were no differences in primary Gleason grade, Gleason score, clinical stage. AAs and EAs had similar rates of first-degree family history of prostate cancer with the majority (∼75%) having no or unknown family history. The self-reported race status was consistent with the distribution of ancestry proportions determined by analysis of AIMs, showing predominant African or European ancestry. Similar results were obtained when association analysis was conducted using self-reported race status versus ancestry-based classification (e.g., ≥80% of African or European ancestry), indicating self-reported race status in PCaP could be a reasonable biological construct of race in the analysis of this population.

Association between genetic variants and prostate cancer characteristics

Association analysis of the SNPs was performed separately in AAs and EAs with four cancer characteristics: prostate cancer aggressiveness, primary Gleason grade, Gleason score, and clinical stage (Supplementary Tables S1 and S2). The results for primary Gleason grade were not included because of no significant SNPs in either AAs or EAs. Across the other three cancer characteristics, all significant SNPs belonged to two genes, *SLCO2A1* and *SLCO5A1* (**Table 2**). The SNP rs9917636, rs9874493, and rs3811662 in *SLCO2A1* were associated with reduced tumor aggressiveness, reduced Gleason score, or both, respectively; and all associations were observed only in AAs, of which associations of rs9917636 and rs3811662 differed significantly by race for tumor aggressiveness ($P_{interaction} < 0.05$). In contrast, four SNPs in *SLCO5A1* (rs16919172, rs4370538, rs4377973, rs10096246) were significantly associated with high clinical stage, but only in EAs. Of the four SNPs in *SLCO5A1*, the association between rs16919172 and clinical stage was significantly different between AAs and EAs ($P_{interaction} < 0.05$). The four SNPs in *SLCO5A1* (rs16919172, rs4370538, rs4377973, rs10096246) were in high LD in EAs but not AAs based on the 1000 Genomes Project (27), explaining the similar associations across the SNPs in EAs.

Comparison of risk alleles/genotypes between AAs and EAs

Three significant SNPs in *SLCO2A1* in AAs had the common homozygote as the risk genotypes: GG of rs3811662, AA of rs9874493, and AA of rs9917636; whereas, four significant SNPs in *SLCO5A1* in EAs had the minor allele as the risk allele: G allele of rs10096246, rs16919172, rs4370538, and rs4377973. A summary of risk alleles or risk genotypes for identified SNPs and their frequencies in AAs and EAs was presented in Supplementary Table S3. The distributions of all SNPs except for rs9917636 in *SLCO2A1* were significantly different between AAs and EAs. The frequencies of risk alleles/genotypes ranged from 0.23 to 0.72 (average 0.52) in AAs and was 0.06 in EAs. An overall higher frequency of risk-alleles/genotypes was observed in AAs.

Joint effect of risk alleles/genotypes on prostate cancer characteristics

Three significant SNPs for *SLCO2A1* in AAs were modeled by combining the number of risk genotypes (0–3) and using the group without any risk genotype as the reference (Supplementary Table S4). A dose-dependent relationship with tumor aggressiveness was observed in AAs; the OR increased to 1.61 (95% CI, 0.90–2.89) with one, 1.96 (95% CI, 1.16–3.30) with two, and 3.49 (95% CI, 1.87–6.53) with three risk-genotypes in comparison with AAs without any risk genotype. Although a similar trend was found for Gleason score and tumor stage, the number of events was too small to assess dose-dependent relationships. In EAs, no significant associations were observed for combined SNPs in *SLCO2A1*with any cancer characteristics, consistent with the results of single SNP analyses (**Table 2**).

The four significant SNPs for *SLCO5A1* in EAs had minor alleles as risk alleles with frequencies about 0.06 and these risk alleles were in high LD, so numbers were not sufficient to test for dose-dependent effects in EAs (Supplementary Table S4). Overall, EAs with at least one risk allele had increased odds for worse cancer characteristics in comparison with EAs without a risk allele in any of the four SNPs; however, only the association with tumor stage reached statistical significance (OR, 6.59; 95% CI, 2.51–17.28). None of these risk alleles in *SLCO5A1* was associated individually with cancer characteristic outcomes in single SNP analyses for AAs (**Table 2**), but a significant association with tumor aggressiveness was appeared among AAs with the presence of three or more of the risk alleles (OR, 1.61; 95% CI, 1.05–2.49) in comparison with AAs without any risk alleles.

A dose-dependent relationship was found in AAs when risk alleles/genotypes in both *SLCO2A1* and *SLCO5A1* were combined for association analyses with prostate cancer aggressiveness (**Fig. 1**; Supplementary Table S4). The odds for the aggressive phenotype of prostate cancer was increased 14% per risk allele or genotype (OR, 1.14; 95% CI, 1.05–1.24). The association was observed in AAs only. For EAs, the significant associations were driven primarily by SNPs in *SLCO5A1*, no joint effect was observed between *SLCO2A1* and *SLCO5A1*.

Expression profile of SLCO transporters at the mRNA level in matched malignant and benign prostate tissues

Expression of all 11 SLCO transporters at the mRNA level was compared between the matched malignant and benign prostate tissue specimen from 50 patients (**Table 3**). *SLCO2A1* and *SLCO5A1* accounted for over 80% of all SLCO transporters expressed in prostate tissue regardless of malignant or benign status. Most other transporters were detected only with cycle threshold values between 35 and 40, and therefore were expressed at very low absolute levels. The overall expression of SLCO transporters at the mRNA level was lower in malignant than benign tissues (1.0 vs. 1.6, $P < 0.001$). *SLCO2A1* and *SLCO5A1* followed the same pattern. However, when the percentages of SLCO2A1 and SLCO5A1 mRNA relative to total SLCO mRNA were compared between malignant and benign tissue, the percentage of SLCO5A1 mRNA was higher in malignant tissue (58.7%) than in benign tissue (50.4%); whereas, the percentage of SLCO2A1 mRNA was lower in malignant tissue (27.1%) than in benign tissue (37.6%). No significant differences were found at the mRNA level of all SLCO transporters by tumor stage or Gleason score. To note, the data were primarily pertinent to EAs as 47 of 50 cases were self-reported non-Hispanic Whites (94%), although the data from the three AA cases showed similar patterns with the predominant expression of *SLCO2A1* and *SLCO5A1* in the prostate and no significant differences in the mRNA levels were observed between AAs and EAs.

In situ expression of SLCO2A1 and SLCO5A1 in prostate cancer

In situ expression of SLCO2A1 and SLCO5A1 at the protein level was examined in serial sections of FFPE prostate cancer tissue specimens using IHC (**Fig. 2A**). SLCO5A1 was expressed predominantly in prostate epithelial cells and SLCO2A1 was expressed in both prostate epithelial and endothelial cells, but at a higher level in endothelial cells. Adjacent serial sections of FFPE prostate cancer tissue specimens were used for *in situ* RNA hybridization for mRNA of SLCO2A1 (RNAScope) and IHC for the endothelial cell marker CD31, respectively, to confirm

Table 2. Differential associations of SNPs in SLCO transporters with prostate cancer characteristics between PCaP AAs and EAs.

			European Americans				African Americans				
Gene	SNP	Genotype	High vs. low[a]	OR (95% CI)[b]	P_{trend}[c]	P_{adj}[d]	High vs. low[a]	OR (95% CI)[b]	P_{trend}[c]	P_{adj}[d]	$P_{interaction}$[e]
Aggressiveness high vs. low/intermediate											
SLCO2A1	rs9917636	AA	33/231	1.00	0.896		62/150	1.00	**<0.001**	**0.03**	**0.004**
		AG	88/417	1.52 (0.98–2.36)			93/367	0.59 (0.41–0.86)			
		GG	34/222	1.04 (0.62–1.74)			44/233	0.45 (0.29–0.71)			
		AG/GG vs. AA	122/639	1.35 (0.89–2.05)			137/600	0.54 (0.38–0.77)			
SLCO2A1	rs3811662	GG	150/861	1.00	0.021		160/519	1.00	**0.001**	**0.039**	**0.004**
		GA	5/10	3.77 (1.22–11.64)			38/210	0.58 (0.39–0.86)			
		AA	0/0	N/A			1/20	0.16 (0.02–1.2)			
		GA/AA vs. GG	5/10	3.77 (1.22–11.64)			39/230	0.54 (0.37–0.8)			
Gleason score ≥8 vs. <8											
SLCO2A1	rs3811662	GG	114/922	1.00	0.574		108/603	1.00	**0.001**	**0.049**	0.218
		GA	2/13	1.55 (0.33–7.23)			22/235	0.52 (0.32–0.84)			
		AA	0/0	N/A			0/22	N/A			
		GA/AA vs. GG	2/13	1.55 (0.33–7.23)			22/257	0.47 (0.29–0.77)			
SLCO2A1	rs9874493	AA	84/671	1.00	0.967		94/518	1.00	**0.001**	**0.049**	0.352
		AG	30/243	1.04 (0.66–1.62)			36/294	0.66 (0.44–1)			
		GG	2/22	0.88 (0.2–3.85)			0/48	N/A			
		AG/GG vs. AA	32/265	1.02 (0.66–1.59)			36/342	0.56 (0.37–0.85)			
Stage 3/4 vs. 1/2											
SLCO5A1	rs16919172	AA	11/914	1.00	**<0.001**	**0.039**	15/729	1.00	0.604		**0.028**
		AG	7/101	6.24 (2.32–16.8)			3/199	0.78 (0.22–2.75)			
		GG	0/3	N/A			0/12	N/A			
		AG/GG vs. AA	7/104	5.98 (2.23–16.1)			3/211	0.74 (0.21–2.64)			
SLCO5A1	rs4370538	AA	10/901	1.00	**<0.001**	**0.035**	6/468	1.00	0.101		0.25
		AG	8/113	6.95 (2.64–18.3)			10/414	2.22 (0.77–6.36)			
		GG	0/3	N/A			2/64	2.85 (0.54–14.9)			
		AG/GG vs. AA	8/116	6.67 (2.54–17.5)			12/478	2.31 (0.83–6.39)			
SLCO5A1	rs4377973	GG	11/908	1.00	**0.001**	**0.039**	6/447	1.00	0.331		0.383
		GC	7/108	5.93 (2.21–15.9)			11/422	2.28 (0.81–6.44)			
		CC	0/3	N/A			1/77	1.08 (0.13–9.32)			
		GC/CC vs. GG	7/111	5.68 (2.12–15.3)			12/499	2.09 (0.75–5.77)			
SLCO5A1	rs10096246	AA	11/911	1.00	**0.001**	**0.039**	10/572	1.00	0.526		0.078
		AG	7/105	6.04 (2.24–16.3)			7/336	1.29 (0.48–3.47)			
		GG	0/3	N/A			1/37	1.69 (0.20–14.0)			
		AG/GG vs. AA	7/108	5.79 (2.15–15.6)			8/373	1.33 (0.51–3.45)			

Note: Significant P values ($P < 0.05$) are indicated in bold.

[a]High versus low indicates the number of events in each cancer characteristic group, for example, number of cases with high aggressiveness versus number of cases with low/intermediate aggressiveness, number of cases with Gleason score ≥8 versus number of cases with Gleason score <8, and number of cases with stage 3/4 versus number of cases with stage 1/2, respectively.

[b]ORs and 95% CIs were estimated from codominant and dominant models adjusting for age at diagnosis (continuous), state (NC or LA), first-degree family history of prostate cancer (yes/no), and African ancestry proportion (continuous).

[c]P_{trend} was estimated for genetic dose response by coding genotypes as 0, 1, 2 according to the number of variant alleles.

[d]P_{adj} was generated from P_{trend} after correction for multiple comparisons by FDR. Only SNPs with P_{adj} less than 0.05 in either AAs or EAs were presented.

[e]$P_{interaction}$ was calculated using Wald test of a multiplicative term for the differences in associations between AAs and EAs.

the expression of SLCO2A1 in endothelial cells (**Fig. 2B**). Cells with more intensive RNAScope signals coincided with the CD31-positive cells, which indicated that *SLCO2A1* was expressed at higher levels in endothelial cells than in epithelial cells. Selective expression of *SLCO5A1* in epithelial cells was also confirmed using RNAScope (Supplementary Fig. S1). Overall, the expression localization pattern was similar between AAs and EAs, showing that SLCO2A1 was expressed predominantly in endothelial cells and at lower levels in epithelial cells, whereas, SLCO5A1 was expressed primarily in epithelial cells.

Expression of SLCO2A1 and SLCO5A1 at the protein level in prostate cancer by race

Expression of SLCO2A1 and SLCO5A1 in malignant and benign prostate epithelial cells was compared between 92 AAs and 92 EAs

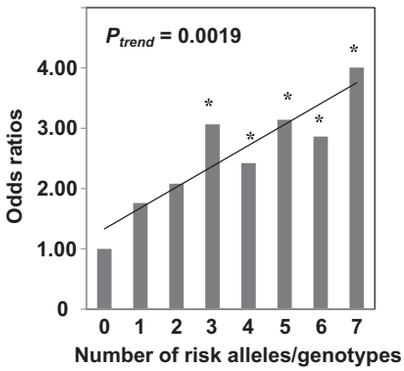

Figure 1.

Increase of odds of aggressive phenotype of prostate cancer with the number of risk allele/genotype in *SLCO2A1* and *SLCO5A1* in AAs. ORs were calculated using logistic regression with adjustment for age at diagnosis (continuous), study site (NC or LA), first-degree family history of prostate cancer (yes or no), and African ancestry proportion (continuous).

(**Fig. 3**). Each data point in the graph represents an average staining score from three cores per patient. Expression levels of SLCO5A1were significantly higher in EAs than AAs regardless of types of tissue, but no differences were found between benign and malignant cells for either AAs or EAs. which indicated that the difference in expression level between AAs and EAs might not be cancer specific. Higher expression of SLCO5A1 was found among EAs in patients with more aggressive phenotypes (**Table 4**) including primary Gleason grade (H-score 210 in ≥ 4 vs. 182 in <4, $P = 0.037$), Gleason score (216 in ≥8 vs. 183 in <8, $P = 0.047$), T stage (181 in T2, 199 in T3, 205 in T4, $P = 0.069$), N stage (243 in N1 vs. 193 in N0, $P = 0.017$), in patients with failure after biochemical treatment (usually androgen deprivation therapy (ADT); 208 with failure vs. 182 without failure, $P = 0.021$), and in patients with radical prostatectomy failure (203 with failure vs. 182 without failure, $P = 0.03$). In contrast, no significant findings for SLCO5A1 were observed in AAs. SLCO2A1 expression was similar between AAs and EAs, but slightly higher in benign than malignant tissue in AAs (**Fig. 3**). However, no significant differences were found across the panel of prostate cancer characteristic phenotypes (**Table 4**). Endothelial cells were not sufficient to evaluate cell type–specific expression of SLCO2A1 due to the limited presence of endothelial cells in the TMA cores. Using Cox proportional hazards regression models for time to biochemical failure and time to radical prostatectomy failure with adjustment of age at surgery, tumor stage, summary Gleason score, presurgical PSA level, similar results were observed, showing that per SD change in staining score of SLCO5A1 expression was associated with biochemical failure (HR, 1.71; 95% CI, 1.10–2.67) and radical prostatectomy failure (HR, 1.69; 95% CI, 1.10–2.61) in EAs, but not in AAs either with biochemical failure (HR, 1.12; 95% CI, 0.77–1.64) or radical prostatectomy failure (HR, 0.98; 95% CI, 0.70–1.39). No significant associations were found for SLCO2A1 in EAs or AAs. The results were aligned with the results of comparisons of SLCO2A1 and SLCO5A1 expression by tumor characteristics in AAs and EAs (**Table 4**). However, due to the small sample size (92 EAs and 92 AAs) and unavailable treatment data (radiation or chemotherapy), further validation is warranted.

Discussion

The study examined the relationships of the genotype and expression of SLCO transporters with phenotypes of prostate cancer aggressiveness in AAs and EAs. At the genomic level, significant associations between SNPs and phenotypes of prostate cancer aggressiveness were observed only for *SLCO2A1* and *SLCO5A1* of all 11 SLCO transporters, and the associations significantly differed between AAs and EAs. SNPs in *SLCO2A1* were associated with reduced prostate cancer aggressiveness and low Gleason score in AAs; whereas, SNPs in *SLCO5A1* were associated with advanced clinical stages in EAs. At the mRNA level, *SLCO2A1* and *SLCO5A1* were the predominantly expressed SLCO transporters in the prostate and the expression levels of the transporters differed significantly between the paired malignant and benign prostate tissues. At the tissue level, SLCO2A1 was expressed predominantly in endothelial cells, and at lower levels, in epithelial cells; whereas, SLCO5A1 was expressed primarily in epithelial cells. At the individual level, expression of SLCO5A1 was significantly higher in EAs than AAs in both malignant and benign prostate epithelial cells, and higher levels of expression were associated with more aggressive phenotypes of prostate cancer only in EAs. Expression of SLCO2A1 was similar between EAs and AAs, and among the panel of tumor characteristics examined in the study. Because SLCO2A1 is expressed

Table 3. SLCO expression in 50 pairs of matched malignant and benign prostate tissues.

	Malignant		Benign		
Gene	**Expression median (range)**	**Percentage (mean)**	**Expression median (range)**	**Percentage (mean)**	***P*[a]**
SLCO1A2	0.01 (0–0.5)	2.0	0.003 (0–0.1)	0.4	0.04
SLCO1B1	0 (0–0.02)	0.0	<0.001	0.0	0.16
SLCO1B3	0.004 (0–1.0)	1.8	0.001 (0–0.1)	0.3	0.001
SLCO1C1	<0.001	0.0	<0.001	0.0	0.07
SLCO2A1	**0.2 (0.02–5.3)**	**27.1**	**0.6 (0.04–6.6)**	**37.6**	**0.004**
SLCO2B1	0.004 (0–0.5)	1.9	0.004 (0–0.5)	1.5	0.33
SLCO3A1	0.03 (0–2.4)	5.0	0.08 (0–1.0)	5.9	0.003
SLCO4A1	0.01 (0–5.3)	3.1	0.03 (0–0.6)	3.5	0.003
SLCO4C1	0.002 (0–0.8)	0.4	0.002 (0–0.1)	0.4	0.13
SLCO5A1	**0.6 (0.1–7.6)**	**58.7**	**0.7 (0.04–6.7)**	**50.4**	**0.006**
SLCO6A1	<0.001	0.0	<0.001	0.0	0.79
Total	**1.0 (0.2–12.8)**		**1.6 (0.3–11.0)**		**<0.001**

[a]*P* values were calculated using Wilcoxon signed–rank test for paired samples. Two dominant SLCO transporters are denoted in bold.

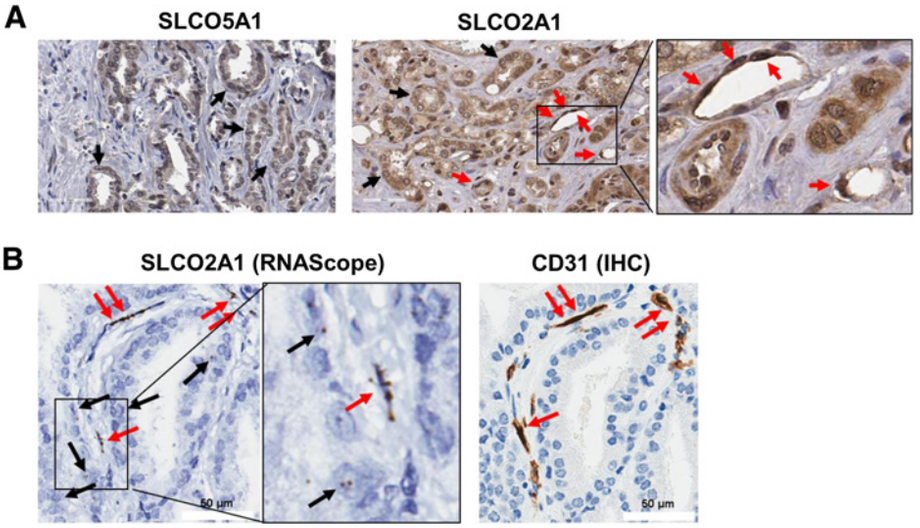

Figure 2.
Differential expression of SLCO5A1 and SLCO2A1 in prostate endothelial cells and prostate epithelial cells. *In situ* expression of SLCO2A1 and SLCO5A1 was examined at the protein level using IHC (**A**) and at the RNA level using RNAScope along with the endothelial cell marker CD31 using IHC (**B**). Black and red arrows point to epithelial and endothelial cells, respectively.

more preferentially in endothelial cells compared to epithelial cells, further investigation of the cell type–specific expression of SLCO2A1 in prostate cancer could help explain the observed differential associations between SNPs in *SLCO2A1* and prostate cancer aggressiveness in AAs and EAs.

SLCO2A1 and SLCO5A1 were among the least studied SLCO family members in prostate cancer. Studies of SLCO transporters in prostate cancer have been focused primarily on SLCO1B3 and SLCO2B1, and to a lesser extent on SLCO1A2, SLCO1B1, and SLCO4A1. These SLCO transporters have been reported as uptake transporters for androgens including testosterone (12, 13) and dehydroepiandrosterone (28), and therapeutic drugs for treatment of prostate cancer (14, 29–37). SLCO transporters are known to have largely overlapping substrate profiles (14, 15), but the substrate specificity and the uptake efficiency for SLCO2A1 and SLCO5A1, particularly on androgen uptake, require further characterization. Interestingly, SNPs in *SLCO1B3* or *SLCO2B1* were reported to be associated with worse response to ADT (13, 20, 29) and shorter prostate cancer–specific (17) and overall survival (12), but none of the SNPs in *SLCO1B3* or *SLCO1B1* was associated with cancer aggressiveness in either AAs or EAs in our study. A possible reason for association of genetic variations in *SLCO1B3* and *SLCO2B1* with prostate cancer progression but not with cancer aggressiveness might be the very low expression of these transporters in the prostate, but their expressions increase as a result of prostate cancer development, as shown in our data between matched benign to malignant tissue and shown in a study by Wright and colleagues between treatment-naïve and metastatic castration-resistant prostate cancer tissue (17). Together, these findings suggest an important, but not yet defined, role of individual SLCO transporters in development and progression of prostate cancer.

One of the intriguing findings of our study is that in comparison with AAs, EAs had significantly higher expressions of SLCO5A1 at the protein level in both malignant and benign prostate epithelial cells but had a lower percentage of more aggressive cancers. The hypothesized function of SLCO transporters is to regulate the availability of androgens to prostate cancer cells, in that higher expression levels could result in more efficient uptake of androgens, and in turn lead to more aggressive prostate cancer. This discrepancy might be explained partly

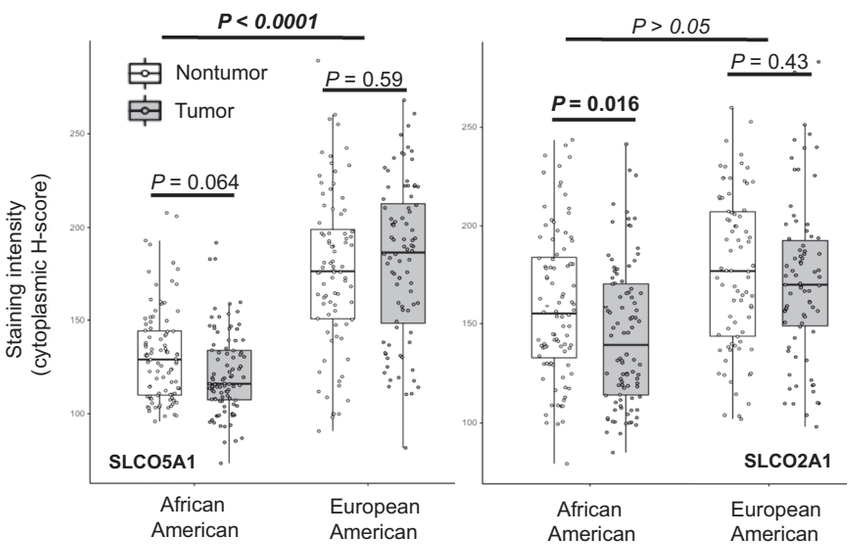

Figure 3.
Comparison of SLCO2A1 and SLCO5A1 expression in malignant and benign prostate tissues between AAs and EAs. Staining intensities, indicated by H-scores, were compared using the Kruskal–Wallis test by rank.

Table 4. Expression of SLCO5A1 and SLCO2A1 in AAs and EAs with prostate cancer.

	SLCO5A1						SLCO2A1					
	AAs			EAs			AAs			EAs		
	N	H-Scores	*P*[a]	*N*	H-Scores	*P*[a]	*N*	H-Scores	*P*[a]	*N*	H-Scores	*P*[a]
Primary Gleason grade												
Low (<4)	71	116 (74–183)	0.748	69	182 (82–261)	**0.037**	69	145 (85–241)	0.248	67	171 (98–283)	0.648
High (≥4)	24	127 (85–192)		16	210 (119–268)		23	122 (96–226)		16	166 (104–240)	
Gleason score												
Low (<8)	82	116 (74–183)	0.439	78	183 (82–261)	**0.0466**	80	145 (85–241)	0.582	76	170 (98–283)	0.941
High (≥8)	13	124 (96–192)		7	216 (130–268)		12	131 (99–226)		7	169 (143–230)	
Presurgical PSA												
Low (< median)	47	115 (74–160)	0.374	41	182 (111–224)	0.1264	44	125 (85–241)	0.206	42	170 (102–247)	0.814
High (≥ median)	47	118 (85–192)		45	189 (82–268)		47	148 (95–228)		42	171 (98–283)	
Age at operation[b]												
<50	16	118 (93–183)	0.486	4	172 (166–194)	0.123	15	134 (106–228)	0.617	4	160 (138–183)	0.349
50–59	47	115 (73–118)		41	176 (82–241)		46	138 (85–211)		41	169 (98–283)	
60–69	31	118 (85–183)		34	192 (111–261)		31	148 (99–241)		33	170 (104–251)	
≥ 70	1	135		7	210 (156–268)		0	N/A		7	186 (151–278)	
Pathologic T stage												
T2	64	116 (74–183)	0.828	60	181 (82–254)	0.0678	62	148 (85–241)	0.276	59	171 (98–283)	0.495
T3	26	118 (85–192)		22	199 (118–268)		25	122 (99–207)		22	168 (110–251)	
T4	5	116 (87–183)		4	205 (157–222)		5	166 (95–185)		4	140 (119–197)	
Biochemical recurrence												
No	84	116 (74–192)	0.34	69	185 (82–268)	0.6733	83	133 (85–241)	0.259	68	170 (98–283)	0.596
Yes	9	124 (107–146)		16	184 (119–261)		7	155 (118–207)		16	166 (102–251)	
Biochemical failure												
No	63	116 (74–183)	0.418	57	182 (82–254)	**0.0212**	62	145 (85–241)	0.478	56	170 (98–283)	0.853
Yes	30	116 (87–192)		28	208 (118–268)		28	127 (95–207)		28	168 (102–251)	
Radical prostatectomy failure												
No	56	116 (74–183)	0.543	55	182 (82–254)	**0.0303**	56	145 (85–241)	0.535	54	170 (98–283)	0.937
Yes	37	116 (87–192)		30	203 (118–268)		34	131 (95–207)		30	168 (102–251)	

[a]*P* values were calculated using Kruskal–Wallis tests by rank. Significant *P* values ($P < 0.05$) are indicated in bold.
[b]Age at operation was significantly correlated with expression of SLCO5A1 ($r = 0.24$) and 2A1 ($r = 0.26$) in EAs but not AAs ($r = 0.03$ and 0.04, respectively).

by our observation of the cell type–specific expression of the two predominant SLCO transporters in prostate cancer: SLCO2A1 in endothelial cells and SLCO5A1 in epithelial cells. The relative expression of the two transporters might coordinately determine the availability of androgens to prostate cancer cells via regulation of the sequential transportation of androgen from the circulation to the prostate tissue microenvironment by the endothelial cell and from the tissue microenvironment to the cancer by the epithelial cell. Higher expression of SLCO5A1 in prostate epithelial cells in EAs may mitigate the reliance of SLCO2A1-mediated androgen transportation by endothelial cells from the circulation into the interstitial space, which explains the lack of association of genetic variations in *SLCO2A1* with prostate cancer aggressiveness in EAs. In comparison, the lower expression of SLCO5A1 in epithelial cells of AAs may rely more on SLCO2A1in endothelial cells or the cooperation of SLCO2A1 and SLCO5A1 between endothelial and epithelial cells to obtain adequate tissue levels by elevated transport of androgen from the circulation. Indeed, a joint effect between risk alleles/genotypes in *SLCO2A1* and *SLCO5A1* was only observed in AAs, while for EAs, the significant associations were driven primarily by SNPs in *SLCO5A1*. Therefore, the race-specific associations of genetic variations in *SLCO2A1* and *SLCO5A1* are aligned with both transporters being the predominant SLCO transporters expressed in prostate cancer tissue, and their cell type–specific expression in prostate cancer endothelial cells and epithelial cells.

The cell type–specific expression of SLCO2A1 and SLCO5A1 in prostate tissue is supported by our recent report of RNA sequencing data generated from prostate epithelial cells and endothelial cells isolated from fresh clinical specimens of prostate tissue (38). However, knowledge on the biological functions and substrates of SLCO2A1 and SLCO5A1 in human prostate tissue is scant. SLCO2A1 was identified as a prostaglandin uptake transporter (39–41). Later identification of multiple SLCO2A1 inhibitors/blockers suggested the presence of other potential physiologic substrates for SLCO2A1 in addition to prostaglandins (42–44). SLCO2A1 was reported to be expressed in endothelial cells (45) and to be involved in tumor angiogenesis contributing to tumor growth in a mouse model of colorectal cancer (46). The observation in colorectal cancer is in line with our findings on a potential role for SLCO2A1 in endothelial cells in prostate cancer aggressiveness. Physiologically relevant substrates for and function of SLCO5A1 are unclear. Some general SLCO substrates were tested and excluded as substrates for SLCO5A1 in SLCO5A1-overexressing *X. laevis* oocytes, including Prostaglandin E_2, estrone-3-sulfate, and dehydroepiandrosterone-3-sulfate (47). However, potential substrates for SLCO5A1 in prostate cancer epithelial cells need to be evaluated *in situ*, because the expression of SLCO5A1 is cell-type specific and its substrate specificity might be a function dependent on the tissue microenvironment context.

Ideally, significant SNPs identified in genotype-phenotype association studies may point out directions for further functional characterization. However, all significant SNPs identified in the study in both *SLCO2A1* and *SLCO5A1* are located in introns, and none are in LD with potentially functional SNPs or coding SNPs based on the 1000

Genome Project and dbGap databases, nor in classical intron-exon juxtapositions that represent mRNA splice junctions. Furthermore, these SNPs are unlikely to affect gene expression in that the risk alleles/genotypes were present much more frequently in AAs (averagely 52%) than EAs (6%), while expression at the protein level was similar (SLCO2A1) or even lower (SLCO5A1) in AAs in comparison with EAs. In a subset of PCaP subjects (50 AAs and 58 EAs) that were genotyped for SLCO transporters and also had tumor tissues constructed in TMAs, IHC examination of SLCO2A1 and SLCO5A1 expression in the TMAs found that none of the significant SNPs was associated with gene expression. Therefore, the data further suggest that the identified SNPs may alter the transport function of SLCO2A1 and SLCO5A1 instead of affecting gene expression levels. Indeed, recent studies showed that a class of genetic variants termed "deep intronic variants," does not exist in the classic splice junctions but nevertheless acts as regulatory sites to change the splicing phenotype (48–50). SNPs in other transporters, such as genes of SLCO1B3 and SLCO2B1, were found to alter the uptake rates of androgens and therapeutic agents (12, 13), which supports a functional consequence of the SNPs identified in *SLCO2A1* and *SLCO5A1* in altering androgen uptake efficiency or substrate specificity. Since our study focused on common tag SNPs with MAF ≥ 0.05, the possibility of linkage with other unidentified rare genetic variants in these genes, in addition to the potential impact on splicing isoforms, cannot be excluded. Sequencing of the whole genes of *SLCO2A1* and *SLCO5A1* could help further delineate the signals and identify functional genetic variants.

In conclusion, we found that among all 11 SLCO family members, SLCO2A1 and SLCO5A1 may play important, but different, roles in prostate cancer aggressiveness in AAs versus EAs. The finding calls for considerations of racial differences in biomarker studies of prostate cancer and calls for further investigations on biological functions of SLCO2A1 and SLCO5A1 in the development and progression of prostate cancer.

Authors' Disclosures

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Authors' Contributions

L. Tang: Conceptualization, resources, data curation, formal analysis, supervision, funding acquisition, investigation, methodology, writing–original draft, project administration, writing–review and editing. **Q. Zhu:** Formal analysis, methodology, writing–review and editing. **Z. Wang:** Data curation, formal analysis, writing–review and editing. **C.M. Shanahan:** Data curation, writing–review and editing. **J.T. Bensen:** Resources, data curation, writing–review and editing. **E.T.H. Fontham:** Resources, data curation, writing–review and editing. **G.J. Smith:** Resources, data curation, writing–review and editing. **E.A. Pop:** Data curation, investigation, writing–review and editing. **G. Azabdaftari:** Data curation, investigation, writing–review and editing. **J.L. Mohler:** Conceptualization, resources, methodology, writing–review and editing. **Y. Wu:** Conceptualization, resources, data curation, formal analysis, supervision, funding acquisition, investigation, methodology, writing–original draft, project administration, writing–review and editing.

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