Original Article

Association between *Cyclin D1* G870A Gene Polymorphism and Risk of Multiple Myeloma

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Abstract

Background: Multiple myeloma (MM) primarily origins from terminally differentiated B cells. The *Cyclin D1* (*CCND1*) gene polymorphism is located on the long arm of chromosome 11 in exon 4, and coding of the *Cyclin D1* protein is reported in MM. This study aimed at investigating the relationship between *CCND1* polymorphism and MM in northern part of Iran. **Materials and Methods:** This case–control study was performed on 87 patients with MM (case group) and 70 healthy individuals (control group). The population was selected from Touba Clinic, the largest referral center in Mazandaran province, Iran. Data analysis was done in SPSS software version 19. **Results:** The participants were aged 18–83 years (mean age: 53.8 ± 16.7 years), including 72 (45.9%) males and 85 (54.1%) females. The adjusted odds ratio (aOR) for GG genotype was found to be significant (aOR = 4.28, confidence interval [CI] 95% = 1.21–15.13). In addition, the presence of G allele compared to allele A increased the odds ratio (OR) of MM (OR = 2.28, CI 95% = 1.45–3.57). The age and sex aOR by age and sex was 2.08, CI 95% = 1.10–3.93. **Conclusion:** The current study elucidated that, unlike most previous surveys on *Cyclin D1 G870A* gene polymorphism that associated AA genotype with various types of malignancies, the GG genotype is a risk factor for MM.

Keywords: *Cyclin D1 gene, multiple myeloma, single-nucleotide polymorphisms*

Introduction

Cancer is one of the major health problems in the world and a major health crisis in Iran.[1,2] Multiple myeloma (MM) is a type of cancer caused by malignant proliferation of plasma cells, a class of white blood cells that normally produces antibodies.[3] This plasma cell neoplasm is characterized by the accumulation of malignant plasma cells within the bone marrow.[4] The disease is responsible for 13% of hematologic malignancies and 1% of cancers.[5] In fact, it is the second most common prevalent hematologic cancer, and the annual incidence of MM is estimated as 86,000 cases worldwide.[6] The clinical manifestations of MM include anemia, hypercalcemia, renal dysfunction, gammopathy, and light chain amyloidosis.[7] While the etiology of MM is largely unknown, there is strong evidence for inherited genetic susceptibility to the disease.[8] The disorder is a hereditary malignancy with high heterogeneity.[9] Evidence suggests that polymorphisms contribute to the development of various diseases.[10] Single-nucleotide polymorphisms (SNPs) are a significant source of genetic variation in humans and are thought to be responsible, at least partially, for individual differences in genetic susceptibility to complex diseases including MM.[11] *Cyclin D1 (CCND1)* is one of the most important cell cycle regulatory proteins that regulates G1 to S-phase transition of the cell cycle and acts as a catalyzer for tumor suppressor proteins, and its overexpression in cancers, including MM, has been reported.[12] This protein can promote cell proliferation or induce growth arrest and apoptosis.[11] The *CCND1* (*G870A*) gene polymorphism is located on the long arm of chromosome 11 in exon 4, and coding of the *Cyclin D1* protein is reported to have a role in developing MM.[12] This study aimed at investigating the association between *CCND1* gene (*G870A*) and MM.

Materials and Methods

The present case–control study was performed on the DNA of 70 cancer-free healthy controls and 87 patients with MM.

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The frequency of SNP of the *CCND1* gene (*G870A*) in patients with MM had been determined to apply polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) methods. The study population was selected from Touba Clinic, the largest referral center in Mazandaran province, in the north of Iran. Venous blood samples (10 mL) were obtained from patients and controls and were kept in an ethylenediaminetetraacetic acid tube. A portion of each blood sample was stored at −20°C for extraction of the DNA genome. Genomic DNA was extracted using a blood DNA extraction kit according to the manufacturer's recommendations (Denazist, Mashhad, Iran) and was stored at −80°C for SNP assessment. *CCND1* (*G870A*) genotyping was performed using the PCR-RFLP method. Primers for amplification of *CCND1* were as follows: 5'-GCA GTG CAA GGC CTG AAC CT-3' (sense) and 5'-GGG ACA TCA CCC TCA CTT AC-3' (antisense). PCR was carried out in 20-μL volumes containing 100-ng genomic DNA isolated from patients, 1 U *Taq* polymerase, 1.5-μM $MgCl_2$ (Denazist, Mashhad, Iran), 200-mM dNTPs (Denazist, Mashhad, Iran), 10 pmol of each primer, and 7-μL nuclease-free water. The 113-bp target DNA fragment containing the CC ↓ NGG site of *CCND1 G870A* was digested with *ScrF*I endonuclease enzyme (Thermo Fisher Scientific, MA, USA) at 37°C for 10 min. The digested products were separated by a 3% agarose gel containing safe stain dye (Denazist, Mashhad, Iran) in (Tris Borate EDTA) TBE (Denazist, Mashhad, Iran) buffer for 40 min at 50 V. The AA genotype produced a single 113-bp band due to loss of *ScrF*I restriction site. The wild-type GG genotype produced two bands (91-bp and 22-bp), and the G/A genotype produced three bands (113-bp, 91-bp, and 22-bp) [Figure 1]. Finally, all the digested segments were visualized under ultraviolet via gel documentation (Kimia Gen, DigiDoc H101, Mashhad, Razavi Khorasan Province, Iran).

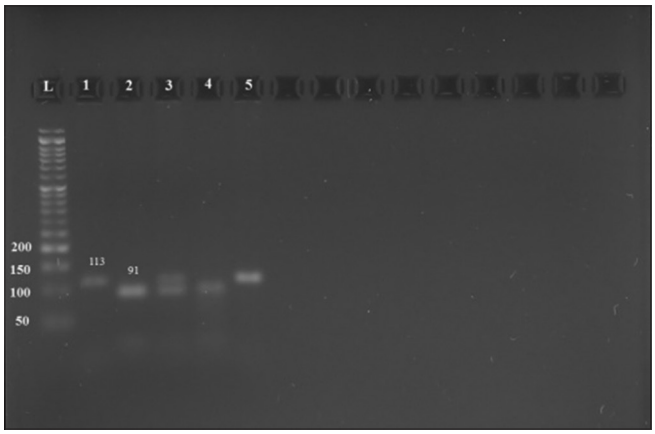

Figure 1: Genotyping analysis by digestion of the amplified product and RFLP. Lane 3 is GA heterozygote; lanes 2 and 4 are homozygote samples for GG, and lanes 1 and 5 are AA homozygote samples. The first lane (L) is 50 bp DNA ladder

Statistical analysis

All statistical analyses were performed using SPSS software V19 (SPSS Inc. Chicago, IL, USA). Student's *t*-test was applied to compare the age of patients in the two groups. Pearson's Chi-squared test or Fisher's exact test (when the expected number in any cell was <5) was used to compare the distribution of *CCND1* genotypes between cases and controls. The associations between *CCND1* polymorphisms and risk of MM were estimated by computing odds ratios (ORs) and 95% confidence intervals (CIs) from unconditional logistic regression analysis with adjustment for possible confounders (sex and age). $P < 0.05$ was considered statistically significant, and all statistical tests were two sided.

Ethical approval and consent to participate

Informed consent was obtained from all patients. The study protocol received ethical approval from the Ethics Committee of the Mazandaran University of Medical Sciences (IR.MAZUMS.REC.95.2733).

Results

The study was performed on 87 patients with MM and 70 healthy controls (mean age: 53.76 ± 16.4 years) [Table 1]. There were 72 (45.9%) males and 85 (54.1%) females. Age categorization of the patients showed that 85 (54.1%) persons were under the age of 60 years and 72 (45.9%) were ≥60 years. Genotyping for *CCND1 G870A* gene polymorphisms indicated that the frequency of AA, GG, and AG genotypes was 15 (17.2%), 32 (36.8%), and 40 (46.0%) in MM patients and 31 (44.3%), 13 (18.6%), and 26 (37.1%) in controls, respectively [Table 2]. In addition, A allele was found in 112 (71.3%) of the total population studied. In order to verify the Hardy–Weinberg equilibrium for different *CCND1 G870A* alleles, the observed frequency and expected frequency were investigated applying Chi-square test. There were 57 (65.5%) males and 30 (34.5%) females in the case group. In the control group, 15 (21.4%) males and 55 (78.6%) females were included ($P < 0.0001$) [Table 2]. In MM group, 64 (73.6%) patients were aged 60 years and older, whereas in the control group, there were only eight (11.4%) persons who were aged ≥60 years ($P < 0.0001$) [Table 2]. Distribution of different genotypes elucidated that the frequency of homozygous AA genotype was 31 (44.3%) and 15 (17.2%) in control and case groups, respectively. The frequency of GG homozygous genotype for cases and controls was 32 (36.8%) and 13 (18.6%), respectively, whereas the frequency of heterozygote AG genotype was 26 (37.1%) in the control group and 32 (36.8%) in patients with MM. These differences were statistically significant ($P = 0.001$) [Table 2]. Furthermore, comparison of GG homozygote genotype with AG/AA genotype was statistically significant ($P = 0.012$) [Table 2]. Homozygous GG variant was statistically significantly associated with an increased

Table 1: Demographic characteristics of patient and control groups

Variable	Total			Mean±SD		*P*-value
	Minimum	Maximum	Mean±SD	Control	MM	
Age (years)	18	83	53.76±16.74	41.76±15.72	63.43±9.91	0.0001**

**Significant at 0.01 level. SD: Standard deviation, MM: Multiple myeloma

Table 2: Demographic parameters of patient and control groups

Variable	MM (*n*=87), *n* (%)	Control (*n*=70), *n* (%)	Total (*n*=157), *n* (%)	*P*-value
		Gender		
Male	57 (65.5)	15 (21.4)	72 (45.9)	0.0001**
Female	30 (34.5)	55 (78.6)	85 (54.1)	
		Age group		
<60	23 (26.4)	62 (88.6)	85 (54.1)	0.0001**
≥60	64 (73.6)	8 (11.4)	72 (45.9)	
		Genotype		
AA	15 (17.2)	31 (44.3)	46 (29.3)	0.001**
GG	32 (36.8)	13 (18.6)	45 (28.7)	
AG	40 (46.0)	26 (37.1)	66 (42.0)	
AA/AG	55 (63.2)	57 (81.4)	112 (71.3)	0.012*
GG	32 (36.8)	13 (18.6)	45 (28.7)	

*Significant at 0.05 level, **Significant at 0.01 level. MM: Multiple myeloma

Table 3: Raw and adjusted odds ratio for different genotypes for multiple myeloma

Genotype	Crude OR			aOR (sex and age)		
	OR	95% CI	*P*-value	aOR	95% CI	*P*-value
A Allele	1		Reference			
G Allele	2.282	1.45-3.57	0.0001**	2.087	1.10-3.93	0.023*
AA	1		Reference			
AG	3.179	1.44-7.00	0.004**	2.684	0.90-8.04	0.078
GG	5.087	2.09-12.41	0.0001**	4.282	1.21-15.13	0.024*
AA/AG	1		Reference			
GG	3.815	1.84-7.91	0.0001**	3.183	1.15-8.84	0.026*

*Significant at 0.05 level, **Significant at 0.01 level. CI: Confidence interval, OR: Odds ratio, aOR: Adjusted OR

risk of MM (OR = 5.087; 95% CI: 2.09–12.41; *P* = 0.0001) [Table 3]. The OR adjusted for confounder variables including sex and age was also found to be statistically significant (adjusted OR = 4.282; 95% CI: 1.21–15.13; *P* = 0.024) [Table 3].

Discussion

MM is a malignancy derived from differentiated plasma cells, characterized by the infiltration of monoclonal cells in bone marrow.[13,14] Cytogenetic abnormalities, deregulation, and increased level of Cyclin D1 (*CCND1*) are reported to be associated with the clinical presentation and progression of MM.[15] *CCND1* is considered a cancer gene that modulates cell development from the G1 phase of the cell cycle to the S phase. Different types of malignancies are related to *CCND1* polymorphisms. Variant polymorphism leads to abnormal production of protein and results in cancer progression.[16] The association between genetic polymorphisms of Cyclin D1 and the risk of MM is poorly investigated. We aimed to investigate the relationship between *CCND1* polymorphisms and MM development. The current study was performed on 87 patients with MM and 70 healthy controls in an Iranian population. The findings showed Cyclin D1 polymorphism to be associated with MM development in the population studied. It was also observed that GG genotype contributed to an increased risk of MM. This is consistent with the study findings of Wang *et al*. who investigated the association between Cyclin D1 G870A polymorphism and the risk of MM in Chinese populations. In that study, a strong correlation was found between GG homozygous genotype of Cyclin D1 and MM (OR = 4.67 and CI 95% = 1.08–5.64). Stratified analysis showed a significantly increased risk of MM in patients over 60 years of age (OR = 3.29 and CI 95% = 1.05–10.26) but not in those younger than 60 years of age.[12] Interestingly, others found a significant relationship between the risk of MM and age over 60 years;[17] however, in the current study, significant association was found between all age groups in developing MM even following sex and age adjustment (OR = 4.28, CI 95% = 1.21–15.13). The differences between the average age of patients with MM in the study by Solomon *et al*. and that of the present study (55.95 ± 10.57 vs. 63.43 ± 9.91) might be due to demographic and environmental factors affecting the development of the disease. There are reports suggesting *CCND1* c.870G>A polymorphism as a genetic factor for stem cell transplantation in MM patients because the polymorphism is believed to be correlated with a specific chromosomal translocation in these patients.[17] The main genetic and molecular mechanism of *CCND1* in the progression of MM remains unclear; however, evidence shows that translocation of the *Cyclin D1* gene with immunoglobulin heavy-chain locus t(11; 14)(q13; q32) leads to the overexpression of *Cyclin D1* RNA, and this polymorphism may be involved in the development of MM.[18-20] The presence of a GG genotype of the *CCND1* G870A in exon 4 on the long arm of the chromosome 11 is believed to have a significant correlation with susceptibility to MM neoplasm. The *CCND1* G870A polymorphism is suggested as a risk factor for MM. These findings are consistent with those of the current results, although previous studies included large number of MM patients. *CCND1* G870A polymorphism was not only reported for the development of MM but was also found in different types of carcinoma. Hryhorowicz *et al*. studied patients with

thyroid carcinoma and healthy controls and reported that the A allele (OR = 1.18, CI 95% = 1.01–1.36) and homozygous AA genotype (OR = 1.41, CI 95% = 1.05–10.98) were more frequent in patients compared with controls.[18] Yuan *et al*. also observed the AA genotype of the *CCND1* G870A polymorphism to be associated with an increased risk of bladder cancer (OR = 1.54, CI 95% = 1.08–2.20), and the risk was even higher in patients over 65 years of age (OR = 1.74, CI 95% = 1.06–2.88), males (OR = 1.67, CI 95% = 1.15–2.44), and smokers (OR = 1.82, CI 95% = 1.12–1.82).[19] Lin *et al*. found *CCND1* GG genotype to be related to lower risk of development of urethral malignancy (OR = 0.44, CI 95% = 0.24–0.81) and bladder cancer (OR = 0.34, CI 95% = 0.15–0.76). In contrast, they reported that the GG genotype was associated with a high risk of muscular invasion (OR = 5.88, CI 95% = 1.08–32.01).[20] In a study in Taiwan on the relationship between *CCND1* genotype and colorectal cancer, significant differences were seen between patients and controls in the distribution of *CCND1 G870A* genotypes. AG and GG genotypes had OR = 0.56, CI 95% = 0.40–0.78 and OR = 0.51, CI 95% = 0.32–0.81, respectively. In addition, the presence of G allele had a protective effect on nonsmoker and nonalcoholic individuals against colorectal cancer.[21] The *CCND1* G870A variant was also found to increase the risk of non-Hodgkin's lymphoma (OR = 1.4, CI 95% = 1.1–1.8) and was associated with poor prognosis.[22] Research on the distribution of *CCND1 G870A* variant in patients with advanced cervical carcinoma and healthy controls showed that the presence of AA and AG genotypes increased the risk of cervical carcinoma (OR = 1.81, CI 95% = 1.15–2.85).[23] In a study on *CCND1* G870A gene polymorphism and the risk for lung cancer, individuals with AG (OR = 0.59, CI 95% = 0.44–0.78) and GG (OR = 0.52, CI 95% = 0.35–0.79) genotypes were found to have a lower risk for lung cancer than those with AA genotype.[24] Some studies did not find any association between *CCND1* gene polymorphism and the risk of laryngeal cancer, esophagus squamous cell carcinoma, and breast cancer development.[25-27] The present study suggests that *CCND1* G870A polymorphism might be a genetic predisposing factor for MM and that GG genotype could be correlated with an increased risk of MM in northern part of Iran. In MM patients, GG genotype was seen as a risk factor, but AA genotype and A allele were detected as the main genetic variants in different types of carcinoma. Therefore, further studies are needed to investigate the molecular mechanism of gene polymorphism on cancer susceptibility. The present study has some limitations that need to be addressed. The sample size was limited, and the study was conducted in a single area of geographical region. Further prospective large-scale comparative studies in greater geographical areas and high-risk cohorts are needed to confirm these results.

Conclusion

CCND1 G870A polymorphism may increase MM development. However, more large-scale and well-designed researches are still a prerequisite to estimate the interaction of *CCND1* G870A polymorphism with MM.

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Conflicts of interest

There are no conflicts of interest.

References

1. Kolahdoozan S, Sadjadi A, Radmard AR, Khademi H. Five common cancers in Iran. Arch Iran Med 2010;13:143-6.
2. Parkin DM, Bray F, Ferlay J, Pisani P. Estimating the world cancer burden: Globocan 2000. Int J Cancer 2001;94:153-6.
3. Specht K, Haralambieva E, Bink K, Kremer M, Mandl-Weber S, Koch I, *et al.* Different mechanisms of cyclin D1 overexpression in multiple myeloma revealed by fluorescence *in situ* hybridization and quantitative analysis of mRNA levels. Blood 2004;104:1120-6.
4. Moreaux J, Hose D, Reme T, Jourdan E, Hundemer M, Legouffe E, *et al.* CD200 is a new prognostic factor in multiple myeloma. Blood 2006;108:4194-7.
5. Raab MS, Podar K, Breitkreutz I, Richardson PG, Anderson KC. Multiple myeloma. Lancet 2009;374:324-39.
6. Mahabadi S, Khadem-Ansari M, Kheradmand F, Maghsoudloo MS. Evaluation of serum level of alpha-1 antitrypsin by capillary zone Electrophoresis in patients with multiple myeloma stage I. Urmia Med J 2016;27:1027-3727.
7. Gerkes EH, de Jong MM, Sijmons RH, Vellenga E. Familial multiple myeloma: Report on two families and discussion of screening options. Hered Cancer Clin Pract 2007;5:72-8.
8. Szemraj-Rogucka ZM, Szemraj J, Grzybowska-Izydorczyk O, Robak T, Jamroziak K. CD38 gene polymorphisms and genetic predisposition to multiple myeloma. Acta Haematol Pol 2013;44:58-62.
9. Pon JR, Marra MA. Driver and passenger mutations in cancer. Annu Rev Pathol 2015;10:25-50.
10. Hedayatizadeh-Omran A, Rafiei A, Khajavi R, Alizadeh-Navaei R, Mokhberi V, Moradzadeh K. Association between ghrelin gene (Leu72Met) polymorphism and ghrelin serum level with coronary artery diseases. DNA Cell Biol 2014;33:95-101.
11. Lu C, Dong J, Ma H, Jin G, Hu Z, Peng Y, *et al. CCND1* G870A polymorphism contributes to breast cancer susceptibility: A meta-analysis. Breast Cancer Res Treat 2009;116:571-5.
12. Wang S, Huang Y, Su R, Fang Z, Han M. Cyclin D1 G870A polymorphism is associated with an increased risk of multiple myeloma. Genet Mol Res 2015;14:5856-61.
13. Anderson KC, Carrasco RD. Pathogenesis of myeloma. Annu Rev Pathol 2011;6:249-74.
14. Morgan GJ, Walker BA, Davies FE. The genetic architecture of multiple myeloma. Nat Rev Cancer 2012;12:335-48.
15. Ihnatova I, Nemec P, Schmitz A, Brødker JS, Kjeldsen MK, Bøgsted M, *et al*. Cell cycle gene sets coordination in multiple

myeloma and plasma cell leukemia. Blood 2013;122:1901.
16. Chen-Kiang S. Cell-cycle control of plasma cell differentiation and tumorigenesis. Immunol Rev 2003;194:39-47.
17. Solomon DA, Wang Y, Fox SR, Lambeck TC, Giesting S, Lan Z, *et al.* Cyclin D1 splice variants. Differential effects on localization, RB phosphorylation, and cellular transformation. J Biol Chem 2003;278:30339-47.
18. Hryhorowicz S, Ziemnicka K, Kaczmarek-Ryś M, Hoppe-Gołębiewska J, Pławski A, Skrzypczak-Zielińska M, *et al. CCND1* gene polymorphic variants in patients with differentiated thyroid carcinoma. Oncol Lett 2015;9:442-8.
19. Yuan L, Gu X, Shao J, Wang M, Wang M, Zhu Q, *et al.* Cyclin D1 G870A polymorphism is associated with risk and clinicopathologic characteristics of bladder cancer. DNA Cell Biol 2010;29:611-7.
20. Lin HH, Ke HL, Hsiao KH, Tsai CW, Wu WJ, Bau DT, *et al.* Potential role of *CCND1* G870A genotype as a predictor for urothelial carcinoma susceptibility and muscle-invasiveness in Taiwan. Chin J Physiol 2011;54:196-202.
21. Huang CY, Tsai CW, Hsu CM, Chang WS, Shui HA, Bau DT. The significant association of *CCND1* genotypes with colorectal cancer in Taiwan. Tumour Biol 2015;36:6533-40.
22. Wang SS, Cozen W, Severson RK, Hartge P, Cerhan JR, Davis S, *et al.* Cyclin D1 splice variant and risk for non-Hodgkin lymphoma. Hum Genet 2006;120:297-300.
23. Warchoł T, Kruszyna L, Lianeri M, Roszak A, Jagodziński PP. Distribution of *CCND1* A870G polymorphism in patients with advanced uterine cervical carcinoma. Pathol Oncol Res 2011;17:133-7.
24. Hsia TC, Liu CJ, Lin CH, Chang WS, Chu CC, Hang LW, *et al.* Interaction of *CCND1* genotype and smoking habit in Taiwan lung cancer patients. Anticancer Res 2011;31:3601-5.
25. Verim A, Ozkan N, Turan S, Korkmaz G, Cacina C, Yaylim I, *et al.* Association of the cyclin D1 G870A polymorphism with laryngeal cancer: Are they really related? Asian Pac J Cancer Prev 2013;14:7629-34.
26. Jang Y, Lu SA, Chen ZP, Ma J, Xu CQ, Zhang CZ, *et al.* Genetic polymorphisms of *CCND1* and PTEN in progression of esophageal squamous carcinoma. Genet Mol Res 2013;12:6685-91.
27. Naidu R, Yip CH, Taib NA. Polymorphisms of HER2 ile655Val and cyclin D1 (*CCND1*) G870A are not associated with breast cancer risk but polymorphic allele of HER2 is associated with nodal metastases. Neoplasma 2008;55:87-95.