

## **CYP1B1 variants are associated with prostate cancer in non-Hispanic and Hispanic Caucasians**

Joke Beuten<sup>1</sup>, Jonathan A.L.Gelfond<sup>2</sup>, John J.Byrne<sup>1</sup>,  
Ivana Balic<sup>3</sup>, AnaLisa C.Crandall<sup>1</sup>, Teresa L.Johnson-Pais<sup>4</sup>,  
Ian M.Thompson<sup>5</sup>, Douglas K.Price<sup>6</sup> and Robin J.Leach<sup>1,4,5,\*</sup>

<sup>1</sup>Department of Cellular and Structural Biology, <sup>2</sup>Department of Epidemiology and Biostatistics, <sup>3</sup>Department of Psychiatry, <sup>4</sup>Department of Pediatrics and <sup>5</sup>Department of Urology, The University of Texas Health Science Center at San Antonio, TX 78229-3900, USA and <sup>6</sup>Medical Oncology Branch, National Cancer Institute, Bethesda, MD 20892, USA

\*To whom correspondence should be addressed. Tel: +1 210 567 6947;  
Fax: +1 210 567 6781;  
Email: leach@uthscsa.edu

**Cytochrome P450 1B1 (CYP1B1) is involved in the activation of many carcinogens and in the metabolism of steroid hormones. We compared allele, genotype and haplotype frequencies of six single-nucleotide polymorphisms (SNPs) within CYP1B1 among non-Hispanic Caucasians (496 cases and 498 controls) and Hispanic Caucasians (153 cases and 240 controls). In the Hispanic Caucasians, the GG genotype for rs1056836 decreased the risk for prostate cancer (PCa) when compared with the CC genotype [odds ratio (OR) = 0.31, *P* = 0.04, 95% confidence interval (CI) = 0.10–0.96]. Among non-Hispanic Caucasian men with more aggressive PCa, the prevalence of several SNPs (rs2567206, rs2551188, rs2617266, rs10012 and rs1056836) was significantly associated with the disease status. A common C-G-C-C-G-A haplotype for rs2567206-rs2551188-rs2617266-rs10012-rs1056836-rs1800440 showed an inverse association with PCa risk in Hispanic Caucasians (OR = 0.19, *P* = 0.04, 95% CI = 0.04–0.95) and with aggressive disease status (i.e. Gleason score  $\geq$ 7) in non-Hispanic Caucasian cases (OR = 0.64, *P* = 0.008, 95% CI = 0.47–0.89). In the non-Hispanic Caucasian cases, a second major haplotype T-A-T-G-C-A was positively associated with the high-grade disease status (OR = 1.77, *P* = 0.002, 95% CI = 1.24–2.53). Our findings suggest that genetic polymorphisms in CYP1B1 may modify the risk for PCa and support the role of CYP1B1 as a candidate gene for PCa.**

### **Introduction**

The diagnosis of prostate cancer (PCa; MIM 176807) is common—in the USA approximately one in six men is diagnosed with PCa in his lifetime (1). PCa is the most common non-skin cancer and the second leading cause of cancer death in men in USA. It is estimated that there would be 218 890 new cases of PCa and 27 050 deaths resulting from PCa in 2007 in USA (1). The underlying etiology of PCa remains poorly understood, with both genetic predisposition and environmental factors likely to play a role. From a large twin study, the proportion of PCa risk accounted for by inheritable factors was estimated to be 42% [95% confidence interval (CI) = 29–50%] (2). Heritable risk factors can involve highly penetrant susceptibility genes with low frequencies and/or low penetrant genes with higher frequencies in the population. A segregation study in 263 PCa families found that the disease is more probable due to the

**Abbreviations:** CI, confidence interval; CYP1B1, cytochrome P450 1B1; MAF, minimum allele frequency; OR, odds ratio; PCa, prostate cancer; SNP, single-nucleotide polymorphism.

contributions of two to four PCa susceptibility genes than one gene (3) which corroborates earlier predictions that the majority of PCa cases most likely involve more common, low- to moderate-penetrant alleles in genes that are components of pathways that influence prostate function (4–6).

The cytochromes P450 (P450) are a very large gene family of constitutive and inducible enzymes with a major role in the oxidative activation and/or deactivation of a wide range of xenobiotics, including many potential carcinogens and several anticancer drugs (7). Cytochrome P450 1B1 (CYP1B1) is a member of the CYP1 gene family and one of the major enzymes involved in the hydroxylation of estrogens, a reaction of key relevance in hormonal carcinogenesis (8). Particularly, CYP1B1 catalyzes the hydroxylation of 17 $\beta$ -estradiol (E2) at the C<sub>4</sub> position (9,10) and testosterone at the C<sub>6 $\beta$</sub>  and C<sub>12 $\alpha$</sub>  positions (11).

The human CYP1B1 gene (CYP1B1, MIM 601771) has been mapped to chromosomal region 2p21–p22 (12) and is composed of three exons and two introns spanning ~12 kb of DNA (13). The mRNA is 5.2 kb and the open reading frame begins at the 5' end of the second exon. The predicted protein sequence is 543 amino acids. Although CYP1B1 is expressed in a wide variety of tissues, expression is particularly high in the prostate, breast and ovary (14–16). Furthermore, no CYP1B1 protein expression was detected in normal prostate tissue in contrast to the overexpression of CYP1B1 protein in prostate carcinoma (17).

More evidence for a potential role of CYP1B1 in carcinogenesis comes from several observations; CYP1B1 interacts with a number of structurally diverse clinically useful anticancer agents in a substrate-dependent manner (18). The importance of CYP1B1 in chemical carcinogens is well illustrated in animal models in which metabolites of CYP1B1 have been shown to induce PCa (19,20). Furthermore, CYP1B1-null mice, created by targeted gene disruption in embryonic stem cells, were protected from 7,12-dimethylbenz(*a*)anthracene-induced malignant lymphomas (21). McGrath *et al.* reported on the correlation of a polymorphism that increases the degradation efficiency of CYP1B1 with decreased cancer risk, suggesting that an increased rate of CYP1B1 protein degradation mediates a protective effect against tumorigenesis (22,23).

Several polymorphisms in the CYP1B1 gene have been described (see <http://www.imm.ki.se/CYPalleles/cyp1b1.htm>), of which four results in amino acid substitutions, including the common single-nucleotide polymorphisms (SNPs) rs10012, rs1056827, rs1056836 and rs1800440. Functional studies suggest that these non-synonymous SNPs may alter enzymatic activity and catalytic specificity of CYP1B1 (24–26). Several studies have evaluated the relationship between CYP1B1 polymorphisms and the risk of cancers, including PCa. An association between rs1056836 and the risk of PCa was found among Caucasians and Japanese, respectively (27,28). Others found that carrying the CYP1B1 rs1056827 T allele was positively associated with PCa in Japanese and in Caucasian cancer cases (29,30). Furthermore, a common haplotype C-G-C-C-G for the rs2567206-rs2551188-rs2617266-rs10012-rs1056827 combination was associated with an increased risk for PCa (31) and the CYP1B1 rs1056827(T)-rs1056836(C) haplotype was positively associated with PCa among men with high aggressive disease (30).

To date, no association study of CYP1B1 variants has been reported in Hispanic Caucasian PCa cases. We therefore performed a case–control study among non-Hispanic Caucasians and Hispanic Caucasians and

determined possible associations of SNPs as well as haplotypes using six polymorphisms in *CYP1B1*.

**Materials and methods**

*Study participants*

Participants used in this study were part of the San Antonio Center for Biomarkers of Risk of Prostate Cancer cohort. San Antonio Center for Biomarkers of Risk of Prostate Cancer is funded by the National Cancer Institute and has been prospectively enrolling healthy male volunteers between 2001 and 2007. On each annual visit, a digital rectal examination was performed and prostate-specific antigen level was determined. From this cohort, 183 incident cases (124 non-Hispanic Caucasians and 59 Hispanic Caucasians) were available. We also included 520 cases with a known history of PCa which are enrolled within the same time period in a parallel study of prevalent PCa from the same metropolitan population and recruited using the same means. The

prevalent cases had a median time period of 3 years (range 0–25 years) between disease diagnosis and enrollment into the study. Cases had biopsy-confirmed PCa and controls consisted of male volunteers of at least 45 years old who had normal digital rectal examinations and prostate-specific antigen levels <2.5 ng/ml on at least two and up to six study visits. Race/ethnicity was self-reported on a questionnaire filled out at a clinic site at the time of recruiting. We used the Hazuda model to define the racial/ethnic groups (32). All participants have established USA residency and are believed to be long-term residents. Clinical characteristics of the study group are shown in Table I. A total of 994 non-Hispanic Caucasians (496 cases and 498 controls) and 393 Hispanic Caucasians (153 cases and 240 controls) were selected for this study. This study received Institutional Review Board approval from the University of Texas Health Science Center at San Antonio and informed consent was obtained from all subjects. For the purposes of determining whether variants within *CYP1B1* are associated with the disease aggressiveness, we subdivided the cases of each ethnic group in two subgroups: men with a low grade of aggressiveness (Gleason score <7) and men with a high grade of aggressiveness (Gleason score ≥7). Gleason score data were available for 392 non-Hispanic Caucasians (225 with low grade and 157 with high grade) and 132 Hispanic Caucasians (69 with low grade and 63 with high grade).

**Table I.** Clinical data of the study group

Subgroup	No. of cases (n = 706)	No. of controls (n = 1334)
Ethnic background, n (%)		
Non-Hispanic Caucasian	536 (76.0)	833 (62.4)
Hispanic Caucasian	170 (24.0)	501 (37.6)
Age, n (%)		
≤50	18 (2.6)	163 (12.2)
51–60	146 (21.0)	503 (37.7)
61–70	313 (44.6)	443 (33.2)
>70	223 (31.8)	225 (16.9)
Mean	66 (SD 8.2)	60.8 (SD 9.1)
Prostate-specific antigen (ng/ml)		
≤4.0	147	1337
4.1–10.0	30	0
10.1–20.0	1	0
>20.0	5	0
Mean	4.67 (SD 19.3)	0.87 (SD 0.46)
Family history of PCa, n (%)		
Negative	504 (71.6)	1084 (81.2)
Positive	200 (28.4)	250 (18.8)
Gleason, n (%)	n = 514	
Non-Hispanic Caucasian		
<7	225 (59)	
7	103 (27)	
>7	54 (14)	
Hispanic Caucasian		
<7	69 (52)	
7	39 (30)	
>7	24 (18)	

SD, standard deviation.

*DNA isolation and genotyping*

DNA was isolated from participants' whole blood cells using a QIAamp DNA Blood Maxi Kit (Qiagen, Valencia, CA). Seven SNPs, rs2567206, rs2551188, rs2617266, rs10012, rs1056827, rs1056836 and rs1800440 were selected within *CYP1B1* based on the following criteria: (i) for replication purposes, we preferentially choose SNPs that have been reported and for which significant data were found; (ii) we used the most common non-synonymous SNPs with a minimum allele frequency (MAF) > 0.1 and (iii) we included intronic SNPs with a MAF > 0.2 to obtain as optimal coverage of the gene as possible. Intronic polymorphisms, either individually or in combination, may affect gene expression or mRNA splicing efficiency (33–35). Table II shows the primer and probe sequences for the seven SNPs analyzed in this study. SNPs rs2567206, rs2551188, rs2617266, rs10012 and rs1056836 were provided by Applied Biosystems (ABI, Foster City, CA) using their assay-by-design method and analyzed using their standard TaqMan® allelic discrimination reaction conditions. Primers and probes for the rs1800440 variant were designed using Primer Express (ABI) and this SNP was genotyped using the TaqMan allelic discrimination assays consisting of 10 ng/μl of genomic DNA, 900 nM of each primer, 200 nM of each probe and 1× TaqMan® Universal Master Mix. Cycling conditions were 95°C for 10 min followed by 40 cycles of denaturing at 95°C for 15 s and annealing/extending at 60°C for 1 min. Genotypes were determined using an ABI 7900 Sequence Detection System using the SDS 2.1 software (ABI, Foster City, CA). For the rs1056827 variant, the target sequences were amplified by polymerase chain reaction in 20 μl reaction mix containing 35 ng of genomic DNA, 750 nM of each primer, 200 nM of each probe, 200 μM deoxyribonucleotide triphosphates 1× Qiagen buffer containing MgCl<sub>2</sub>, 1× Q-solution and 1 U Qiagen *Taq* polymerase. After initial denaturation at 95°C for 2 min, reactions were carried out for 35 cycles at 94°C for 30 s, 51°C for 30 s and 72°C for 30 s. The polymerase chain reaction products of 635 bp were digested without further purification with 2 units of *NaeI* restriction enzyme (New England Biolabs3, Ipswich, MA) at 37°C for 2 h. After digestion, samples were run on a 3% agarose gel and the digested products were classified into

**Table II.** Position, nucleotide variation, primer and probe sequences of seven SNPs within *CYP1B1*

SNP	dbSNP ID	Location	Chromosome position	AA change	Alleles	F and R primer sequences	Probe sequences
–1001C/T	rs2567206	Promoter	38215182		C/T	F: GGTAACCGCGCTTCATCAC R: TGTACCGAGCGTGGTTCTG	CTCCAATCA/GAGGCCG
–263G/A	rs2551188	Intron 1	38214445		A/G	F: CGCAAGGCGCGTAACG R: CGCCACCGCCTCGAT	TCCCCAG/AATTGCA
–13C/T	rs2617266	Intron 1	38214195		C/T	F: CTGCACCCCTGAGTGTC A R: GGCTGGTGCCCATGCT	CCTTCTCCTC/TTCTGTCC
142C/G (R48G)	rs10012	Exon 2	38214041	Arg/Gly	C/G	F: GGTGCTGAGGCAACGGA R: CAGTGGCCACGCAAACG	CAGCTCC/GGGTCCG
355G/T (A119S)	rs1056827	Exon 2	38213828	Ala/Ser	G/T	F: AGACCACGCTCTGCTACTC R: GAACCTCTCGTTGTGGCTGA	
4326C/G(L432V)	rs1056836	Exon 3	38209854	Leu/Val	C/G	F: TGTCAACCAGTGGTCTGTGAATC R: TGGATCAAAGTTCTCCGGGTTA	ATGACCAC/GTGAAGTG
4390A/G(N453S)	rs1800440	Exon 3	38209790	Asn/Ser	A/G	F: ACTTTGATCCAGCTCGATTCTTG R: CGCCTTTTGCCCACTGAA	CTCATCAG/ACAAGGAC

F, forward; R, reverse.

homozygotes for the *Ala/Ala* alleles (340 and 295 bp fragments), homozygotes for the *Ser/Ser* alleles (635 bp fragment) and heterozygotes (635, 340 and 295 bp fragments). To ensure quality control of the genotyping results, ~5% of the samples were randomly selected and re-genotyped for each SNP.

#### Statistics

The allele frequency for each SNP was determined for the two ethnic groups individually and the frequencies among the case-control groups or cases with and without family history were compared using the chi-square test. Association analyses were performed using R statistical software version 2.5.1 and were stratified by ethnicity. The odds ratio (OR) and its 95% CI was estimated by unconditional logistic regression as a measure of the associations between genotypes and PCa risk and also to compare associations of Gleason grade (low grade <7 versus high grade ≥7) among PCa cases. The homozygous carriers of the common allele as determined in the whole sample group represented the reference group in the SNP analysis. Study age among controls was the age at last follow-up, and age among cases was the age at PCa diagnosis. Our control group was younger than our PCa cases with a mean age (standard deviation) of 60.8 (9.1) years for the control samples and a mean age (standard deviation) of 66 (8.2) for the cases ( $P < 0.0001$ ). Because of this difference and the fact that PCa risk increases with age, all the ORs were adjusted for age. Logistic regression was used to calculate the ORs of the haplotypes using the method implemented in the *haplo.ccs* package (36). Only major haplotypes, with an estimated frequency of >5%, are considered in this report. The model was fit for each major haplotype so that the OR of each major haplotype was computed relative to a reference group consisting of all other haplotypes including rare haplotypes. Three genetic models (additive, dominant and recessive) were tested, with age as a covariate. All statistical tests were two-sided and significance was set at  $P < 0.05$ . To measure linkage disequilibrium and define the block structure between the markers for each race/ethnicity, we used Haploview version 4 beta 15 [(37); <http://www.broad.mit.edu/mpg/haploview/>].

## Results

#### Allele frequencies

To decrease confounding because of population admixture, we analyzed the two race/ethnic groups separately. All allelic distributions in the Hispanic Caucasian samples were in Hardy-Weinberg equilibrium. In the non-Hispanic Caucasians marker rs1056827 showed a deviation from Hardy-Weinberg equilibrium ( $P = 0.001$ ). Upon re-genotyping this marker in our samples, we found a high error rate in both ethnic groups (whereas for the other SNPs the rate was <1.5%) and therefore this marker was left out in further analyses (data not shown). No significant differences were found in the allelic frequencies between the cases and controls of each ethnic group (Table III). We also looked at the allelic distributions between cases with and without a family history but did not find significant differences between the two groups (data not shown).

#### Association of SNPs with PCa risk or disease aggressiveness

When each of the SNPs was analyzed separately, age-adjusted logistic regression analysis demonstrated that Hispanic Caucasian carriers of the GG genotype when compared with the CC genotype showed a marginally significant decreased risk for PCa (OR = 0.31,  $P = 0.04$ , 95% CI = 0.10–0.96; Table IV). No significant relationships between any of the SNPs and PCa were observed in non-Hispanic Caucasians (Table IV). However, among non-Hispanic Caucasian

men with more aggressive PCa (Gleason grade ≥7), variants rs2567206, rs2551188, rs2617266, rs10012 and rs1056836 were directly correlated with the disease aggressiveness (Table V). Positive associations with aggressive disease were found for four SNPs with ORs ranging from 1.64 (95% CI = 1.05–2.57;  $P = 0.03$  for the AG genotype at rs2551188) to 3.38 (95% CI = 1.51–7.57;  $P = 0.003$  for the TT genotype at rs2617266). An inverse association for disease aggressiveness was found for men carrying the GG genotype at rs1056836 as compared with the CC genotype (OR = 0.45, 95% CI = 0.24–0.84,  $P = 0.01$ ) (Table V). In the Hispanic Caucasian cases, marker rs2567206 showed a marginally significant decrease in the risk for disease aggressiveness (OR = 0.44,  $P = 0.04$ , 95% CI = 0.20–0.97; Table V).

#### Association of haplotypes with PCa risk or disease aggressiveness

Haplotype block structure was determined using the genotype data from each race/ethnic group and defined according to the default settings proposed by Gabriel *et al.* (38). In the non-Hispanic Caucasians, the pairwise  $D'$  values were >0.98 and all six SNPs were part of one haplotype block. In the Hispanic Caucasians, high linkage disequilibrium was found between the rs2567206-rs2551188-rs2617266-rs10012 polymorphisms ( $D'$  values >0.98), and these four SNPs are part of one haplotype block. A second haplotype block encompassing rs1056836 and rs1800440 was present in this race/ethnic sample group. Therefore, there is no independent risk association for the six SNPs in the Hispanic Caucasians and for the four strongly linked SNPs in the non-Hispanic Caucasians.

Interestingly, when measuring the effect of PCa risk, a common C-G-C-C-G-A haplotype (frequency of 22.9% in the study group) for SNPs rs2567206-rs2551188-rs2617266-rs10012-rs1056836-rs1800440 was found to decrease the risk for PCa in the Hispanic Caucasian samples under the recessive model (OR = 0.19,  $P = 0.04$ , 95% CI = 0.04–0.95; Table VI). No statistically significant results were found under the additive or dominant model in the Hispanic Caucasian samples. Furthermore, we did not find significant results in the non-Hispanic Caucasian samples when measuring the effect of the risk of PCa (data not shown).

However, among non-Hispanic Caucasian men with more aggressive PCa, this C-G-C-C-G-A haplotype (frequency of 42.5% in the study group) showed a significant inverse association with disease aggressiveness under the additive model (OR = 0.64,  $P = 0.008$ , 95% CI = 0.47–0.89; Table VI). In this ethnic sample group, a second major haplotype T-A-T-G-C-A (28.0%) for the six-SNP combination was found to have an increased risk; this haplotype has the opposite alleles at all, but the last, SNPs as compared with the protective haplotype discussed above and was found to significantly increase the risk in cases with a high grade of PCa under the additive model (OR = 1.77,  $P = 0.002$ , 95% CI = 1.24–2.53; Table VI). No significant results were found when measuring the effects of disease aggressiveness in the cases of the Hispanic Caucasian samples (data not shown).

## Discussion

The prostate is a hormone-responsive organ in which androgens are believed to stimulate growth and secretory functions whereas

**Table III.** Allele frequencies for the more common allele by race/ethnicity and case-control status

SNP	dbSNP rs number	Common allele	Non-Hispanic Caucasians			Hispanic Caucasians			NCBI reported Europeans
			Cases (n = 496)	Controls (n = 498)	$P^a$	Cases (n = 153)	Controls (n = 240)	$P^a$	
–1001C/T	rs2567206	C	0.72	0.73	0.76	0.73	0.72	0.76	0.72
–263G/A	rs2551188	G	0.72	0.73	0.60	0.71	0.69	0.54	0.72
–13C/T	rs2617266	C	0.72	0.73	0.85	0.73	0.71	0.64	0.77
142C/G (R48G)	rs10012	C	0.72	0.73	0.54	0.71	0.69	0.50	0.84
4326C/G(L432V)	rs1056836	C	0.57	0.56	0.69	0.78	0.73	0.12	0.59
4390A/G(N453S)	rs1800440	A	0.80	0.80	0.80	0.84	0.86	0.30	0.83

<sup>a</sup>Assumes Hardy-Weinberg equilibrium.

**Table IV.** Association of *CYP1B1* polymorphisms with PCa in Hispanic and non-Hispanic Caucasians

SNP	Genotype	Hispanic Caucasians					Non-Hispanic Caucasians						
		Cases (n = 153), n (%)	Controls (n = 240), n (%)	OR <sup>a</sup>	95% CI	P	Trend P value	Cases (n = 496), n (%)	Controls (n = 498), n (%)	OR <sup>a</sup>	95% CI	P	Trend P value
rs2567206	C/C	80 (52)	126 (52)	1.00	—	—	0.97	266 (54)	275 (55)	1.00	—	—	0.74
	T/T	10 (7)	21 (9)	0.85	0.37–1.96	0.70		44 (9)	46 (9)	1.00	0.64–1.57	0.99	
	C/T	63 (41)	93 (39)	1.12	0.72–1.74	0.61		186 (38)	177 (36)	1.08	0.83–1.42	0.54	
	T/T + C/T			1.07	0.70–1.63	0.75				1.07	0.83–1.38	0.60	
rs2551188	G/G	76 (50)	114 (48)	1.00	—	—	0.75	263 (53)	275 (56)	1.00	—	—	0.60
	A/A	11 (7)	22 (9)	0.81	0.36–1.82	0.60		44 (9)	46 (9)	1.01	0.64–1.58	0.98	
	A/G	66 (43)	104 (43)	1.01	0.65–1.57	0.95		189 (38)	174 (35)	1.14	0.87–1.49	0.34	
	A/A + A/G			0.98	0.64–1.49	0.92				1.11	0.86–1.43	0.41	
rs2617266	C/C	80 (52)	123 (51)	1.00	—	—	0.92	265 (53)	270 (55)	1.00	—	—	0.82
	T/T	10 (7)	21 (9)	0.80	0.35–1.86	0.61		43 (9)	46 (9)	0.97	0.61–1.53	0.89	
	C/T	63 (41)	95 (40)	1.10	0.70–1.70	0.69		188 (38)	176 (36)	1.09	0.83–1.42	0.54	
	T/T + C/T			1.04	0.68–1.59	0.84				1.06	0.82–1.37	0.64	
rs10012	C/C	76 (49)	113 (47)	1.00	—	—	0.74	262 (53)	275 (56)	1.00	—	—	0.53
	G/G	11 (8)	22 (9)	0.80	0.36–1.82	0.60		45 (9)	46 (9)	1.04	0.66–1.63	0.87	
	C/G	66 (43)	105 (44)	1.01	0.65–1.57	0.96		189 (38)	175 (35)	1.14	0.87–1.49	0.35	
	G/G + C/G			0.96	0.64–1.49	0.91				1.12	0.87–1.44	0.39	
rs1056836	C/C	83 (58)	130 (55)	1.00	—	—	0.12	164 (33)	161 (33)	1.00	—	—	0.71
	G/G	4 (3)	22 (9)	<b>0.31</b>	<b>0.10–0.96</b>	<b>0.04</b>		96 (20)	101 (20)	0.93	0.65–1.34	0.70	
	C/G	55 (39)	85 (36)	0.93	0.59–1.47	0.76		231 (47)	234 (47)	0.98	0.74–1.31	0.90	
	G/G + C/G			0.82	0.53–1.26	0.36				0.97	0.74–1.27	0.81	
rs1800440	A/A	104 (70)	170 (74)	1.00	—	—	0.36	299 (65)	309 (64)	1.00	—	—	0.80
	G/G	4 (3)	3 (1)	1.70	0.35–8.24	0.51		19 (4)	22 (5)	0.90	0.47–1.70	0.74	
	A/G	41 (27)	57 (25)	1.19	0.73–1.93	0.48		142 (31)	148 (31)	0.99	0.75–1.31	0.94	
	G/G + A/G			1.22	0.76–1.95	0.41				0.98	0.75–1.28	0.87	

<sup>a</sup>Adjusted for age. Significant results are shown in bold.

**Table V.** Association of *CYP1B1* polymorphisms with disease aggressiveness in Hispanic and non-Hispanic Caucasians

SNP	Genotype	Hispanic Caucasians					Non-Hispanic Caucasians						
		High grade (n = 59), n (%)	Low grade (n = 61), n (%)	OR <sup>a</sup>	95% CI	P	Trend P value	High grade (n = 149), n (%)	Low grade (n = 215), n (%)	OR <sup>a</sup>	95% CI	P	Trend P value
rs2567206	C/C	36 (61)	27 (44)	1.00	—	—	0.27	66 (44)	125 (58)	1.00	—	—	<b>0.002</b>
	T/T	5 (8)	3 (5)	1.24	0.26–5.75	0.78		20 (13)	12 (6)	<b>3.14</b>	<b>1.44–6.84</b>	<b>0.004</b>	
	C/T	18 (31)	31 (51)	<b>0.44</b>	<b>0.20–0.97</b>	<b>0.04</b>		63 (43)	78 (36)	1.52	0.97–2.38	0.07	
	T/T + C/T			0.52	0.25–1.08	0.08				<b>1.74</b>	<b>1.14–2.66</b>	<b>0.01</b>	
rs2551188	G/G	33 (56)	27 (44)	1.00	—	—	0.50	64 (43)	125 (58)	1.00	—	—	<b>0.002</b>
	A/A	5 (8)	3 (5)	1.35	0.29–6.29	0.70		19 (13)	12 (6)	<b>3.08</b>	<b>1.40–6.75</b>	<b>0.005</b>	
	A/G	21 (36)	31 (51)	0.57	0.26–1.22	0.14		66 (44)	78 (36)	<b>1.64</b>	<b>1.05–2.57</b>	<b>0.03</b>	
	A/A + A/G			0.64	0.31–1.33	0.23				<b>1.84</b>	<b>1.20–2.81</b>	<b>0.005</b>	
rs2617266	C/C	35 (59)	28 (46)	1.00	—	—	0.41	64 (43)	126 (59)	1.00	—	—	<b>0.001</b>
	T/T	5 (8)	3 (5)	1.32	0.29–6.13	0.72		19 (13)	11 (5)	<b>3.38</b>	<b>1.51–7.57</b>	<b>0.003</b>	
	C/T	19 (33)	30 (49)	0.52	0.24–1.12	0.09		66 (44)	78 (36)	<b>1.66</b>	<b>1.06–2.59</b>	<b>0.03</b>	
	T/T + C/T			0.59	0.28–1.24	0.16				<b>1.87</b>	<b>1.22–2.86</b>	<b>0.004</b>	
rs10012	C/C	33 (56)	26 (43)	1.00	—	—	0.40	64 (43)	125 (58)	1.00	—	—	<b>0.002</b>
	G/G	5 (8)	3 (5)	1.30	0.28–6.05	0.74		19 (13)	13 (6)	<b>2.85</b>	<b>1.32–6.15</b>	<b>0.008</b>	
	C/G	21 (36)	32 (52)	0.53	0.24–1.13	0.10		66 (44)	77 (36)	<b>1.66</b>	<b>1.06–2.61</b>	<b>0.03</b>	
	G/G + C/G			0.59	0.29–1.24	0.16				<b>1.84</b>	<b>1.20–2.81</b>	<b>0.005</b>	
rs1056836	C/C	35 (65)	34 (61)	1.00	—	—	0.57	58 (39)	60 (29)	1.00	—	—	<b>0.01</b>
	G/G	1 (2)	2 (3)	0.47	0.04–5.68	0.55		21 (14)	49 (23)	<b>0.45</b>	<b>0.24–0.84</b>	<b>0.01</b>	
	C/G	18 (33)	20 (36)	0.87	0.39–1.94	0.73		69 (47)	102 (48)	0.71	0.44–1.14	0.16	
	G/G + C/G			0.83	0.38–1.83	0.65				<b>0.62</b>	<b>0.40–0.98</b>	<b>0.04</b>	
rs1800440	A/A	38 (66)	41 (71)	1.00	—	—	0.26	85 (63)	129 (66)	1.00	—	—	0.97
	G/G	3 (5)	0 (0)	ND	ND	ND		4 (3)	10 (5)	0.59	0.18–1.96	0.39	
	A/G	17 (29)	17 (29)	1.12	0.49–2.54	0.79		46 (34)	58 (29)	1.20	0.75–1.94	0.45	
	G/G + A/G			1.31	0.59–2.92	0.51				1.11	0.70–1.76	0.65	

High grade includes case with Gleason score ≥7, Low grade includes case with Gleason score <7, ND, not determined.

<sup>a</sup>Adjusted for age. Significant results are shown in bold.

**Table VI.** Association of common *CYP1B1* haplotypes with PCa risk in Hispanic Caucasians or disease aggressiveness in non-Hispanic Caucasians

SNP combination	Hispanic Caucasians					Non-Hispanic Caucasians						
	PCa risk					Disease aggressiveness						
	Frequency (%)	No. of haplotypes		OR <sup>a</sup>	95% CI	P <sup>b</sup>	Frequency (%)	No. of haplotypes		OR <sup>a</sup>	95% CI	P <sup>c</sup>
Cases (n = 153)		Controls (n = 240)	High grade (n = 149)					Low grade (n = 215)				
rs2567206-rs2551188-rs2617266-rs10012-rs1056836-rs1800440												
C-G-C-C-G-A	22.9	2	14	<b>0.19</b>	<b>0.04–0.95</b>	<b>0.04</b>	42.5	81	138	<b>0.64</b>	<b>0.47–0.89</b>	<b>0.008</b>
T-A-T-G-C-A	27.3	10	19	0.79	0.35–1.79	0.57	28.0	77	81	<b>1.77</b>	<b>1.24–2.53</b>	<b>0.002</b>
C-G-C-C-C-G	14.8	4	3	1.82	0.35–9.46	0.47	19.2	50	64	1.14	0.77–1.71	0.51
C-G-C-C-C-A	31.8	16	24	1.04	0.52–2.08	0.91	9.3	22	35	0.79	0.47–1.34	0.38

High grade includes cases with Gleason  $\geq 7$ , Low grade includes case with Gleason  $< 7$ . Only common haplotypes ( $> 5\%$ ) are shown.

<sup>a</sup>OR is age adjusted.

<sup>b</sup>Recessive model.

<sup>c</sup>Additive model. Significant results are shown in bold.

estrogens act as growth inhibitors (39). Substantial evidence for a potential role of *CYP1B1* in PCa comes from the observations that *CYP1B1* protein is overexpressed in prostate carcinoma whereas no *CYP1B1* protein expression is detected in normal prostate tissue (17). In addition, *CYP1B1* has been implicated as an important gene up-regulated in PCa (40) and experiments with *CYP1B1*-knockout mice show the critical role of this enzyme in 7,12-dimethylbenz(a)anthracene-induced tumorigenesis (21). Furthermore, *CYP1B1* is also known to activate several carcinogens that are suspected to be involved in PCa development (20) and expression of *CYP1B1* was increased in humanized mouse models after the removal of androgen signaling via chemical or surgical castration (41).

In humans, *CYP1B1* is genetically polymorphic and several common SNPs have been identified, four of which change the amino acid sequence. A number of studies have investigated possible associations of SNPs in the human *CYP1B1* gene with the risk of PCa in Caucasian (27,30,31) and in Japanese men (28,29). However, association analysis of polymorphisms within *CYP1B1* indicated that the significant findings are controversial, and/or several studies have failed to reveal an association. Furthermore, no study to date has examined the role of *CYP1B1* variants in the Hispanic Caucasian men with PCa. To confirm previous findings in non-Hispanic Caucasians and to test the hypothesis that *CYP1B1* variants are associated with PCa risk in Hispanic Caucasians, we performed a case-control genotype analysis of six polymorphisms within *CYP1B1* in 994 non-Hispanic Caucasians and 393 Hispanic Caucasians.

No significant differences were observed for allele frequencies between cases and controls of each race/ethnic group. In addition, the allele distributions between cases with and without a family history of PCa were not significantly different excluding a possible bias in genetic predisposition. In the Hispanic Caucasians, the GG genotype at rs1056836 showed a marginally significant decrease in the risk for PCa when compared with the CC genotype. Moreover, a common C-G-C-C-G-A haplotype (frequency of 22.9%) for the six consecutive SNPs was found to be protective for PCa in the Hispanic Caucasian samples. This is to our knowledge the first report on the significant association of *CYP1B1* genotypes and haplotypes with the risk to develop PCa in Hispanic Caucasian men.

Among non-Hispanic Caucasian men with more aggressive PCa, four of the six variants analyzed (rs2567206, rs2551188, rs2617266 and rs10012) were associated with the highest grade disease stage whereas variant rs1056836 showed an inverse association with disease aggressiveness. Furthermore, carrying the common C-G-C-C-G-A haplotype for the six SNPs decreased the risk of PCa among non-Hispanic Caucasian men with aggressive disease. In this sample group, a second haplotype T-A-T-G-C-A (frequency of 28.0%) was positively associated with the disease aggressiveness. This risk haplotype shows the opposite allele at each SNP, except for the rs1800440

variant, as compared with the haplotype that is found to be protective in the Hispanic Caucasians and in the non-Hispanic Caucasians cases with high-grade PCa. The reported significance levels are nominal, in particular in the Hispanic Caucasians, and were not adjusted for multiple comparisons. This inflates the probability of a false discovery above the nominal *P* value, and tests of marginal significance should be interpreted cautiously given the number of hypotheses tested. A Bonferroni correction applied across the SNPs is too conservative here given that the SNPs are in strong linkage disequilibrium and are not statistically independent. In order to lessen the bias towards positive findings, both positive and negative results are reported.

Our marginally significant association results of rs1056836 with PCa risk in the Hispanic Caucasians and significant results in non-Hispanic Caucasians with high aggressive disease status suggest a protective effect of the G allele at rs1056836 rather than a risk effect. The protective haplotype C-G-C-C-G-A that is significantly associated with PCa risk also showed the presence of the G allele at rs1056836. This is in contrast with other studies that report on an inverse association between PCa and the rs1056827(T)-rs1056836(C) haplotype (31) or a positive association with the rs1056836G/G genotype alone (27). Moreover, our haplotype results are the opposite of the findings of Chang *et al.* (31) who reported on a frequent C-G-C-C-G haplotype for rs2567206-rs2551188-rs2617266-rs10012-rs1056827 that increased the risk for PCa in Caucasian men. These discrepancies might be seen because the allelic distribution of the *CYP1B1* polymorphisms is different among different ethnicities, which could result in the observed differences in the rate and development of PCa across populations (42,43). Other possible explanations for between-study variability could be that most of the reported association studies are based on small sample sizes and might not give enough power to detect true associations.

Functional studies indicate that certain combinations of alleles can drastically alter the kinetic properties of *CYP1B1* in the formation of both the 2- and 4-hydroxylation of 17 $\beta$ -estradiol. Particularly, combinations that contain the R48G, A119S and L432V amino acid substitutions had the highest *K<sub>m</sub>* and the lowest *V<sub>max</sub>*, indicating that haplotype combinations of these SNPs might have important effects on protein folding (44). However, the observed differences in catalytic activity in some studies were relatively modest and/or variations between laboratories exist (24–26,45,46). Furthermore, no differences at the expression level of the protein were found with allelic changes (47). Since no study has evaluated *CYP1B1* estrogen metabolism in human cells, there currently is no evidence on whether allelic changes, either alone or in combination, can actually modulate the catalytic activity of *CYP1B1*.

A direct role of *CYP1B1* variants on the risk for PCa could be supported by our findings suggesting that interindividual differences in the activation of procarcinogens or in the metabolism of estrogen

due to the genetic variants within *CYP1B1* may contribute to the susceptibility of human cancers. However, *CYP1B1* might contribute in the risk for PCa in combination with other factors such as exposure to carcinogens and carcinogenic metabolism, hormonal activation causing genotoxicity and other genes that participate in *CYP1B1*-mediated pathways. Alternatively, there may be a causal variant residing on or near the corresponding haplotype that affects the risk of PCa development. Additional studies are needed to clarify the functional significance of our findings in the vulnerability/etiology of PCa.

Some of the limitations of this study are that race/ethnicity is self-reported and the significant differences seen in the genotype and haplotype frequencies with PCa or disease aggressiveness could be due to the population stratification. However, we believe that possible misclassifications are equally likely in cases and controls and therefore should not have a substantial impact on the outcome. Another limitation is that both incidence and prevalence cases were used in this study. The prevalent cases were recruited within the same time period from the same metropolitan population and using the same means as the incidence cases, such that both groups of cases corresponded as much as possible. The power of this study is limited by the sample size (994 non-Hispanic Caucasians and 393 Hispanic Caucasians), the minor allele frequencies (MAF) (28–43%), the baseline incidence of disease (~6%) and the unknown OR of a genetic risk factor. Assuming a Type I error of 0.05, an OR of 1.5 and a MAF of 30%, we estimated the power of the study with the method of Slager and Schaid to be 99% and 73% in non-Hispanic Caucasians and Hispanic Caucasians, respectively.

In conclusion, we confirm the likely involvement of *CYP1B1* in the etiology of PCa and we further provide the first evidence for an association of the *CYP1B1* gene with PCa in Hispanic Caucasian men. The risk of *CYP1B1* polymorphisms and PCa seems to be population-type dependent and further studies are warranted that address the discrepant findings between different ethnicities.

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