

Risk of Prostate Cancer and *Cyclin D1 A870G* polymorphism; a Study of Correlation

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Abstract

Cyclin D1 (CCND1) is a critical gene in regulating the progression of cell cycle from G1 to S phases. Like other cyclins, cyclin D1 is frequently dysregulated in multiple cancers. Various clinical and epidemiological studies have suggested the possible association of *cyclin D1 A870G* polymorphism with the development of various cancers. Hence, we investigated the role of *cyclin D1 A870G* polymorphism in modulating the risk of prostate cancer (CaP) in a Kashmiri population. We examined a case-control study in which 129 CaP cases were studied for *cyclin D1 A870G* polymorphism against 221 controls taken from the general population by employing the polymerase chain reaction-restriction length fragment polymorphism technique. We observed the *cyclin D1 A* allele was more frequently present in the CaP group than the control group. Furthermore, men with AA genotype have an increased risk for developing CaP as compared to the control groups. We found AA genotype statistically significantly associated with dwelling, lymph node metastases, histopathological grade, and PSA levels. Therefore, our findings suggest that A870G polymorphism is a risk factor for CaP development. Furthermore, men with AA genotype have an increased risk of developing CaP.

Keywords: CaP; *Cyclin D1*; Genetic polymorphism

Introduction

Prostate cancer (CaP) is a complex disease caused by multiple factors, and is one of the most frequent malignant diseases among men [1]. Accumulating studies have revealed the association of various genetic elements in the development of sporadic CaP [2,3], which might be used as potential therapeutic targets for CaP therapy. Hence, studying the role of genetic variants including single nucleotide polymorphic is of great significance in understanding the susceptibility CaP.

An unbalance in the cell cycle regulation has been found to play a key role in a various type of cancers, and is often associated with the onset of metastasis, in part, by negatively modulating the cell's ability to respond appropriately to DNA damage [4,5]. Reports have suggested that a strong relation between the alleles of these genes and CaP susceptibility [6-10]. *Cyclin D1*, also known as *CCND1*, is confined to the nucleus during G1 phase, and plays a pivotal role in the progression from the G1 to S phase of cell cycle [11]. During the CaP development, the expression of *cyclin D1* gene has often been seen overexpressed and is associated to poor prognosis of CaP [12-14]. These findings indicate the importance of *cyclin D1* (as a cell-cycle regulator) in carcinogenesis and progression of CaP [15].

Cyclin D1 mRNA is alternatively spliced into two different transcripts, which get translated into two different proteins [16,17]. The *A870G* polymorphism appears to modulate the splicing at codon 242 within conserved donor site of exon 4 of the gene [16-18]. Recent studies have suggested that the variant allele encoding A is a major source of variant transcript b in several kinds of cancer cells [16-18].

Moreover, cyclin D1 genotypes have been frequently found associated to variety of cancers [16,18,19-21].

Therefore, considering the importance of *cyclin D1* in the development of CaP, the present study was conducted to investigate the association between the *Cyclin D1 A870G* polymorphism and the susceptibility to CaP or its disease status.

Materials and Methods

Prostate cancer patients

A cohort of 129 CaP tissue samples were collected, with the histopathological diagnosis of the CaP. All the samples included in this study consisted of tumor and adjacent normal tissues. Only the tissue samples confirmed by histopathological studies to be cancerous were included in the study. The patient participation was obtained through informed consent and after approval from the Ethics Committee of Sher-I-Kashmir Institute of Medical Sciences.

The patients underwent histopathological diagnosis in the Department of Histopathology of our institution. Fifty prostate tumor samples and 45 benign hyperplasia (BHP) samples were collected. Samples from 80 healthy males over 50 years of age served as the controls. We also obtained prostate sextant biopsy specimens from 10 patients with elevated levels of serum PSA. Prostate cancer tissue samples consisting of tumor tissues and adjacent normal tissue were collected. Only histo-pathologically confirmed tumors were included in the study. The study was approved by the Ethical Committee of the Sher-i-Kashmir Institute of Medical Sciences.

Controls

Patients attending the Department of general medicine at Sher-I-Kashmir Institute of Medical Sciences (SKIMS) for general checkup were screened. A total of 260 patients visited the SKIMS, out of 260 patients only 221 agreed to take part in the present study, henceforth written informed consent was obtained from all patients for their participation.

A870G gene polymorphism

The *A870G* polymorphism lies within the conserved splice donor site of exon 4 of *cyclin D1 gene*. This SNP was detected by restriction fragment length polymorphism of PCR-amplified fragments. PCR reactions were carried out in a final volume of 25 µL containing 50 ng genomic DNA template, 1X PCR buffer (Biotools) with 2 mM MgCl₂, 0.4 µM of each primer (Genescript), 50 µM dNTPs (Biotools), and 0.5 U DNA polymerase (Biotools). For PCR amplification, the standard program was used as follows: After an initial denaturation step at 94°C for 7 min, followed by 35 denaturation cycles of 30s at 94°C, 30s of annealing at 54°C, and 30s of extension at 72°C, followed by a final elongation cycle at 72°C for 5 min. The amplified product was digested overnight with the *ScrFI* enzyme at 37°C and electrophoresed on a 3.0% agarose gel. Restriction fragments obtained were 146 and 22 bp for the G homozygote, and the unrestricted fragment was 167 bp for the A homozygote, while as the heterozygote displayed both the two bands.

Statistical analysis

Observed frequencies of genotypes in CaP patients were compared to controls using chi-square or Fisher exact tests when expected frequencies were small. The chi-square test was used to verify whether genotype distributions were in Hardy-Weinberg equilibrium. Statistical significance was set at P<0.05. Statistical analyses were performed with PASW version 18 Software.

Results

We determined the *A870G* polymorphism in 129 CaP patients; 22.4% (29 of 129) of the patients were homozygous for G/G variant, 27.9% (36 of 129) were heterozygous for A/G and 49.61% (64 of 129) were homozygous for A/A variant. Whereas the pattern of *A870G* polymorphism in 221 healthy controls is 11.31% (25 of 221) of the patients were homozygous for A/A variant, 48.86% (108 of 221) were heterozygous for A/G and 20.81% (46 of 221) were homozygous for G/G variant.

The present study consisted of 129 CaP patients, out of which 116 patients were above or equal to 65 years of age and 13 were of 50 years of age. Most of our patients (72) had grade I & II tumor, and 57 had grade III & IV tumor status. 104 CaP patients had widely or moderately differentiated histo-pathological grade and 25 had poorly differentiated histo-pathological grade. Men aged 65 years or over formed the greater part of the CaP cases. Table 1 show clinico-pathological characteristics related to the GG, AG and AA variants. A larger number of patients included in our study were having tumor stage I and II, whereas the patients having tumor stage III and IV were relatively small.

In the *cyclin D1 A870G* polymorphism, the AA genotype was statistically significantly associated with the dwelling, PSA levels, tumor stage, and histopathological grade (Table 1). The genotype was

not statistically significantly associated with the mean age at the time of diagnosis.

Variables	Cases (n=129)				P Value ; X ²
	Total	GG	A/G	AA	
	N=129 (%)	29 (22.4%)	36 (27.9%)	64 (49.61%)	
Age					
>50	13 (10.0%)	03 (23.07%)	04 (30.7%)	06 (46.15%)	0.56; 1.12
≤ 65	116 (90.0%)	25 (21.55%)	32 (27.5%)	59 (50.86 %)	
Dwelling					
Rural:	45 (34.6%)	08 (17.77%)	12 (26.66%)	25 (55.55%)	0.52; 1.21
Urban:	84 (65.4%)	23 (27.38%)	28 (33.33%)	33 (39.28%)	
Pesticide Exposure					
Low	44 (34.6%)	05 (11.3%)	09 (20.4%)	30 (68.1%)	0.06; 4.98
High	85 (65.4%)	20 (23.5%)	29 (34.1%)	36(42.3%)	
PSA Levels					
Low (4-8)	35 (27.7%)	05 (14.2%)	06 (17.1%)	24 (68.5%)	0.04; 6.02
High (8-13)	94 (72.3%)	23 (24.4%)	32 (34.0%)	39 (41.4%)	
Tumor Stage					
I +II (a+b)	72 (55.4%)	19 (26.3%)	20 (27.7%)	33(45.8%)	0.05; 4.96
III (a+b)+ IV	57 (44.6%)	09 (16.7%)	19 (33.3%)	29 (50.8%)	
Histopathological Tumor Grade					
PD					
MD+WD	25 (96.0%)	07 (28.0%)	10 (40.0%)	08 (32.0%)	0.04; 5.32
	57+47 (24.1%)	21 (20.1%)	28 (26.9%)	55 (52.8%)	

Table 1: Association between Cyclin D1 A870G and clinico-pathologic characteristics.

In this study, we observed genotype frequencies in cases and controls were in Hardy-Weinberg equilibrium. The genotype frequencies of *Cyclin D1 A870G* in cases and controls were observed and it was found that AA genotype is significantly associated with the CaP cases (p value=0.001) (Table 2).

<i>Cyclin D1 A870G</i> Genotype	Cases	Controls	OR (95% CI); p value
	(n=129)	(n=221)	
GG	29 (22.4%)	46 (20.81%)	0.72; 0.41-1.26; 0.31
AG	36 (27.9%)	108 (48.86%)	0.27; 0.18-0.42; 1.31
AA	64 (49.61%)	25 (11.31%)	2.56; 1.47-5.49; 0.001

Table 2: Genotype frequencies of *cyclin D1 A870G* polymorphism in cases & controls and their associations with the risk of Prostate Cancer.

Discussion

CaP has become a very common disease worldwide. CaP ranges from indolent localized to aggressive metastatic disease [22-24]. The current study found a strong association of the *cyclin D1 A870G* polymorphism and the susceptibility of development the CaP. *Cyclin D1* plays a key role in regulating various important cellular processes including cell proliferation, differentiation and apoptosis [25]. *Cyclin D1* has been also known to play an important role in the transition from the G1 to S phase of the cell cycle [16], and its deregulation has been seen involved in the pathogenesis of several types of cancers [26,27]. Although many polymorphisms of *cyclin D1* have been seen associated to different to types of cancer, but *G870A* polymorphism is most commonly studied. Till date, numerous studies have reported the role of *cyclin D1 G870A* polymorphism as a risk factor for CaP [8,10,28].

As the role of *cyclin D1 G870A* polymorphism has been as risk factor for prostate cancer in various cases [29]. Therefore, we attempted to study the cyclin D1 G870A polymorphism in CaP patients and control subjects of Kashmiri population. It has been already reported that the *Cyclin D1 G870A* (Present in splice variant of exon 4) polymorphism is associated to the risk of CaP [30]. Thus, indicating a possible role of *cyclin D1 G870A* polymorphism in the development of CaP. The present study included a total of 129 CaP patients; 22.4% (29 of 129) of the patients were homozygous for G/G variant, 27.9% (36 of 129) were heterozygous for A/G and 49.61% (64 of 129) were homozygous for A/A variant. Whereas the pattern of A870G polymorphism in 221 healthy controls is 11.31% (25 of 221) of the patients were homozygous for A/A variant, 48.86% (108 of 221) were heterozygous for A/G and 20.81% (46 of 221) were homozygous for G/G variant.

We also found a significant association between AA variant and PSA levels, suggesting that men who underwent a prostate biopsy due to abnormal (increased) serologic PSA, ultra-sonographic, or clinical findings later presented CaP in subsequent biopsies.

A significant association was found between the AA variant and rural dwelling, which depicts that in our population, patients from rural areas are at more risk than the urban ones. Furthermore, we found a significant association between the AA variant and the pesticide exposure, which might be because of the patient's occupation. Patients like farmers, which have high pesticide exposure, have shown a significant susceptibility of developing CaP.

We also found a significant association between AA variant and tumor grade III & IV, which might suggest its role in harboring a higher malignant behavior.

A significant correlation was found between the AA variant and poorly differentiated histopathological grade as has been reported by various earlier studies, suggesting that *cyclin D1 A870G* SNP may be involved in different mechanisms and possibly also various stages of tumor development.

We also found that the genotype frequencies of *A870G* in cases and controls, where AA genotype is significantly associated with the CaP cases (p value=0.001), suggesting that the CaP patients carrying a AA allele are at much higher risk for CaP.

Conclusion

In conclusion, our results support the idea of *cyclin D1 A870G* genotypic variance do have a strong association with the CaP development. The AA genotype was associated with increased of CaP in Kashmiri men as compare to GG genotype.

Conflict of Interest

The contributing Authors have no financial or any non-financial competing interests.

References

- Peters ME, Ostrander EA (2001) CaP: simplicity to complexity. Nat Genet 27: 134-135.
- Lichtenstein P, Holm NV, Verkasalo PK, Iliadou A, Kaprio J, et al. (2000) Environmental and heritable factors in the causation of cancer—analyses of cohorts of twins from Sweden, Denmark, and Finland. N Engl J Med 343: 78-85.
- Zhang Q, Zheng M, Qi XL, Liu F, Mao ZJ, et al. (2014) Effect of NQO1 C609T polymorphism on CaP risk: a meta-analysis. Onco Targets Ther 7: 907-914.
- Kibel AS, Isaacs WB (2000) G (1)/S cell cycle proteins as markers of aggressive prostate carcinoma. Urology 55: 316-322.
- Tomlins SA, Mehra R, Rhodes DR, Cao X, Wang L, et al. (2007) Integrative molecular concept modeling of CaP progression. Nat Genet 39: 41-51.
- Chang BL, Zheng SL, Isaacs SD, Wiley KE, Turner A, et al. (2004) A polymorphism in the CDKN1B gene is associated with increased risk of hereditary CaP. Cancer Res 64: 1997-1999.
- Henner WD, Evans AJ, Hough KM, Harris EL, Lowe BA, et al. (2001) Association of codon 72 polymorphism of p53 with lower CaP risk. Prostate 49: 263-266.
- Kibel AS, Suarez BK, Belani J, Oh J, Webster R, et al. (2003) CDKN1A and CDKN1B polymorphisms and risk of advanced prostate carcinoma. Cancer Res 63: 2033-2036.
- Suzuki K, Matsui H, Ohtake N, Nakata S, Takei T, et al. (2003) A p53 codon 72 polymorphism associated with CaP development and progression in Japanese. J Biomed Sci 10: 430-435.
- Wang L, Habuchi T, Mitsumori K, Li Z, Kamoto T, et al. (2003) Increase risk of CaP associated with AA genotype of cyclin D1 gene A870G polymorphism. Int J Cancer 103: 116-120.
- Sherr CJ (1996) Cancer cell cycles. Science 274: 1672-1677.
- Han EK, Lim JT, Arber N, Rubin MA, Xing WQ, et al. (1998) Cyclin D1 expression in human prostate carcinoma cell lines and primary tumors. Prostate 35: 95-101.
- Chen Y, Martinez LA, LaCava M, Coghlan L, Conti CJ (1998) Increased cell growth and tumorigenicity in human prostate LNCaP cells by overexpression to cyclin D1. Oncogene 16: 1913-1920.
- Drobnjak M, Osman I, Scher HI, Fazzari M, Cordon-Cardo C (2000) Overexpression of cyclin D1 is associated with metastatic CaP to bone. Clin Cancer Res 6: 1891-1895.

15. Agus DB, Cordon-Cardo C, Fox W, Drobnjak M, Koff A, et al. (1999) CaP cell cycle regulators: response to androgen withdrawal and development of androgen independence. J Natl Cancer Inst 91: 1869–1876.
16. Betticher DC, Thatcher N, Altermatt HJ, Hoban P, Ryder WD, et al. (1995) Alternate splicing produces a novel cyclin D1 transcript. Oncogene 11: 1005–1011.
17. Sawa H, Ohshima TA, Ukita H, Murakami H, Chiba Y, et al. (1998) Alternatively spliced forms of cyclin D1 modulate entry into the cell cycle in an inverse manner. Oncogene 16: 1701–1712.
18. Wang L, Habuchi T, Takahashi T, Mitsumori K, Kamoto T, et al. (2002) Cyclin D1 gene polymorphism is associated with an increased risk of urinary bladder cancer. Carcinogenesis 2002 23: 257–264.
19. Matthias C, Branigan K, Jahnke V, Leder K, Haas J, et al. (1998) Polymorphism within the cyclin D1 gene is associated with prognosis in patients with squamous cell carcinoma of the head and neck. Clin Cancer Res 4: 2411–2418.
20. Kong S, Amos CI, Luthra R, Lynch PM, Levin B, et al. (2000) Effect of cyclin D1 polymorphism on age of onset of hereditary nonpolyposis colorectal cancer. Cancer Res 60: 249–252.
21. Simpson DJ, Fryer AA, Grossman AB, Wass JA, Pfeifer M, et al. (2001) Cyclin D1 (CCND1) genotype is associated with tumour grade in sporadic pituitary adenomas. Carcinogenesis 22: 1801–1807.
22. Bracarda S, de Cobelli O, Greco C, PrayerGalletti T, Valdagni R, et al. (2005) Cancer of the prostate. Crit Rev Oncol Hematol 56: 379–396.
23. Mahon KL, Henshall SM, Sutherland RL, Horvath LG (2011) Pathways of chemotherapy resistance in castration-resistant CaP. Endocr Relat Cancer 18: R103–123.
24. Zhang L, Jiao M, Li L, Wu D, Wu K, et al. (2012) Tumorspheres derived from CaP cells possess chemoresistant and cancer stem cell properties. J Cancer Res Clin Oncol 138: 675–686.
25. Evan GI, Vousden KH (2001) Proliferation, cell cycle and apoptosis in cancer. Nature 411: 342–348.
26. Knudsen KE, Diehl JA, Haiman CA, Knudsen ES (2006) Cyclin D1: polymorphism, aberrant splicing and cancer risk. Oncogene 25: 1620–1628.
27. Musgrove EA, Caldon CE, Barraclough J, Stone A, Sutherland RL (2011) Cyclin D a therapeutic target in cancer. Nat Rev Cancer 11: 558–572.
28. Mandal RK, Mittal RD (2012) Are cell cycle and apoptosis genes associated with CaP risk in North Indian population? Urol Oncol 30: 555–561.
29. Zheng, Wan, He, Qi, Liu, et al. (2015) World. J Surg Oncol 13: 55.
30. Wang, Habuchi, Kenji (2003) Increased risk of CaP associated with aa genotype of cyclin d1 gene a870g polymorphism. Int J Cancer 103: 116–120.