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Effect of *SLCO1B3* haplotype on testosterone transport and clinical outcome in Caucasian patients with androgen-independent prostatic cancer

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Abstract

Purpose—The organic anion transporter OATP1B3, encoded by *SLCO1B3*, is involved in the transport of steroid hormones. However, its role in testosterone uptake and clinical outcome of prostatic cancer is unknown. This study examined: 1) the *SLCO1B3* genotype in cancer cells as well as the uptake of testosterone by cells transfected with genetic variants of *SLCO1B3*; 2) the expression of OATP1B3 in normal prostate, benign prostatic hyperplasia and prostatic cancer, and 3) the role of *SLCO1B3* haplotype on clinical outcome of Caucasian patients with androgen-independent prostatic cancer.

Experimental design—*SLCO1B3* genotype was assessed in the NCI-60 panel of tumor cells by sequencing, while testosterone transport was analyzed in Cos-7 cells transfected with wild-type (WT), 334G and 699A *SLCO1B3* variants. OATP1B3 expression in prostatic tissues was examined by fluorescence microscopy and the relationship between *SLCO1B3* haplotypes and survival was examined in patients.

Results—Cells transfected with WT (334T/699G) *SLCO1B3*, or with a vector containing either the 334G or 699A variants, actively transported testosterone, while its uptake was impaired in cells transfected with a gene carrying both 334G and 699A SNPs. Prostatic cancer over-expresses OATP1B3 compared to normal or benign hyperplastic tissue; patients with *SLCO1B3* 334GG/699AA

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haplotype, showed longer median survival (8.5 vs. 6.4 years, p=0.020) and improved survival probability at 10 years (42 vs. 23%, p<0.023) than patients carrying TT/AA and TG/GA haplotypes.

Conclusions—The common *SLCO1B3* GG/AA haplotype is associated with impaired testosterone transport and improved survival in patients with prostatic cancer.

Introduction

Steroid hormones have been implicated in prostate carcinogenesis and much work has been done to evaluate the relationship between the concentration of testosterone in biological fluids and tissues versus clinical outcome of patients with prostate cancer.(1-8) Indeed, polymorphisms in the genes that code for enzymes involved in the androgen regulatory pathway have been proposed to influence the individual's risk of developing prostate cancer.(3,9,10)

The lipophilic nature of steroid hormones would suggest that they cross cell membranes by passive diffusion. However, several members of uptake transporter families localized to the cell membrane may enhance steroid hormone uptake, and these same transporters may also be expressed within the prostate where they could increase the capability of neoplastic cells to utilize testosterone.

One large group of uptake carriers is the organic anion transporting polypeptide (OATP) superfamily encoded by *SLCO* genes.(11-14) Endogenous substances, such as bile acids and steroid hormones, are substrates of OATPs,(15,16) which are localized to human hepatocytes. OATP1B3, a member of this family, is expressed by cancer cells derived from gastric, colorectal, pancreatic, lung, and breast tumors.(16,17) OATP1B3 is involved in the uptake of steroid anions, including estradiol-17β-glucuronide (E2-17βG), dehydroepiandrosterone-3-sulfate (DHEA-S) and estrone-3-sulfate (E1-S).(16) However, it is not known whether testosterone is a substrate for OATP1B3, and no information is available on its expression as it relates to prostate cancer.

SLCO1B3 is a polymorphic gene with two major SNPs in exon 3 (334T>G) and 6 (699G>A), which are in complete linkage disequilibrium, resulting in a serine to alanine change at amino acid 112 (S112A), and methionine to isoleucine change at amino acid 233 (M233I) respectively.(18,19) Polymorphic variants in *SLCO1B3* have shown differences in transport characteristics of several substrates,(16,17,20) and these polymorphisms could effect testosterone uptake in the prostate, potentially influencing carcinogenesis and tumor aggressiveness.

Based on this evidence, the present study was designed to analyze: 1) the genotype of *SLCO1B3* in cancer cells as well as the effect of wild-type (WT) and polymorphic variants of *SLCO1B3* on testosterone uptake, 2) the expression of OATP1B3 in normal prostate, benign prostatic hyperplasia and prostatic cancer, and, 3) the association between *SLCO1B3* haplotypes and clinical outcome of Caucasian patients with androgen-independent prostatic cancer.

Materials and Methods

Genotyping of *SLCO1B3* in cancer cell lines

The analysis of *SLCO1B3* genotype was performed on the NCI-60 panel of tumor cell lines (Developmental Therapeutics Program, NCI[A]). Genomic DNA was extracted from cells using

[A]www.dtp.nci.nih.gov

either the QiAamp Ultrasens Viral DNA or QIAamp DNA Blood kits (Qiagen, Valencia, CA). Primer pairs (Table 1) were designed on the basis of the *SLCO1B3* gene sequence available at GenBank (accession number NM_019844.1). PCR reactions were carried out in a 50-μl reaction mixture containing 200 ng of genomic DNA, 1X PCR buffer (Invitrogen, Carlsbad, CA), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.8 pmol/μl of each primer, and 1.25 U Platinum Taq DNA polymerase (Invitrogen, Carlsbad, CA). PCR amplifications were carried out using the GeneAmp PCR system 9700 (PE Applied Biosystems, Foster City, CA) with the following thermal profile (primary PCR): 20 cycles of denaturation at 94°C for 30 sec, annealing at 62° C for 30 sec, and extension at 72°C for 30 sec. Secondary PCR amplifications were performed by 40 cycles at the same thermal profile with the exception of the annealing temperature that was 60°C. After amplification, the quality of the amplified PCR products was verified by agarose gel electrophoresis.

Secondary PCR reaction products were sequenced in both directions using the internal primer set reported in Table 1. Sequence analyses were carried out using the Big Dye Terminator Cycle Sequencing Kit on an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA). *SLCO1B3* genotypes were compared to mRNA levels analyzed by an Affymetrix microarray platform[B].

Cell transfection and transport assay of testosterone

Cos-7 cells (American Type Culture Collection, Manassas, VA) were cultured in DMEM (GIBCO, Carlsbad, CA) with 10% FBS in an atmosphere of 5% CO2-95% air at 37°C. Cells were transfected with the pCMV6-XL4 DNA vector containing WT *SLCO1B3* (TG), single (GG, TA) or double (GA) variants. The plasmid containing the polymorphisms was obtained by site-directed mutagenesis and transiently transfected by Lipofectamine (Invitrogen, Carlsbad, CA). The human *SLCO1B3* cDNA cloned into the pCMV6-XL4 plasmid vector was purchased from OriGene Technologies (Rockville, MO). Nucleotide exchange was introduced into the cDNA of *SLCO1B3* using the QuikChange Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA). The primer pairs used for the exchange of 334G>T and 669A>G are reported in Table 1. Successful mutagenesis was verified by sequencing.

Cellular uptake of testosterone was measured in *SLCO1B3*-transfected Cos-7 cells incubated in DMEM with 10% FBS containing 20 nM [^{3}H]-testosterone (76 Ci/mmol; Sigma-Aldrich) for 1 hour. The monolayers were rapidly washed thrice with buffer (142 mM NaCl, 5 mM KCl, 1 mM K_2HPO_4, 1.2 mM $MgCl_2$, 5 mM D-glucose, and 12.5 mM HEPES, pH 7.2, at 4°C), solublized in 0.5 ml of 1 N NaOH, and the amount of radioactivity was measured by the LS 6000IC scintillation counter (Beckman Coulter, Fullerton, CA).

Analysis of OATP1B3 in prostate tissue by immunofluorescence microscopy

MaxArray™ Prostate Cancer & BPH Tissue Microarray Slides (Zymed Labs, San Francisco, CA) were used to assess OATP1B3 expression by immunofluorescence. The slide arrays, which contain tissue specimens from prostate cancer (20 cases), benign prostate hyperplasia (BPH, 19 cases), and one normal control, were deparaffinized and rehydrated, and then blocked and incubated with goat polyclonal OATP1B3 primary antibody (Santa Cruz Biotech, Santa Cruz, CA) at a final concentration of 20 μg/mL overnight at 4°C. Arrays were incubated with the secondary Alexa Fluor® 568 labeled donkey anti-goat IgG (Invitrogen, Carlsbad, CA) at 10 μg/mL, for 30 min at 25°C. Immunofluorescence microscopy was performed on Zeiss Axio Imager A1 (Carl Zeiss, Jena, Germany).

[B]see http://dtp.nci.nih.gov/mtweb, Pattern Id: GC91812 by Developmental Therapeutics Program, NCI, NIH

Study population and genotyping

Genomic DNA and clinical history was available from 180 Caucasian patients with androgen-independent prostate cancer enrolled in IRB approved trials, including ketoconazole-alendronate,(21) suramin,(22-24) thalidomide-docetaxel,(25) thalidomide,(26) and sorafenib (protocol identifier at ClinicalTrials.gov: NCT00093431, last accessed on 8-13-2007), within the intramural program of the NCI/NIH. Details of study population are given in Table 2. Prior to enrollment, patients were treated with androgen deprivation therapy consisting of either surgical castration, or luteinizing hormone-releasing hormone (LHRH) agonist with or without non-steroidal anti-androgens as the initial hormone therapy. DNA was also obtained from 131 healthy Caucasian individuals (median age = 37 years). Informed consents were obtained from all subjects prior to trial participation. DNA was extracted from serum or white blood cell buffy coat layers; sequencing was performed as described above for cell lines to stratify patients on the basis of the *SLCO1B3* TT/GG (WT), TG/GA and and GG/AA haplotypes.

Statistical analysis

The comparison of testosterone uptake between cells transfected with WT and genetic variants of *SLCO1B3* was performed by Tukey's multiple comparison test. Association between the *SLCO1B3* genotypes and clinical outcome was evaluated by the exact Kruskal-Wallis test. Patients who had deceased were assessed as complete, whereas those who remained alive were censored at the date of last follow-up. The probability of survival as a function of time was determined by the Kaplan-Meier method. The statistical significance of the differences in overall survival and survival probability were determined by the log-rank and permutation tests.(27) A power analysis was not conducted due to the pilot nature of this study, and all samples available to us were genotyped.

Results

Frequency distribution of *SLCO1B3* genotype in NCI-60 cell lines

The uncommon 1564T variant was not detected in cell lines. The 334T>G and 699G>A SNPs were in complete linkage disequilibrium, with the exception of RXF393 renal tumor cells carrying the TT/GA haplotype. The majority of cell lines displayed the variant haplotype (GG/AA, n=49), the remaining the WT (TT/GG, n=3) and the heterozygous TG/GA (n=6) haplotypes. The genotype and the expression levels of *SLCO1B3* were unrelated, as no specific genotype was associated with high or low expression of *SLCO1B3* (data not shown).

In vitro uptake of testosterone by *SLCO1B3* transfected Cos-7 cells

The effect of transfection of Cos-7 cells with WT and variants of *SLCO1B3* on testosterone uptake is shown in Figure 1. Accumulation of testosterone was markedly induced by transfection with WT *SLCO1B3* TG or variant genotypes GG and TA, with no significant differences among them. However, testosterone uptake by the *SLCO1B3* GA was similar to vector control cells, thus providing evidence that while both *SLCO1B3* WT and single variants actively transport testosterone into the cell, the double mutant lost most of this capability.

Expression of OATP1B3 protein in prostatic tissue

As shown in Figure 2, the expression of OATP1B3 protein in tissues from normal prostate of BPH was almost absent, while it was markedly over-expressed in all prostate cancer tissue samples examined. The distribution of the OATP1B3 protein on the cell membrane was evidenced by the peripheral staining of the cells (Figure 2).

Survival analysis

Statistical analysis of the genotype prevalence did not show significant differences between patients and healthy volunteers, nor were differences observed in the frequencies of the *SLCO1B3* genotypes relative to Gleason scores (p=0.31) or age at diagnosis (p=0.42) in men with prostate cancer (Table 3). There was a near-significant difference in clinical stage at diagnosis (p=0.052). However, this was entirely due to a disproportionate number of TT/GG patients with early clinical stage (stage B) tumors. Odd ratios of *SLCO1B3* haplotypes for prostate cancer patients versus healthy controls crosses 1.0, thus suggesting the lack of association between *SLCO1B3* polymorphisms and the development of prostate cancer in Caucasian patients (Table 3).

Since the 334T>G and 699G>A polymorphisms are in linkage disequilibrium, we used the 334T>G as the tagging SNP for the correlation of genotype with clinical outcome of patients, as shown in Figure 3. Overall, the estimated median survival for all patients was 8.0 years (95% confidence interval [CI] 6.8 to 8.8), but the median survival for the 45 patients with both the WT TT/GG or TG/GA haplotypes was 6.4 years (95% CI 4.1 to 8.1), compared to 8.5 years (95% CI 7.2 to 10.2) for 135 patients with the GG/AA variant haplotype, the survival between the two groups being significantly different by logrank test (p=0.020, HR 1.57; 95% CI 1.11 to 2.24). The estimated survival probability at ten years was 23% (95%CI, 13% to 37%) in TT/GG or TG/GA haplotypes, and 42% (95%CI, 34% to 50%) in patients carrying the GG/AA haplotype, the difference being statistically significant (p=0.023 by the permutation test). These results suggest that survival was significantly longer for subjects with double variant haplotype.

Discussion

The present study provides evidence that testosterone uptake by cells occurs via OATP1B3 and its presence increases from undetectable levels in normal prostatic tissue and benign hyperplasia to marked expression in prostatic cancer. The present study also demonstrates that the capability of testosterone transport by OATP1B3 is dependent on its genotype, the WT being most active and the variant haplotype 334GG/699AA being less efficient. Thus, OATP1B3 may play a key role in prostate cancer biology and this finding presents a novel mechanism whereby cancer cells can actively utilize testosterone to support their own growth, perhaps even in the presence of depleted circulating levels of the hormone, as a result of medical/surgical castration. As a confirmation of preclinical data, the *SLCO1B3* double variant GG/AA haplotype was also associated with better survival of patients with prostatic cancer. Furthermore, there is no significant difference in the proportion of high Gleason grade tumors with the TT/GG or TG/GA versus the GG/AA haplotype. While there is a near-significant difference in the proportion of patients with the TT/GG haplotype with early clinical stage tumors at diagnosis, this difference, if anything, may result in an *underestimation* of the survival difference attributable to the SLCO1B3 haplotype. While we characterized patients with respect to Gleason score and stage at diagnosis, other prognostic variables for survival such as PSA and PSA doubling time at diagnosis were not available. We should emphasize that this is a retrospective study with relatively small numbers of patients and requires further confirmation with prospective studies. Nonetheless, these results are promising and may shed new light on prognostication of the clinical course for patients with prostate cancer.

The results of the present study are consistent with the research field on the genetics of enzymes involved in steroid hormone biosynthesis, including CYP17,(9,28) CYP3A4,(29) CYP11,(30) and CYP19,(10) which were reported to be associated with the risk of developing prostate cancer or with its clinical outcome.(31) However, the role of transporters of steroid hormones was not addressed in detail previously, although it is well documented that members of the OATP/SLCO family may transport steroid hormones in normal tissues,(11)·(32)·(33) and that

OATP1B3 is expressed in cancer cells derived from gastric, colon, pancreatic, lung, and breast tumors.(11)

Indeed, the transfection of Cos-7 cells with a vector carrying the 334G/699A alleles was associated with a significantly reduced ability of cells to uptake testosterone, compared to the WT vector carrying the wild-type 334T/699G haplotype. Although a previous report by Letschert *et al.* provides evidence that the 334T>G SNP affects the transport activity *in vitro* of steroid metabolites, such as E2-17βG, DHEA-S and E1-S,(17) however, that study did not take into account that the 334T>G SNP is in linkage disequilibrium with the 699G>A, thus the steroid transporting activity of the double polymorphic variant may be different from the results obtained by Letschert *et al*. This finding would have a marginal impact if the transporter was only marginally expressed on cancer cells; on the contrary, we suggest that its role is substantial, since the OATP1B3 protein was overexpressed in prostatic cancer tissue when compared to benign prostate. Collectively, these data suggest that the overexpression of OATP1B3 is part of a complex stepwise process of cancer progression, which allows the prostate cancer cells to gain a biological advantage, by up-regulating testosterone uptake capability and proliferative activity through increased androgen import.

Among the possible explanations for the different uptake activity of double variant *SLCO1B3*, impaired gene transcription may be ruled out since no significant associations were observed between the *SLCO1B3* genotype and the expression level of *SLCO1B3* mRNA in the NCI-60 panel of cell lines. On the contrary, it may be suggested that these SNPs may impair transport kinetics or alter substrate specificity of OATP1B3 since the amino acids affected by the change are located in the third transmembrane helix domain and the third extracellular loop, respectively.(17)

Since substrates of OATP1B3 include DHEA-S, E1-S, and E2-17βG, we hypothesized that functional polymorphisms affecting the uptake activity of OATP1B3 may play an important role in the biology of prostate cancer and, hence, in the clinical outcome of patients. As a confirmation of this hypothesis, the data of the present study demonstrate that Caucasian patients with androgen-independent prostatic cancer bearing the GG/AA genotype had a significantly longer survival when compared to subjects carrying GG/AA or TG/GA genotypes. To our knowledge, this study is the first report to demonstrate a statistically significant association of a specific *SLCO1B3* haplotype with survival of patients with prostatic cancer. The association between *SLCO1B3* genotype with the clinical outcome of prostatic cancer is likely to be more substantial in the Black African and African American populations, as the allele frequency of at-risk genotypes is approximately 80-90%,(18) perhaps explaining the greater mortality associated with prostatic cancer in the black population living in countries with high incidence of this type of cancer. This possibility should be evaluated in future studies evaluating such populations.

However, the mechanism of OATP1B3 overexpression remains to be elucidated. It can be speculated that the androgen responsive elements (ARE) present on *SLCO1B3* gene may be involved in this process. Indeed, it is known that the expression of mouse *oatp1* gene in the kidney is regulated by androgens(34) and that testosterone-mediated expression of *oatp* mRNA is tissue specific(35). Furthermore, testosterone transported into prostate cells is converted to dehyrotestosterone (DHT), and both of them bind to and activate the androgen receptor (AR). AR transclocates to the cell nucleus, binds to the TGTTCT(36) consensus sequence of the androgen responsive elements (ARE), and regulates gene transcription. Indeed, we have identified 7 of them in the *SLCO1B3* promoter (AC011604, data not shown). This suggests a possible positive feedback, as OATP1B3 can increase the influx of androgens, and through this mechanism, induce its own expression further increasing the ability of the cell to accumulate testosterone. Furthermore, the *SLCO1B3* promoter also contains several hypoxia

responsive elements suggesting that the upregulation of *SLCO1B3* in tumors may also be related to anoxic conditions.(11) This mechanism, if true, may be responsible for failure of androgen-deprivation therapy in early treatment of prostate cancer.

The cornerstone of first-line therapy for advanced prostate cancer is androgen deprivation therapy, most commonly administered with medical castration.(37) However, prostatic cancer almost always becomes resistant and progresses to an androgen-independent state. What has become increasingly clear is that the transition to hormone-independence is associated with 1) reactivation of AR by gene amplification, 2) ligand-independent activation by various growth factors through a phosphorylation-dependent mechanism, 3) modifications in the expression of cofactors that are involved in AR function and 4) mutations in AR that allow steroid ligands, other than androgens, to bind and activate AR.(38) Gain-of-function in the androgen pathway involves sensitization of cells to low serum levels of androgens,(39) and the development of androgen-independent disease is associated with upregulation of genes that are involved in the synthesis of androgens(40). Since the WT allele of OATP1B3 is more active than the variant allele in mediating intracellular transport of testosterone, the enhanced uptake of androgens in patients may shorten the time to androgen independence. We are actively investigating this possibility by examining the association between *SLCO1B3* genotype and the development of androgen-independent tumors.

In conclusion, the present study demonstrates the important functional and biological role of OATP1B3 in prostate cancer and its significant association with survival of Caucasian patients with prostate cancer. Therefore, *SLCO1B3* genotyping may be become a useful tool for the stratification of prostatic cancer patients and for treatment decision-making. The effect on survival of the variant haplotype should be further evaluated in a larger sample size or in a population where its prevalence is higher than Caucasian, such as among African Americans, thus explaining, at least in part, the ethnic difference in prostate cancer outcome.

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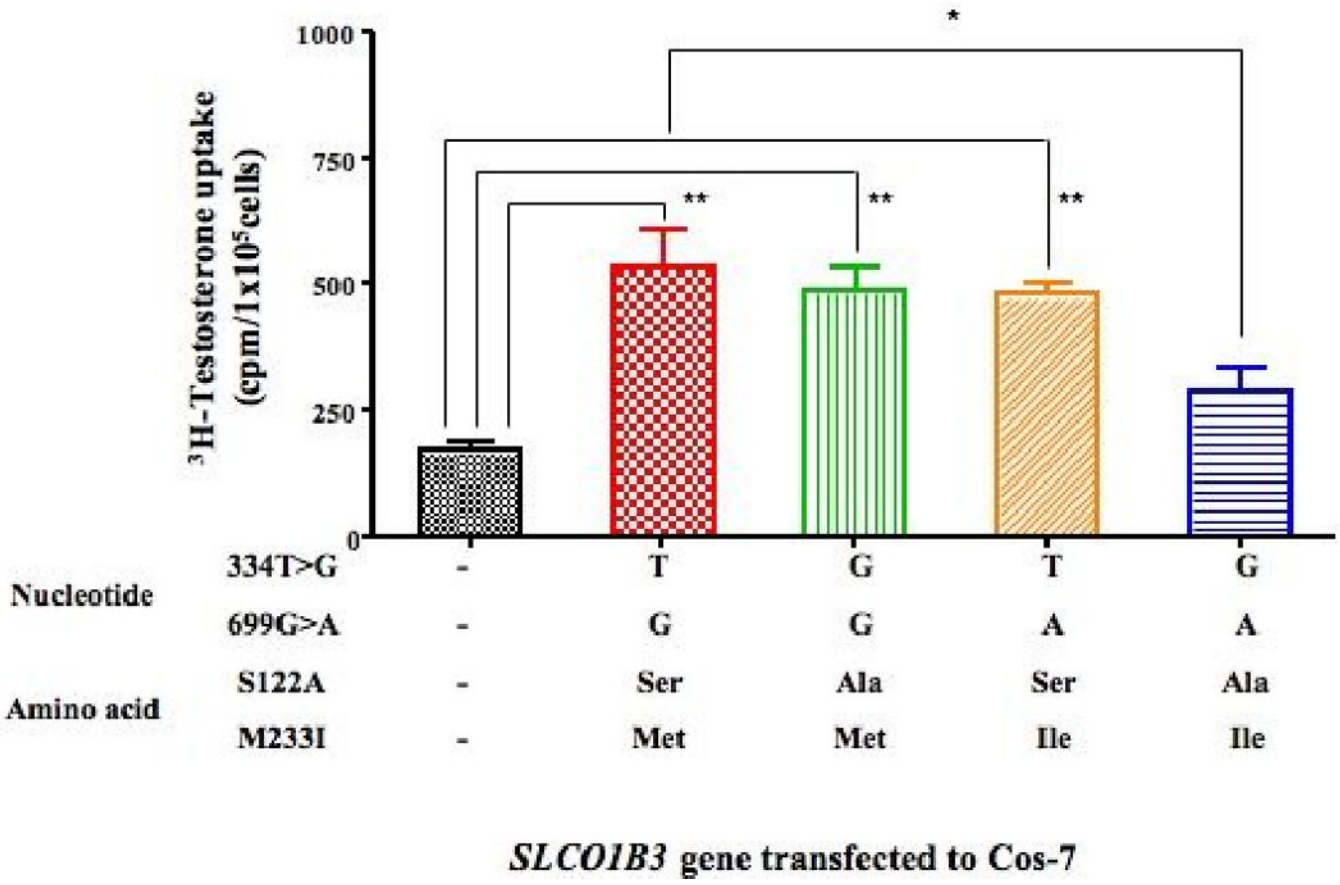

Figure 1.

Influence of *SLCO1B3* polymorphism on testosterone transport by Cos-7 transfected with vectors containing SLCO1B3 WT and single/double mutants. Error bars represent the SEM. **, $p<0.01$ (vector control vs WT, 334G or 699A) and *, $p<0.05$ (334G/699A vs WT); differences were analyzed by the Tukey's multiple comparison test.

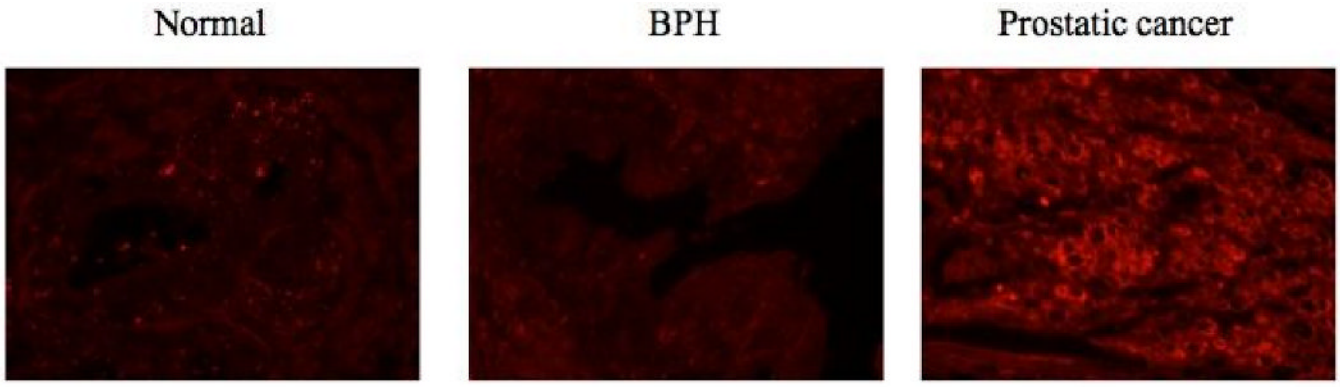

Figure 2.
Immunofluorescent localization of OATP1B3. Absent to very faint immunoreactivity was observed in normal tissue and benign prostatic hyperplasia (BPH), while in prostatic cancer the immunoreactivity was strongly increased. (Original magnification × 200)

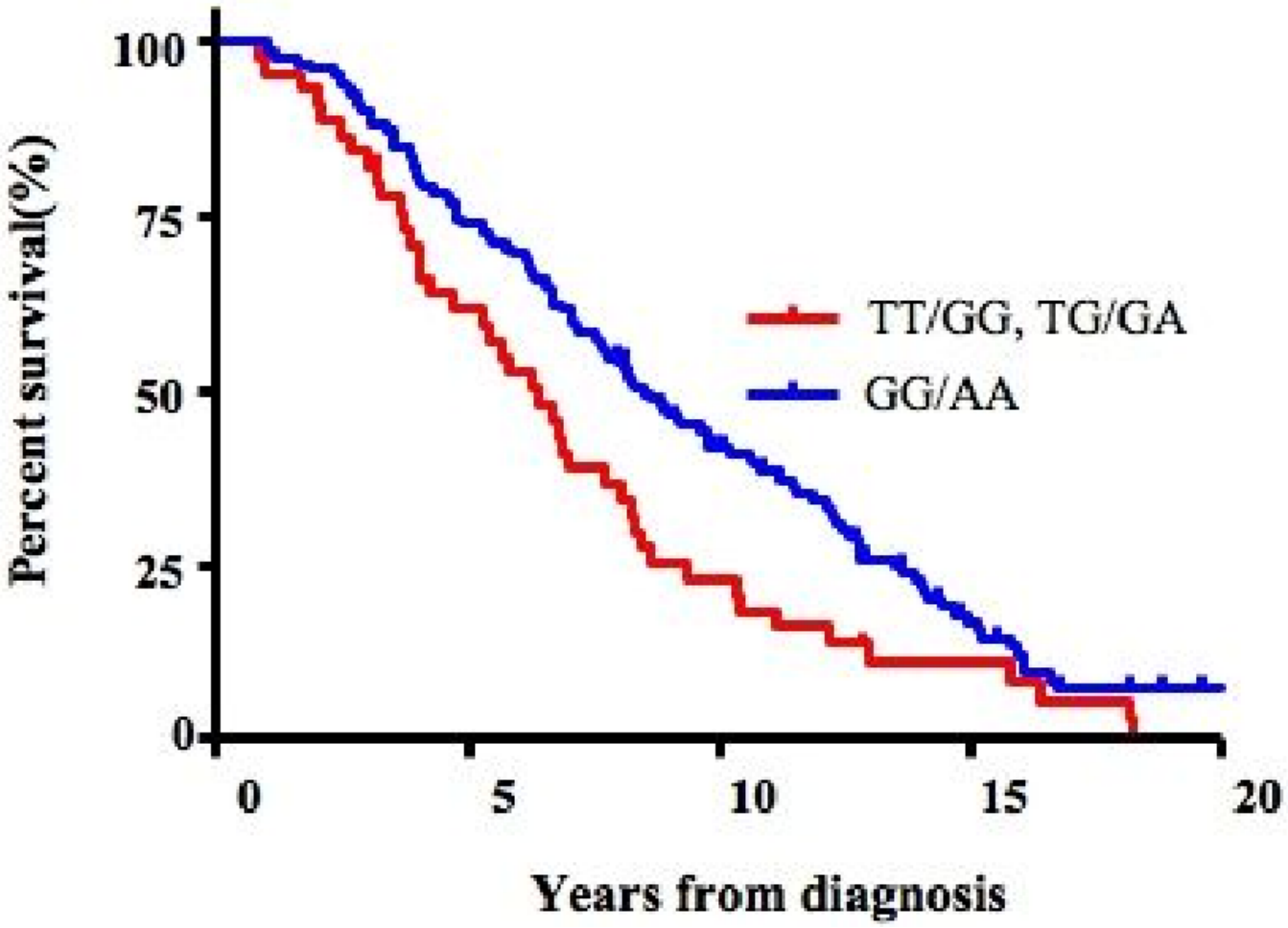

Figure 3.
Kaplan-Meier survival curves in Caucasian prostatic cancer patients grouped by *SLCO1B3* haplotypes (variant GG/AA vs. WT TT/GG and heterozygous TG/GA).

Table 1

Primer sequences for PCR, sequencing and site-directed mutagenesis

SNP	Use	Primer sequence
334T>G	PCR-I	(F) CCTTCACAGTTAAATTACATGGTC
	PCR-I	(R) TATTCATTTCATATAAAACTGTATACC
	PCR-II	(F) GGGCATATTTGCATTCATTTGGG
	PCR-II	(R) CATGATAAATAAAGAAATACATGATG
	Seq	(F) CACTAAGTCATATCAACATAATTTTG
	Seq	(R) GCATACCTATAGGTATTCCTCTC
	Mut	(F) TGGGAACTGGAAGTATTTTGACATCTTTACCACATTTCTTCATG
	Mut	(R) CATGAAGAAATGTGGTAAAGATGTCAAAATACTTCCAGTTCCCA
669G>A	PCR-I	(F) TCCTTGTATTTAGGTAACGTACAG
	PCR-I	(R) TCAAGTTTGGTTATTTTGGATCAAG
	PCR-II	(F) GATCTACCCTTGAAATAATAATGTC
	PCR-II	(R) GTAAAAGCAAAGTATAAATAGGAGC
	Seq	(F) TAAAAGCATGTTAAATGAAAACCAAG
	Seq	(R) AAATAGTAAACAAAGAACTATTGAAAG
	Mut	(F) GGGATCTCTGTTTGCTAAAATGTACGTGGATATTGGATATGTAG
	Mut	(R) CTACATATCCAATATCCACGTACATTTTAGCAAACAGAGATCCC
1564G>T	PCR-I	(F) ATATACAGAATTTCATACACTAATTTC
	PCR-I	(R)AATTCTAAGAAAATGCATTCTCAAAG
	PCR-II	(F) TATTTTGCCTTCACTATTAAGCAA
	PCR-II	(R) AATATGAATTTGAGCTCAAAATACAG
	Seq	(F) GGAATGTATTCATAGCCCTGTTG
	Seq	(R) ATGACAATGTTTTACAGGATCATA

F, forward; R, reverse; PCR-I, primary PCR; PCR-II, secondary PCR; Seq, sequencing; Mut, site-directed mutagenesis

Table 2

Demographic and clinical characteristics of patients

Total	**180**
Age at Diagnosis	
< 49	19
50 – 59	57
60 – 69	78
> 70	26
Gleason score	
4 – 6	24
7	49
8 - 10	93
unknown	14
Clinical Stage	
B	48
C	45
D1	14
D2	71
Unknown	2
Hormone therapy	
Surgical castration	4
LHRH analogues (leuprolide, goserelin)	9
CAB	167
Clinical study treatment	
Ketoconazole/alendronate	47
Suramin	45
Thalidomide /docetaxel	40
Thalidomide	36
Others	12

CAB: combined androgen blockade (LHRH analogue plus androgen receptor antagonist)

Table 3

Associations of *SLCO1B3* haplotypes with selected clinical characteristics and risk of prostate cancer for Caucasian prostatic cancer patients and healthy subjects

	TT/GG	TG/GA	GG/AA	*P*
Age at diagnosis				0.42
< 49	0	4	15	
50 – 59	4	10	43	
60 – 69	7	13	58	
> 70	2	5	19	
Gleason score[a]				0.31
2 – 6	1	4	19	
7	5	6	38	
8 – 10	6	19	68	
unknown	1	3	10	
Clinical stage at diagnosis				
B	6	5	37	0.052
C	4	10	31	
D1	2	3	9	
D2	1	14	56	
Unknown	0	0	2	
Controls (n=131)[b]	9	26	96	0.75[d]
Prostatic cancer (n=180)	13	32	13	0.95[e]
OR (95% CI)[c]	1	1.17 (0.43-3.17)	1.03 (0.42-2.50)	

[a] Mildly aggressive (2-6), moderately aggressive (7) or highly aggressive (8-10)

[b] Healthy American Caucasian. Genotype frequencies are similar to previously published Caucasian controls (p=0.34) (18).

[c] Odds ratio (OR) and 95% confidence interval (CI) of prostatic cancer vs. controls

[d] TG/GA vs. TT/GG

[e] GG/AA vs. TT/GG