Title Page

Genetic Variations of Genes Involved in Testosterone Metabolism are Associated to Prostate Cancer Progression: a Spanish Multicenter Study

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ABSTRACT

Background. Prostate cancer (PCa) is an androgen-dependent disease. Nonetheless, the role of single nucleotide polymorphisms in genes encoding androgen metabolism remains an unexplored area.

Purpose. To investigate the role of germ-line variations in cytochrome P450 17A1 (*CYP17A1*), steroid-5α-reductase, α-polypeptide 1 and 2 (*SRD5A1*, *SRD5A2*) genes in PCa.

Patients and Methods. 494 consecutive Spanish patients diagnosed with non-metastatic localized PCa were included in this multicenter study and were genotyped for 32 single nucleotide polymorphisms (SNPs) in *SRD5A1*, *SRD5A2* and *CYP17A1* genes using a Biotrove OpenArray® NT Cycler. Clinical data were available. Genotypic and allelic frequencies, as well as haplotype analyses, were determined using the web-based environment SNPator. All additional statistical analyses comparing clinical data and SNPs were performed using PASW Statistics 15.

Results. The call rate obtained (determined as the percentage of successful determinations) was 97.3% of detection. Two SNPs in *SRD5A1* – rs3822430 and rs1691053 – were associated to PSA level at diagnosis. Moreover, G carriers for both SNPs were at higher risk of present initial PSA levels > 20 ng/mL (Exp(B) = 2.812, confidence interval (CI) 95% (1.397 – 5.657), p = 0.004) than those AA – AA carriers. Haplotype analyses showed that PCa patients non-homozygous for the haplotype GCTTGTAGTA were at higher risk of present bigger clinical tumor size (Exp(B) = 3.823, CI95% (1.280 – 11.416), p = 0.016), and bigger Gleason score (Exp(B) = 2.808, CI95% (1.134 – 6.953), p = 0.026).

Conclusions. Single nucleotide polymorphisms in *SRD5A1* seem to condition the clinical characteristics of Spanish PCa patients.

Keywords: SNP; *SRD5A1*; *SRD5A2*; *CYP17A1*; prostate cancer; OpenArray

1. INTRODUCTION

Prostate cancer (PCa) is an androgen-dependent disease [1]. The synthesis of testosterone (T) is mediated by the enzyme 17-hydroxylase/17,20-lyase, encoded by *CYP17A1* gene. Dihydrotestosterone (DHT) is considered the active metabolite of testosterone. T is converted into DHT through the 5α-reductase pathway, thus conditioning tumour availability of the hormone and subsequent tumour development and progression [2].

Single nucleotide polymorphisms (SNPs) are simple genetic variations in the DNA sequence which would modify the efficacy of encoded enzymes [3]. Genetic alterations in the testosterone metabolism pathway are expected to alter hormonal homeostasis and likely influence PCa development and progression.

The association between *CYP17A1* gene polymorphisms and prostate cancer is controversial [4-6]. Although it has been suggested that some polymorphisms in *CYP17A1* are associated with PCa risk [7] and survival [8], it is not clear how SNPs in that gene would affect clinical variables of PCa such as tumor size, prostate specific antigen (PSA) levels or Gleason score. In any case, *CYP17A1* is considered an important factor for PCa progression since it has become a new therapeutic target. Thus, abiraterone acetate is a new drug very active in the treatment of certain PCa through specific *CYP17A1* blockade [9], although the role of SNPs in *CYP17A1* in response to abiraterone acetate has not been explored.

5α-reductase enzymes are encoded by the steroid-5α-reductase, α-polypeptide 1 (*SRD5A1*) and steroid-5α-reductase, α-polypeptide 2 (*SRD5A2*) genes. The role of *SRD5A1* and *SRD5A2* polymorphisms in PCa has been little studied, although it has been shown positive associations of several *SRD5A1* and *SRD5A2* variations as independent predictors of PCa outcome in terms of biochemical recurrence [10]. Anyhow, *SRD5A1/A2* are also therapeutic targets, and the specific blockade of these enzymes with dutasteride or finasteride have showed that decreasing

production of DHT using 5α-reductase inhibitors decrease the incidence of clinically localized PCa [11, 12].

At a clinical level, tumor size, Gleason score, and pre-treatment serum level of PSA are the most important prognostic factors [13]. Nonetheless, although these are key factors conditioning the outcome, the heterogeneity in clinical behavior requires the expansion of knowledge about the disease [14].

Since the presence of genetic variations in the androgen biosynthesis and metabolism genes may alter hormone bioavailability, we hypothesized here that certain polymorphisms in the testosterone synthesis pathway may be important to define the biological characteristics of the disease, possibly influencing the classic prognostic factors of prostate cancer. For the first time, we conducted a study to explore the association between germ-line variations in *SRD5A1/2* and *CYP17A1* and prostate cancer progression in a cohort of Spanish Caucasian PCa patients.

2. MATERIALS AND METHODS

2.1. Patients

A total of 601 patients with non-metastatic localized PCa were initially included in the study. PCa were recruited from four different regions of Spain, as has been previously published [15]. Since genotypic and allelic frequencies varies among subjects from these regions, and Andalusian patients showed the greatest differences [15, 16], we excluded these subset of patients from further analyses to avoid bias. Patients who were initially operated were also excluded to form a homogenous group of prostate cancer patients. Thus, a total of 494 subjects were included in the present study. All patients were from Spanish origin and all of them received written informed consent before blood sample collection. The study was approved by the Research and Ethics Committee of each institution participant in the study: Hospital Universitario de Gran Canaria Dr. Negrín (Las Palmas de Gran Canaria), Hospital de la Esperanza. Parc de Salut Mar (Barcelona), Hospital Universitari de Bellvite (L'Hospitalet de

Llobregat), Onkologikoa (Guipuzcoa), Institut Català d'Oncologia (L'Hospitalet de Llobregat), and Hospital de la Santa Creu i Sant Pau (Barcelona).

Clinical tumor size (cT), initial prostate specific antigen (PSA) value, and Gleason score were recruited for all PCa. Clinical tumor size was assessed by digital rectal examination (DRE) followed by transrectal ultrasonography (TRUS) and magnetic resonance imaging (MRI); PSA serum levels were assessed by chemiluminescence in an Architect i2000 analyzer (Abbott Laboratories, IL, USA); Gleason score was determined in the biopsy specimen by a pathologist. After collecting demographic and clinical data, a blood sample was taken after the signature of informed consent.

2.2. DNA isolation and quantification

All the blood samples were sent to the Hospital Universitario de Gran Canaria Dr. Negrín for DNA extraction and genotyping analyses. DNA was isolated from 300 µl of whole-blood in an iPrep™Purification Instrument using the iPrep™ PureLink™ gDNA Blood Kit (Invitrogen, by Life Technologies, Carlsbad,CA). DNA integrity was determined by NanoDrop ND-1000 (NanoDrop Technologies, Wilmington, DE).

2.3. Selection criteria for SNPs

SNPs were selected using data of individuals with European ancestry (CEU) from the HapMap Project (available at: www.hapmap.org). Pairwise linkage disequilibrium (LD) tagging was achieved with Haploview v4.2 software (free downloaded from http://www.broadinstitute.org/scientific-community/science/programs/medical-and-population-genetics/haploview/haploview) [17]. This strategy is based in the haplotype blocks formed by clustered SNPs [18]. By selecting a modest number of SNPs within a block is possible to recover up to 95% of the heterozygosity present at a locus. Haploview tool allows to select tagged SNPs based on haplotype blocks identified using the existing genotype data from the HapMap Project. The R^2

given by Haploview was >0.95 for all genes. Thus, a total of 32 SNPs located in three genes (*SRD5A1*, 10 SNPs; *SRD5A2*, 12 SNPs; and *CYP17A1*, 10 SNPs) were studied (Table 1).

2.4. Genotyping

The SNP genotyping was performed in a Biotrove OpenArray® NT Cycler (Applied Biosystems, Foster City,CA) [19]. DNA samples loaded in OpenArray (OA) had A260/A280 and A260/230 ratios of 1.7-1.9 and were adjusted to 50ng/µl. A total of 300 ng of genomic DNA was used. A final amount of 150 ng was incorporated into the array with the autoloader and genotyped according to the manufacturer's recommendations. A non-template control (NTC) consisting of DNase-free double-distilled water was introduced within each assay. When the DNA and master mix were transferred, the loaded OA plate was filled with an immersion fluid and sealed with glue. The multiplex TaqMan assay reactions were carried out in a Dual Flat Block (384-well) GeneAmp PCR System 9700 (Applied Biosystems) with the following PCR cycle: an initial step at 93°C for 10 minutes followed by 50 cycles of 45 seconds at 95°C, 13 seconds at 94°C and 2 minutes, 14 seconds at 53°C; followed by a final step during 2 minutes at 25ºC and holding at 4ºC.

The fluorescence results were read using the OpenArray® SNP Genotyping Analysis software version 1.0.5. (Applied Biosystems). The genotyping analysis was made with the TaqMan Genotyper software version 1.0.1. (available at: ttp://www.invitrogen.com/site/us/en/home/Global/forms/taqman-genotyper-software-download-reg.html) using autocalling as the call method. The quality value of the data points was determined by a threshold above 0.95.

2.5. Statistical Analysis

Genotype and allelic frequencies were determined using the web-based environment SNPator (SNP Analysis To Results, from the Spain's National Genotyping Center and the National Institute for Bioinformatics) [20]. Relative excess heterozygosity was determined to check compatibility of genotype frequencies with Hardy-Weinberg equilibrium (HWE). Thus, p-

values from the standard exact HWE lack of fit test were calculated using SNPator. Comparisons of genotypic and allelic frequencies, as well as haplotype analyses were also done in SNPator. Haplotype analysis was also performed using the SNPator tool.

All additional statistical analyses comparing clinical data and SNPs were performed using PASW Statistics 15 (IBM Corporation, Armonk, NY, USA).

3. RESULTS

All the genotyped samples met the quality criteria stated above and all samples were genotyped with the same batch of material and at the same time. A total of 494 PCa patients were genotyped for 32 SNPs. Of the 15,808 possible determinations, 97.33% were successfully genotyped. The genotypic and allelic frequencies are shown in Table 1. All SNPs were in HWE.

Distribution of clinical variables is detailed in Table 2. The majority of PCa patients were cT1a – cT2a (54.7%), PSA < 10 ng/mL (61.9%), and Gleason score < 7 (45.7%). We did not observed different proportion of aggressiveness of prostate cancer among different regions of Spain.

Among the 32 SNPs studied, only two of them (rs3822430 (minor allele frequency (MAF) = 0.41) and rs1691053 (MAF = 0.07), both located in *SRD5A1*) were significantly different distributed among PCa patients according to the initial PSA level (Table 3). Thus, among the 157 PCa patients showing initial PSA levels < 10 ng/mL, 92 (58.6%) carried the genotype rs3822430 – AA; while among the 180 PCa patients showing initial PSA levels ≥ 10 ng/mL, 115 (63.9%) carried the G allele for this SNP (chi square test, p = 0.015). A similar result was observed for the SNP rs1691053 (p = 0.008). We explored the combined role of both SNP in relation to the initial PSA level (Table 3), and we observed that PCa patients who carried the G allele showed highest levels of initial PSA than those AA – AA carriers (73% vs. 27%, p = 0.002). Moreover, G carriers for SNPs rs3822430 and rs1691053 were at higher risk of present

initial PSA levels > 20 ng/mL (Exp(B) = 2.812, confidence interval (CI) 95% (1.397 – 5.657), p = 0.004; binary logistic regression test) than those AA – AA carriers (Data not shown). No other polymorphism showed significant associations with clinical variables.

We explored the role of the three most frequent haplotypes detected in our series in chromosomes (chr) 2 (encoding *SRD5A2* gene), 5 (encoding *SRD5A1* gene) and 10 (encoding *CYP17A1* gene) (Table 4). For chr 2, 70.9% of PCa patients presented one of the three most frequent haplotypes; for chr 5, 54.9% of PCa patients presented one of the three most frequent haplotypes; and for chr 10, 62% of PCa patients presented one of the three most frequent haplotypes. We observed that among 23 patients homozygous for the haplotype GCTTGTAGTA (located in chr 5), 19 (82.6%) were cT1a – cT2a, compared with the 55.4% (251 of 453) observed among PCa patients non-homozygous for this haplotype (chi square test, p = 0.018, data not shown). A similar result was observed for Gleason score < 7 (69.6% vs. 44.9%, p = 0.032). Moreover, as shown in Table 5, we observed that PCa patients non-homozygous for the haplotype GCTTGTAGTA were at higher risk of present bigger clinical tumor size (Exp(B) = 3.823, CI95% (1.280 – 11.416), p = 0.016). In the same way, PCa patients non-homozygous for the haplotype GCTTGTAGTA were at higher risk of present bigger Gleason score (Exp(B) = 2.808, CI95% (1.134 – 6.953), p = 0.026). No other haplotypes in chr 5, or in chromosomes 2 and 10 were significantly associated to the clinical variables considered in the study.

4. DISCUSSION

The role of 32 SNPs located in three key genes involved in testosterone metabolism (*SRD5A1*, *SRD5A2* and *CYP17A1*) has been explored in a cohort of Spanish prostate cancer patients, on the assumption that inter-individual levels of testosterone and dihydrotestosterone may be influenced by germ line polymorphisms in those genes, and this fact could determine clinical characteristics of the tumor, a phenomenon previously observed in relation to SNPs located in genes involved in DNA repair [21].

We have observed in our series that certain SNPs in *SRD5A1* seem to be associated to clinical variables of PCa such as initial PSA levels. Thus, G carriers for SNPs rs3822430 and rs1691053 were at higher risk of present initial PSA levels > 20 ng/mL. This finding may have an important influence in daily clinical practice. PSA levels decrease in PCa patients treated with 5α-reductase inhibitors [22]. Moreover, it has been suggested that DHT serum level may serve as a potential diagnostic marker of intraprostatic 5α-reductase activity during treatment of patients with 5α-reductase inhibitors [23]. Thus, the effect of 5α-reductase inhibitors in terms of PSA and DHT levels would be highly influenced by the presence of certain genotypes in *SRD5A1*. Interestingly, there are not studies exploring the role of SNPs in *SRD5A1* in relation to the response to 5α-reductase inhibitors. It is known that specific SNPs located in *SRD5A1* are associated to low enzymatic activity [24], but in any case, the knowledge of *SRD5A1* polymorphisms in prostate cancer is very limited and contradictory. In a recent study, men with AG or GG versus AA genotypes in rs1691053 are at higher risk of develop prostate cancer [25], which supports our results and suggests a role for this specific SNP in prostate cancer. With respect to rs3822430, it has been studied in the context of biochemical failure, with no significant associations reported [10]. The role of SNPs in *SRD5A1* in PCa seems to be reinforced by the fact that a specific haplotype in that gene (GCTTGTAGTA) was strongly associated to tumor size and Gleason score. The consequences that this specific haplotype may have in the function of the enzyme have to be explored. Recently, a systematic analysis of both constitutional and somatic (prostate cancer) variants of steroid 5α-reductase type II indicates significant pharmacogenetic variation for both finasteride and dutasteride response, and allow to map areas of the wild-type enzyme that are responsible for the time-dependent inhibition for either (or both) enzyme inhibitor(s) [26]. SNPs are the basics of pharmacogenetics, and it is possible that individual SNPs or specific haplotypes can explain this heterogeneity. However, we did not observe any SNP or haplotype in *SRD5A2* associated to any clinical variable in our series. Nonetheless, other authors have published a role of SNPs rs4952197 or rs523349 (both included in our analyses) in terms of biochemical recurrence [10]. In fact, rs523349 is one of the best characterized polymorphisms in *SRD5A2* gene, and it has been associated to poor prognosis

in PCa patients in terms of PSA failure [27] or extracapsular invasion [28]. Shibata et al. and Jaffe et al. published their results in smaller cohorts of patients than our series; and Audet-Walsh et al. included subjects from different ethnic origins. We have recently published that there is an important influence of ethnics in the analysis of genetic variations [15, 16]. Together with differences in the genotype determination techniques or in the strategies for SNP analyzes, these could be plausible reasons to explain the differences observed between our results and those reported by others.

In our series, none of the polymorphisms in *CYP17A1* was statistically associated to the clinical variables considered. It has been reported an association between the risk of disease development and SNPs rs743572, rs619824 and rs2486758 [5, 7]. However, the results are contradictory and conditioned by the ethnic group [2]. An association with PCa survival has been recently reported for rs10883783 polymorphism after a median of follow-up of 13.2 years [8]. The role of this gene in prostate cancer appears to be limited to developing thereof, not altering the biological characteristics of the tumor once debuted. This hypothesis seems to be reinforced by the fact that abiraterone acetate shows the best results when the testicular production of testosterone is abolished by hormonal treatment, otherwise, the specific inhibition of *CYP17A1* is not success [27].

Functional polymorphisms in *SRD5A1* gene may affect the production rate of dehydrotestosterone and the subsequent local exposure to androgens of androgen-responsive cells of the prostate, thus influencing biological characteristics of the tumor assessable by tumor size, Gleason score or diagnostic PSA level. How these SNPs and haplotype may influence the function of the enzyme and the levels of testosterone is a question that must be answered. In any case, our results should be considered at a clinical level and would influence the therapeutic management of prostate cancer patients, particularly in relation to hormone treatment. Thus, those PCa patients carrying certain polymorphisms or haplotypes associated to higher levels of diagnostic PSA (such as G carriers for SNPs rs3822430 and rs1691053) should have a proper

hormone deprivation treatment to ensure the control of PSA level and minimize the risk of early biochemical relapse.

The present study has some weaknesses that need to be highlighted: i) although 494 PCa patients seem sufficient to obtain statistically reliable results, it is possible that some results may be of stochastic nature, especially for those SNPs with lower MAF; ii) other factors associated to prostate cancer (i.e. age, familiar aggregation, toxic habits or some kind of diets) have not been taken into account in the present study; iii) since it is a multicenter study, clinical endpoints were obtained by different physicians (including pathologists, urologists and radiation oncologists), and there may be an inter-observer bias in the determination of any of these parameters; and iv) observations should be confirmed in an independent cohort of patients including pathology data from patients who have been treated with radical prostatectomy. However, the present study provides a number of advantages that contribute to their credibility. First, the high confidence of the genotyping system and the clinical endpoints considered (of total routine in daily clinical practice) minimize the possibility of inferring results due to the subjectivity of the observations. Second, the present study has been performed in a series of Spanish prostate cancer patients which was homogeneous regarding to ethnicity, a factor that has been shown to be important in association studies with polymorphisms. And third, all the determinations (15,808 in total) were performed with the same methodology (OpenArray, Applied Biosystems), with the same batch of chips and by the same investigator, thus minimizing biases from technical origin.

5. CONCLUSSIONS

This study reveals the importance of single nucleotide polymorphisms in relation to the biological characteristics of prostate cancer at diagnosis, defining novel actions of *SRD5A1* gene in a cohort of Spanish prostate cancer patients. Future experiments are warranted to explore the role of the genotypes in the follow-up and prognosis.

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TABLES

Table 1. Genotypic and allelic frequencies among Spanish prostate cancer patients

Gene/SNP	n	Genotypic frequencies						Allelic frequencies			
SRD5A1											
rs166050	479	AA	0.59	AG	0.36	GG	0.05	A	0.77	G	0.23
rs501999	488	CC	0.28	CT	0.47	TT	0.24	C	0.52	T	0.48
rs518673	476	AA	0.09	AG	0.41	GG	0.50	A	0.29	G	0.71
rs3822430	481	AA	0.33	AG	0.51	GG	0.16	A	0.59	G	0.41
rs500182	489	GG	0.01	GT	0.18	TT	0.82	G	0.09	T	0.91
rs8192120	483	AA	0.10	AC	0.45	CC	0.46	A	0.32	C	0.68
rs4702378	490	CC	0.06	CT	0.31	TT	0.63	C	0.21	T	0.79
rs1691053	488	AA	0.87	AG	0.13	GG	0.00	A	0.93	G	0.07
rs39848	472	CC	0.14	CT	0.47	TT	0.39	C	0.38	T	0.62
rs3797179	482	AA	0.04	AG	0.26	GG	0.70	A	0.17	G	0.83
SRD5A2											
rs2208532	475	AA	0.30	AG	0.49	GG	0.21	A	0.54	G	0.46
rs12470143	488	CC	0.30	CT	0.52	TT	0.17	C	0.57	T	0.43
rs2281546	490	GG	0.02	GT	0.24	TT	0.74	G	0.14	T	0.86
rs3754838	480	CC	0.02	CT	0.20	TT	0.78	C	0.12	T	0.88
rs4952222	490	AA	0.00	AC	0.00	CC	1.00	A	0.00	C	1.00
rs7562326	483	CC	0.02	CT	0.21	TT	0.77	C	0.13	T	0.87
rs2300702	477	CC	0.18	CG	0.49	GG	0.33	C	0.42	G	0.58
rs4952197	485	AA	0.06	AG	0.33	GG	0.61	A	0.22	G	0.78
rs676033	487	CC	0.51	CT	0.40	TT	0.09	C	0.71	T	0.29
rs523349	398	CC	0.40	CG	0.43	GG	0.17	C	0.62	G	0.38
rs9332975	487	CC	0.02	CT	0.20	TT	0.78	C	0.12	T	0.88
rs7594951	489	CC	0.78	CT	0.20	TT	0.02	C	0.88	T	0.12
CYP17A1											
rs3781287	485	GG	0.21	GT	0.47	TT	0.32	G	0.45	T	0.55

rs1004467	486	AA	0.79	AG	0.20	GG	0.02	A	0.89	G	0.11
rs743572	465	AA	0.32	AG	0.48	GG	0.20	A	0.56	G	0.44
rs10883782	477	AA	0.65	AG	0.32	GG	0.04	A	0.81	G	0.20
rs619824	487	AA	0.22	AC	0.50	CC	0.28	A	0.47	C	0.53
rs2486758	482	CC	0.04	CT	0.29	TT	0.67	C	0.18	T	0.82
rs17115100	490	GG	0.80	GT	0.19	TT	0.01	G	0.89	T	0.11
rs4919686	492	AA	0.48	AC	0.41	CC	0.10	A	0.69	C	0.31
rs10786712	491	CC	0.33	CT	0.47	TT	0.20	C	0.57	T	0.43
rs6163	474	AA	0.19	AC	0.48	CC	0.32	A	0.43	C	0.57

Table 2. Description of clinical variables

Clinical	**n**	**(%)**
Clinical tumor size (cT)		
cT1a – cT2a	270	(54.7)
cT2b – cT2c	141	(25.8)
cT3 – cT4	66	(13.4)
NA	17	(3.4)
Initial PSA (ng/mL)		
< 10	306	(61.9)
10 – 19.99	103	(20.9)
> 20	79	(16.0)
NA	6	(1.2)
Gleason score		
< 7	226	(45.7)
7	195	(39.5)
> 7	71	(14.4)
NA	2	(0.4)

Abbreviations: PSA, prostate specific antigen; NA, not available.

Table 3. Significant associations between initial PSA and SNPs

SNP	Genotypes	Initial PSA (ng/mL)			P#
		< 10	10-19.99	> 20	
rs3822430	AA	92	44	21	0.015
	AG	44	44	14	
	GG	21	50	7	
rs1691053	AA	272	89	59	0.008
	AG	30	13	18	
	GG	0	1	0	
rs3822430 - rs1691053	AA + AA	80	38	10	0.002
	G carriers	213	64	66	

Chi square test.

Table 4. Frequency of detection (%) of the three most frequent haplotypes located in chromosomes 2 (*SRD5A2*), chromosome 5 (*SRD5A1*), and chromosome 10 (*CYP17A1*) in our series

	chr2	freq	chr5	freq	chr10	freq
Hap1	TTTGGACTCCTC	32.8	GCTTGTAGTA	21.6	AAGCAGTAGT	28.1
Hap2	TTCACGCTCGTT	21.0	ACCTATGGCA	18.2	CGGAATCCAT	17.9
Hap3	TTCGGGCTCCTC	11.1	GATTACAGTA	15.1	CAGAATCCAC	16.0

Abbreviations: hap, haplotype; chr, chromosome; freq, frequency.
Haplotypes in chromosome 2 are formed by the SNPs: rs9332975, rs2281546, rs12470143, rs4952197, rs2300702, rs2208532, rs7594951, rs7562326, rs4952222, rs523349, rs3754838, rs676033.
Haplotypes in chromosome 5 are formed by the SNPs: rs518673, rs8192120, rs501999, rs500182, rs166050, rs4702378, rs3822430, rs3797179, rs39848, rs1691053.
Haplotypes in chromosome 10 are formed by the SNPs: rs619824, rs10883782, rs17115100, rs4919686, rs1004467, rs3781287, rs10786712, rs6163, rs743572, rs2486758.

Table 5. Haplotypes significantly associated to clinical variables

Haplotype	**chr**	**Exp(B)**	**C.I. 95%**	**P**
		cT1-T2a vs. cT2b-T4		
Homozygous for GCTTGTAGTA#	5	1		
Other haplotypes		3.823	(1.280-11.416)	0.016
		Gleason score (<7 vs. ≥7)		
Homozygous for GCTTGTAGTA#	5	1		
Other haplotypes		2.808	(1.134-6.953)	0.026

Abbreviations: chr, chromosome; C.I., confidence interval
Reference category (binary logistic regression test)