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Single-nucleotide Polymorphisms in p53 Pathway and Aggressiveness of Prostate Cancer in a Caucasian Population

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Abstract

Purpose—The tumor suppressor p53 plays a crucial role in maintaining genomic stability and tumor prevention. Mdm2, Mdm4 and Hausp are all critical regulators of the p53 protein. Despite the importance of p53 pathway in prostate cancer development and progression, little is known about the association of functional SNPs in the p53 pathway genes and prostate cancer aggressiveness.

Experimental Design—In this study, we analyze the association of SNPs in p53, Mdm2, Mdm4 and Hausp genes with prostate cancer clinicopathologic variables in a large hospital-based Caucasian prostate cancer cohort (N = 4073).

Results—We found that the Mdm2 SNP 309 T allele was associated with earlier onset prostate cancer ($P = 0.004$), higher Gleason scores ($P = 0.004$) and higher stages men undergoing a radical prostatectomy (RP) ($P = 0.011$). Both the Mdm4 and Hausp SNPs (rs1380576 and rs1529916) were found to be associated with higher D'Amico risk prostate cancer category at the time of diagnosis ($P = 0.023$ and $P = 0.046$, respectively). Mdm4 SNP was also found to be associated with higher Gleason score at RP ($P = 0.047$). We did not observe any statistically significant association between the p53 Arg72 Pro polymorphism and prostate cancer aggressiveness or pathologic variables.

Conclusions—These results suggested the importance of these p53 regulators in prostate cancer development and progression.

Keywords

Prostate cancer; TP53; MDM2; MDM4; HAUSP; Single-nucleotide Polymorphisms

Introduction

Tumor suppressor p53 lies at critical point of a complex signaling network for response to stress. The normal functioning p53 protein is involved in cell cycle arrest, DNA repair, apoptosis and maintenance of genetic integrity (1). Therefore, it is regarded as a potent barrier to cancer. Malfunction of the p53 pathway is an almost universal hallmark of human tumors (2). p53 is also found as one of the most commonly mutated genes in all types of human cancer. Mutations involving p53 are considered as late events during multi-step prostate carcinogenesis

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(3). Apparently, loss of the wild-type p53 function can contribute to the hormone-resistance of prostate cancer cells (4).

The Mdm2 oncoprotein is an established regulator of p53 via effects on p53 degradation and negative feedback inhibition (5). The level of the Mdm2 protein in a cell or organism has a significant impact on cancer formation. Mdm2 can also reduce androgen receptor signaling in prostate cancer via p53 inhibition and is implicated in ubiquitination and degradation of androgen receptor by the Akt pathway (6). Mdm2 protein is over-expressed in about 30–45% of analyzed prostate cancers (7,8). This over-expression was associated with advanced tumor stage or increased cell proliferation (7).

Mdm4, which is a structural homolog of Mdm2, binds to the amino terminus of p53, functioning as a major inhibitor of p53 activity (9,10). Hausp (herpesvirus-associated ubiquitin-specific protease) stabilizes Mdm2, Mdm4, and p53 via its specific deubiquitinase activity (11). The Mdm2, Mdm4 and Hausp proteins thus maintain p53 level and activity.

In humans, functional single nucleotide polymorphisms (SNPs) have been identified in both p53 and its negative regulators. The p53 SNP rs1042522, which is located at codon 72 in the putative Src homology 3 (SH3) binding domain, results in G to C change and an Arg to Pro amino acid substitution, influencing binding capacity and thereby functional properties of p53. The p53 Pro is a stronger inducer of target gene transcription than p53 Arg, whereas the p53 Arg seems to induce apoptosis with faster kinetics and suppresses transformation more efficiently than the p53 Pro variant (12). The relationship of p53 Arg72Pro with cancer susceptibility, reproduction and aging has been well-studied (13–16).

The pivotal role of Mdm2, Mdm4 and Hausp in the control of p53 function argues that polymorphisms at these loci may be important for the modulation of p53 function. A SNP (309 T > G) (rs2279744) in the promoter region of Mdm2, increases affinity for binding stimulatory protein (Sp) 1 leading to increased Mdm2 expression and the subsequent attenuation of the p53 pathway (17). This SNP has been associated with susceptibility of certain types of cancer, including breast, lung, gastric and colon cancer (18–20).

Recent studies on the haplotype SNP structure on Mdm4 and Hausp indicated the presence of candidate SNPs that influence p53 function and subsequently confer cancer risk. Mdm4 gene SNP (rs2279744) was reported under positive evolutionary selection and associated with risk of breast and ovarian cancers, or human fertility in Caucasian population (21,22). Hausp gene SNP (rs1529916) was also associated with human fertility (21).

Several studies have investigated the association of p53 and Mdm2 polymorphisms with prostate cancer susceptibility (23–30). However, results among these studies are inconsistent and inconclusive. Moreover, Mdm4 and Hausp polymorphisms have never been studied in prostate cancer. One of the most important questions in prostate cancer research is to identify people at risk of developing aggressive forms of the disease. Therefore, we are interested in the study of genetic variants in p53 pathway that are associated with more aggressive and/or with a better response to current therapies. By studying a large well-defined homogenous ethnic background patient cohort, we evaluated the association between candidate p53 pathway SNPs and prostate cancer aggressiveness in Caucasian Americans.

Materials and Methods

Study population

The details of the Dana-Farber Harvard Cancer Center SPORE (Gelb Center) Prostate Cancer cohort have been previously described (31). Briefly, all patients seen at DFCI and Brigham

and Women's Hospital with a diagnosis of prostate cancer are approached to participate. The consent rate for patients is 86%. A total of 4073 prostate cancer patients diagnosed between 1976 and 2007, who had been consented during 1993 to 2007 to provide information and tissue and had blood collected for research purposes, were included in this study cohort.

To control the quality of the ethnicity information from the self-reported data, we sampled three percent of self-reported Caucasian (N = 180) and performed genotyping using 26 SNPs which can distinguish Caucasian population from Non-Caucasian populations (32); the genotyping data showed that none of the tested samples were in discordance. This confirmed the reliability of self-reported Caucasian ethnicity. For all individuals who ambiguously reported their ethnicity, such as reported as "American", or who do not have the ethnicity information, their Caucasian identity was determined by genotyping using the same set of 26 SNPs. Only reliably self-reported or SNP confirmed Caucasians were eligible for this study. Age at diagnosis was calculated from the date of the first positive biopsy. Using the D'Amico risk classification criteria, prostate cancer patients were identified as at low, intermediate or high risk of clinical recurrence after primary therapy (33). Since original D'Amico risk classification was set to predict biochemical outcome of localized patients, in this study, patients who diagnosed with N1 or M1 diseases were regarded as high D'Amico risk class. Within entire cohort, 1716 out of 4073 patients received radical prostatectomy (RP) as the primary treatment. RP Gleason scores and pathological stages of RP specimen were acquired by reviewing pathology reports.

Selection of SNPs

Two well-studied SNPs, p53 Arg72Pro (rs1042522) and Mdm2 SNP 309 (rs2279744), which have been shown to modify the activity or the levels of the p53 protein and influence cancer susceptibility, were chosen for this study (12,17). For Mdm4 gene, we chose to study SNP rs1380576, which is located in Mdm4 gene intron 1 region, and is in complete linkage disequilibrium (LD) ($r^2 = 1$) with a previously described SNP (rs2279744) which was reported under positive evolutionary selection and associated with risk of certain cancer (21,22). We also included the reported Hausp SNP (rs1529916), which is under evolutionary selection pressure and associated with human fertility in Caucasian (21).

DNA, SNPs and Genotyping assays

All DNA samples were extracted from peripheral whole blood using QIAamp DNA Blood mini kit (QIAGEN Inc, Valencia, CA). Genotyping was performed with Sequenom iPLEX matrix-assisted laser desorption/ionization-time of flight mass spectrometry technology. For quality control, about 5% random selected duplicates were included. No discrepancy between duplicates was observed in the genotyping data of all 4 SNPs. All SNPs had greater than 99% genotype passing rates.

Statistical methods

We analyzed each SNP as a categorical variable with a common homozygote, a rare homozygote, and a heterozygote. Observed genotype distributions were tested for departure from Hardy-Weinberg equilibrium using Pearson's goodness-of-fit test. No SNP violated Hardy-Weinberg equilibrium (all P value > 0.10).

To investigate the association between genotypes and early onset prostate cancer ($\leq$ 60 yrs), we estimate Odds Ratios (ORs) and their 95% confidence intervals (CIs) using unconditional logistic regression. Prostate cancer aggressiveness at diagnosis was categorized using D'Amico risk classes (low, intermediate or high risk) with criteria described previously. Generalized logistic regression was employed to evaluate its relationship with SNPs with the low risk group as reference for comparison. In a sub-cohort of patients who received RP, we also examined

the association between p53 pathway SNPs and RP Gleason Score or pathologic stages with unconditional logistic regression. The analyses, with the exception of those for early onset prostate cancer, were adjusted for age at diagnosis.

All statistical tests were performed using SAS version 9.1 (SAS Institute Inc, Cary, NC) and $P < 0.05$ (two-sided) was considered statistically significant.

Results

Subject characteristics

Selected clinical characteristics of study participants are described in Table 1. Briefly, all participants are Caucasian, and their mean age at diagnosis is 61.3 years (range: 42 to 91 years). Among patients with sufficient information for modified D'Amico risk classification, 1004 (30%) patients were low-risk, 1357 (40%) patients were intermediate-risk and 986 (30%) patients were high-risk. In patients received RP; 1161 (68%) men had organ-confined (T1/T2) disease at the time receiving surgery, while 475 (28%) men had extraprostatic tumor (T3/T4) and 80 (4%) men had metastatic tumor (N1 or M1). The post-RP Gleason score was <7 in 652 (39%), of 7 in 769 (45%) patients and ≥7 in 271 (16%) patients.

Correlation of SNPs with Age at diagnosis

We first estimate associations between the genotypes of p53 Arg72Pro, Mdm2 SNP 309, Mdm4 SNP (rs1380576) and Hausp SNP (rs152 9916) and the risk of developing an early onset prostate cancer (Table 2). Compared with the p53-72 Pro/Pro genotype, patients carrying one 72 Arg allele or two 72 Arg alleles had an OR of 1.42 (95% CI, 1.10–1.85) or 1.29 (95% CI, 1.00–1.66), respectively, of developing prostate cancer at or before 60 years. This result indicated that the p53 SNP had a borderline association with the age at diagnosis ($P = 0.021$). Compared with Mdm2 GG genotype, a moderate increase of OR was found in the association of the Mdm2 309 GT heterozygous genotype (OR, 1.23; 95% CI, 1.02–1.49) with the age at diagnosis. The Mdm2 309 TT genotype increased the likelihood early onset prostate cancer (OR 1.38; 95% CI, 1.14–1.68). However, we did not find a significant association of polymorphisms in the Mdm4 or Hausp genes with age at diagnosis.

Correlation of SNPs with D'Amico risk classification

In this analysis, we found that Mdm4 intron 1 SNP (rs1380576) was associated with D'Amico category ($P = 0.023$, Table 3). Comparing with Mdm4 GG genotype, the CG genotype or CC genotyping had ORs of 1.47 (95% CI, 1.11–1.95) or 1.31 (95% CI, 0.99–1.74) for developing intermediate risk prostate cancer, and ORs of 1.38 (95% CI, 1.01–1.89) or 1.50 (95% CI, 1.10–2.04) for developing high risk disease, respectively. We also observed that Hausp gene SNP (rs1529916) minor allele T allele conferred an increased risk of developing intermediate or high risk prostate cancer in a recessive manner. The Hausp AA genotype exhibited an OR of 1.44 (95% CI, 1.06–1.97) for developing intermediate risk prostate cancer, and an OR of 1.39 (95% CI, 0.99–1.94) for developing high risk prostate caner comparing with GG genotype. We did not observe any statistically significant association between p53 or Mdm2 genotypes and disease aggressiveness.

Correlation of SNPs with RP Gleason score

In the patients who underwent RP, cases with RP Gleason score ≥ 7 were compared with RP Gleason score < 7 (Table 4). The Mdm2 309 TT genotype had an OR of 1.51 (95% CI, 1.11–2.05) of having tumor RP Gleason score ≥ 7 at the time of receiving surgery compared with GG genotype. Similarly, Mdm4 GG genotype had an OR of 1.50 (95% CI, 1.07–2.09) to

developing higher RP Gleason score prostate cancer by the time of the surgery. The p53 and Hausp SNPs had no statistically significant association with the RP Gleason score.

Correlation of SNPs with Pathologic stages

We also classified men as either having evidence of extraprostatic (T3/T4) or metastatic (N1 or M1) disease or localized disease (T1/T2) at prostatectomy. In this analysis (Table 5), we only found Mdm2 309 TT genotype was correlated with the development of extraprostatic or metastatic prostate cancer (OR, 1.53; 95% CI, 1.10–2.12), compared with GG genotype.

Discussion

The p53 Arg72 Pro (rs1042522) polymorphism has been well characterized in both functional analyses and association studies (12–16). In a limited number of studies in prostate cancer, no consistent conclusion has been made about the association between the p53 polymorphism and prostate cancer risk or clinicopathological variables (23–26,28). This may be due to small sample sizes (< 200 prostate cancer cases) and/or mixed ethnic populations. In our present large homogeneous case-case analysis, we did not observe any statistically significant association between P53 Arg72 Pro polymorphism and prostate cancer aggressiveness or pathologic variables.

The Mdm2 SNP309 T > G (rs2279744) is a functional SNP that increases Mdm2 expression levels and attenuates the p53 pathway (17). Only a few studies have addressed an association of Mdm2 SNP 309 with prostate cancer in relatively small cohorts (27–30). Intriguingly, previous studies have implicated that G allele of Mdm2 SNP 309 was the high-risk allele in many other kinds of carcinoma (17,19,20). Our current study found that the T allele was associated with earlier onset prostate cancer (≤ 60 yrs) in the entire cohort, higher RP Gleason (≥ **7**) and advanced pathologic stages in RP patients. Our data is not the first to note that the T allele is associated with an increase risk or aggressiveness of prostate cancer. Kibel et al (29) examined the Mdm2 SNP309 polymorphism in a European-American cohort of 186 patients with advanced prostate cancer and 220 cancer-free controls and found that the T allele of Mdm2 SNP 309 was associated with an increased risk of advanced prostate cancer. In addition, studies from German (27) or Japan (30) suggested no correlation between a certain allelic variant of Mdm2 and an increased prostate cancer risk.

While speculative, these conflicting observations in different cancers raise the possibility that each variant (T or G) may be associated with increased risk for a particular disease (17–20). Furthermore, increased cancer risk could be associated with a specific geographic or genetic background (18). The effect of variance may also be influenced by the hormonal environment. Bond et al. demonstrated that the 309G variant is bound more efficiently by the transcriptional factor Sp1 (a co-activator for many hormone receptors) than 309 T allele. The estrogen receptor also binds the Mdm2 promoter in the region of SNP 309 (34). Hu and colleagues determined that, in estrogen-responsive cells, estrogen preferentially induced the transcription of Mdm2 form the SNP309 promoter and that the levels of Mdm2 in SNP 309 GG cells were higher than in heterozygous GT or TT cells (35). Bond et al hypothesize that women carrying a G allele would preferentially benefit from lower estrogen level which could retard the progression of their disease. They further proposed that Mdm2 SNP 309 G allele accelerates tumor formation in a gender-specific and hormone-dependent manner (36). While no evidence to date on androgen signaling was presented, given the importance of androgen axis in prostate tumorigenesis and progression, our observation suggests the possibility that the effect of Mdm2 309 G allele may also be dependent on hormonal signaling.

Mdm4 and Hausp are two important p53 regulators (11). A recently described haplotype structure analysis indicated the presence of candidate functional SNPs in Mdm4 and Hausp

(21,22). Kulkarni et al. performed a case-only study of breast cancer patients, and found that the including SNP in Mdm4 haplotype analysis (rs1563828) was associated with early age at diagnosis of ER negative, but not ER positive, breast cancers (37). Subsequently, they hypothesized that Mdm4 SNP may influent expression of different isoforms of Mdm4, or affect hormone signaling pathway in a cooperative way with Mdm2 (37). In our study, we evaluated the association between prostate cancer clinicopathologic variables and another intronic SNP rs1380576, which was located in intron 1 regulatory region of Mdm4 gene and in complete LD with rs1563828. We also included the reported candidate SNP in Hausp (rs1529916). Interestingly, both Mdm4 and Hausp SNPs were found to be associated with increased risk of intermediate or high D'Amico category prostate cancer at the time of diagnosis, indicating that they are associated with more aggressive prostate cancer. Furthermore, Mdm4 dominant genotype GG was also found to be associated with higher RP Gleason score in subset RP patients. These results implicated that Mdm4 and Hausp SNPs contribute to the risk of aggressive prostate cancer, probably through influencing their crucial roles in p53 network or affecting hormonal signaling.

In summary, we found that alleles in p53 gene regulators, Mdm2, Mdm4 and Hausp, instead of SNPs in p53 itself, are associated with earlier age at diagnosis or more aggressive prostate cancer patients in a Caucasian population. These results suggest the importance of these p53 regulators in prostate cancer development, while the biologic functions of these SNPs need further exploration.

Translational Relevance

P53, and its regulators, Mdm2, Mdm4, and Hausp, contain functional single nucleotide polymorphisms that attenuate the p53 pathway. Our study analyzes the role of four common polymorphisms in the p53 pathway, the P53 Arg72Pro (rs1042522), the Mdm2 SNP309 (rs2279744), the Mdm4 rs1380576 and the Hausp rs1529916, on the risk of developing aggressive prostate cancer. The results show that alleles in p53 gene regulators, Mdm2, Mdm4 and Hausp, instead of SNPs in p53 itself, are associated with either earlier age at diagnosis or more aggressive prostate cancer. These findings could point to the relevance of this pathway in the development of aggressive prostate cancer and lead to consideration of using these genetic variants as part of a multigenic model for identifying high-risk subgroups who may benefit from intensive therapeutic strategies.

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Abbreviations

RP	Radical Prostatectomy
SNPs	Single Nucleotide Polymorphisms
ORs	Odds Ratios
CIs	Confidence Intervals
LD	Linkage Disequilibrium

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Table 1

Clinical Characteristics of Study Participants

Cases (n)	4073
Ethnicity	Caucasian
Age at diagnosis (n)	3983
Mean (yrs)	61.3
Median (Q1, Q3)	61 (55, 67)
Biopsy Gleason Score at diagnosis (n)	3750
<7 (%)	1771 (47.2)
7 (%)	1272 (33.9)
>7 (%)	707 (18.9)
Clinical stage (n)*	3056
T1–T2 (%)	2807 (91.9)
T3–T4 (%)	65 (2.1)
N1 (%)	46 (1.5)
M1 (%)	138 (4.5)
PSA at diagnosis (n)	3518
Median (ng/mL)(Q1, Q3)	6 (5,11)
D' Amico risk classification (%)	3347
Low	1004 (30.0)
Intermediate	1357 (40.5)
High	986 (29.5)
RP sub-cohort (n)	1716
RP Gleason Score (n)	1692
<7 (%)	652 (38.5)
7 (%)	769 (45.5)
>7 (%)	271 (16.0)
Pathologic stage (n)*	1716
T1–T2 (%)	1161 (67.7)
T3–T4 (%)	475 (27.7)
N1 (%)	76 (4.4)
M1 (%)	4 (0.2)

*:T1–T2 represented for T1–T2, N0 or Nx, M0 or Mx; T3–T4 represent for T3–T4, N0 or Nx, M0 or Mx; N1 represent for T1–T4 or Tx, N1, M0 or Mx; M1 represent for T1–T4 or Tx, N0 or Nx, M1 according to AJCC staging.

Table 2

The Association Between p53 Pathways Genotypes and Age at Diagnosis

	All patients	> 60 (yrs)	≤ 60 (yrs)			
SNP	**N (%)**	**N (%)**	**N (%)**	**OR* (95% CI)**		***P* value****
p53 Arg72Pro (rs1042522)						
CC	274 (6.9)	161 (7.9)	113 (6.0)	Ref		0.021
CG	1593 (40.4)	797 (38.9)	796 (42.0)	1.42	(1.10–1.85)	
GG	2080 (52.7)	1092 (53.2)	988 (52.0)	1.29	(1.00–1.66)	
total	3947 (100.0)	2050 (100.0)	1897 (100)			
Mdm2 SNP 309 (rs2279744)						
GG	557 (14.1)	320 (15.6)	237 (12.5)	Ref		0.004
GT	1842 (46.7)	964 (47.1)	878 (46.3)	1.23	(1.02–1.49)	
TT	1546 (39.2)	764 (37.3)	782 (41.2)	1.38	(1.14–1.68)	
total	3945 (100.0)	2048 (100.0)	1897 (100.0)			
Mdm4 (rs1380576)						
GG	408 (10.3)	214 (10.4)	194 (10.1)	Ref		0.945
CG	1674 (42.1)	871 (42.2)	803 (42.0)	1.02	0.82–1.26	
CC	1894 (47.6)	978 (47.4)	916 (47.9)	1.03	0.83–1.28	
total	3976 (100.0)	2063 (100.0)	1913 (100.0)			
Hausp (rs1529916)						
GG	1968 (49.4)	1046 (50.7)	922 (48.1)	Ref		0.074
GA	1652 (41.5)	822 (39.8)	830 (43.3)	1.15	(1.01–1.31)	
AA	360 (9.1)	196 (9.5)	164 (8.6)	0.95	(0.76–1.19)	
total	3980 (100.0)	2064 (100.0)	1916 (100.0)			

* Odds Ratio of having early onset prostate cancer (≤ 60 yrs).

** *P* values for Wald Chi-square tests.

Table 3

Genotype frequencies, odds ratios and 95% CI comparing D'Amico Risk Classes

		N (%)		OR (95% CI)*				
SNP	**Low**	**Intermediate**	**High**	**Intermediate vs. Low**		**High vs. Low**		***P* value†**
p53 Arg72Pro (rs1042522)								
CC	70 (7.0)	91 (6.8)	79 (8.2)	Ref		Ref		0.938
CG	407 (40.7)	548 (40.7)	395 (40.8)	1.11	(0.79–1.56)	0.93	(0.65–1.33)	
GG	523 (52.3)	709 (52.6)	494 (51.0)	1.09	(0.78–1.53)	0.88	(0.62–1.25)	
total	1000 (100.0)	1348 (100.0)	968 (100.0)					
Mdm2 SNP 309 (rs2279744)								
GG	142 (14.2)	194 (14.4)	125 (12.8)	Ref		Ref		0.810
GT	452 (45.4)	626 (46.5)	454 (46.7)	1.03	(0.80–1.32)	1.19	(0.90–1.58)	
TT	402 (40.4)	526 (39.1)	394 (40.5)	0.98	(0.76–1.28)	1.17	(0.88–1.55)	
total	996 (100.0)	1346 (100.0)	973 (100.0)					
Mdm4 (rs1380576)								
GG	122 (12.1)	126 (9.3)	90 (9.1)	Ref		Ref		0.023
CG	396 (39.5)	597 (44.1)	397 (40.4)	1.47	(1.11–1.95)	1.38	(1.01–1.89)	
CC	485 (48.4)	632 (46.6)	496 (50.5)	1.31	(0.99–1.74)	1.50	(1.10–2.04)	
total	1003 (100.0)	1355 (100.0)	983 (100.0)					
Hausp (rs1529916)								
GG	517 (51.5)	669 (49.4)	472 (47.9)	Ref		Ref		0.046
GA	413 (41.1)	550 (40.6)	420 (42.6)	1.04	(0.87–1.24)	1.12	(0.93–1.35)	
AA	74 (7.4)	136 (10.0)	94 (9.5)	1.44	(1.06–1.97)	1.39	(0.99–1.94)	
total	1004 (100.0)	1355 (100.0)	986 (100.0)					

*adjusted by the age at diagnosis.

†*P* values for Wald Chi-square tests.

Table 4

Genotype frequencies, odds ratios and 95% CI comparing RP Gleason <7 and ≥ 7

	Gleason < 7	Gleason ≥ 7			
SNP	**N (%)**	**N (%)**	**OR (95%CI) ***		***P* value†**
p53 Arg72Pro (rs1042522)					
CC	43 (6.6)	72 (7.0)	Ref		0.923
CG	266 (41.0)	425 (41.4)	1.05	(0.69–1.61)	
GG	340 (52.4)	529 (51.6)	0.98	(0.64–1.48)	
total	649 (100.0)	1026 (100.0)			
Mdm2 SNP 309 (rs2279744)					
GG	107 (16.6)	137 (13.3)	Ref		0.004
GT	312 (48.4)	450 (43.8)	1.12	(0.83–1.51)	
TT	226 (35.0)	441 (42.9)	1.51	(1.11–2.05)	
total	645 (100.0)	1028 (100.0)			
Mdm4 (rs1380576)					
CC	89 (13.6)	109 (10.5)	Ref		0.047
CG	281 (43.1)	434 (41.7)	1.31	(0.94–1.84)	
GG	282 (43.3)	497 (47.8)	1.50	(1.07–2.09)	
total	652 (100.0)	1040 (100.0)			
Hausp (rs1529916)					
GG	332 (50.9)	509 (48.9)	Ref		0.628
GA	264 (40.5)	430 (41.4)	1.04	(0.84–1.29)	
AA	56 (8.6)	101 (9.7)	1.24	(0.86–1.80)	
total	652 (100.0)	1040 (100.0)			

* adjusted by the age at diagnosis.

† *P* values for Wald Chi-square tests.

Table 5

Genotype frequencies, odds ratios and 95% CI comparing Pathologic Stage in RP patients

	T1 or T2	T3 or T4 or N1 or M1			
SNP	**N (%)**	**N (%)**	**OR (95%CI)** *		***P* value**†
p53 Arg72Pro (rs1042522)					
CC	78 (6.8)	40 (7.3)	Ref		0.850
CG	482 (41.8)	222 (40.6)	1.10	(0.71–1.71)	
GG	593 (51.4)	285 (52.1)	1.13	(0.73–1.74)	
subtotal	1153 (100.0)	547 (100.0)			
Mdm2 SNP 309 (rs2279744)					
GG	176 (15.3)	67 (12.3)	Ref		0.011
GT	538 (46.7)	231 (42.3)	1.09	(0.78–1.52)	
TT	438 (38.0)	248 (45.4)	1.53	(1.10–2.12)	
subtotal	1152 (100.0)	546 (100.0)			
Mdm4 (rs1380576)					
GG	130 (11.2)	55 (9.9)	Ref		0.721
CG	487 (42.0)	235 (42.3)	1.16	(0.81–1.66)	
CC	544 (46.8)	265 (47.8)	1.17	(0.82–1.66)	
subtotal	1161 (100.0)	555 (100.0)			
Hausp (rs1529916)					
GG	587 (50.6)	265 (47.7)	Ref		0.534
GA	465 (40.0)	237 (42.7)	1.13	(0.91–1.41)	
AA	109 (9.4)	53 (9.6)	1.15	(0.80–1.66)	
subtotal	1161 (100.0)	555 (100.0)			

* adjusted by the age at diagnosis.

** *P* values for Wald Chi-square tests.