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ORIGINAL ARTICLE

PDCD1 and *PDCD1LG1* polymorphisms affect the susceptibility to multiple myeloma

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Abstract

Single-nucleotide polymorphisms (SNPs) of the programmed cell death protein-1 (*PDCD1*), programmed cell death protein-1 ligand-1 (*PDCD1LG1*), and cytotoxic T lymphocyte-associated antigen-4 (*CTLA4*) genes are implicated in the pathogenesis of some cancers. We investigated the role of *PDCD1*, *PDCD1LG1*, and *CTLA4* SNPs in MM pathogenesis and the susceptibility to and clinical features of multiple myeloma (MM). We obtained genomic DNA from 124 patients with MM and 211 healthy controls and detected *PDCD1* (rs36084323, rs41386349, and rs2227982), *PDCD1LG1* (rs2297136 and rs4143815), and *CTLA4* (rs733618, rs11571316, rs231775, and rs3087243) genotypes using the polymerase chain reaction–restriction fragment length polymorphism method or the TaqMan allelic discrimination real-time PCR method. The patients with MM had a significantly higher frequency of the PDCD1 GCC/GCC haplotype (rs36084323/rs41386349/rs2227982) compared with the healthy controls. *PDCD1* rs2227982 CC genotype was associated significantly with a higher frequency of bone lesions. Patients with *PDCD1LG1* rs2297136 TT and TC types (high-expression types) showed lower albumin level than those with CC genotype. In addition, the *PDCD1LG1* rs4143815 CC and CG types (high-expression types) were associated significantly with higher frequency of patients who were treated with thalidomide and/or bortezomib. However, there was no statistical significance between *CTLA4* polymorphisms and clinical variables of patients with MM. There were no significant differences between all the polymorphisms and OS. Our study indicates that the *PDCD1* haplotype is associated with a susceptibility to MM. The *PDCD1* rs2227982 and *PDCD1LG1* rs2297136 affect the clinical features of multiple myeloma patients.

Keywords Programmed cell death protein-1 · Programmed cell death protein-1 ligand-1 · Multiple myeloma · Polymorphism

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Introduction

Multiple myeloma (MM) is a lymphoproliferative disorder characterized by the proliferation of malignant plasma cells, lytic bone lesions, and the presence of monoclonal immunoglobulins in serum and/or urine. The incidence of MM increases with age; it is more common in the elderly and is rare in patients under 40 years of age [1]. Recently, novel agents, including immunomodulatory drugs (IMiDs) such as thalidomide and proteasome inhibitors such as bortezomib, have been used for treating this disease [2]. These agents have improved the prognosis of MM patients; however, MM remains an incurable disease. Moreover, the interactions between MM cells and their bone marrow (BM) microenvironment such as immune cells, endothelial cells, and

mesenchymal stromal cells play important roles in myeloma cell growth and survival, and the development of resistance to therapy [3–5].

Immune checkpoint pathways are critical modulators of the immune system, allowing the initiation of the immune response and preventing the onset of autoimmunity. Programmed cell death protein-1 (PD-1, encoded by *PDCD1*) and cytotoxic T lymphocyte-associated antigen-4 (CTLA-4, encoded by *CTLA4*) play central roles in immune checkpoint pathways. PD-1 is expressed in the activated T cells and has been reported to suppress activation of lymphocytes and cytokine production by interacting with its ligands, PD-1 ligand-1 (PD-L1, encoded by *PDCD1LG1*) and PD-1 ligand-2 (PD-L2) [6, 7]. CTLA-4 is homologous to CD28 and a competitive antagonist for B7 (CD80 and CD86) on the surface of antigen-presenting cells. CTLA-4 has greater affinity for B7 than CD28 and is responsible for T cell inactivation. In some cancers, these immune checkpoint molecules often inhibit the anti-tumor immune response. Compared to healthy controls, overexpression of CTLA-4 was observed in the BM of MM patients [8]. Moreover, PD-L1 molecules on myeloma cells not only induce T cell down-regulation, but also enhance aggressive characteristics of myeloma cells that include high proliferative ability and drug resistance [9]. However, the effect of PD-1-blocking antibody (nivolumab) was insufficient in MM patients compared with other B cell malignancies in phase I trial [10]. Thus, further understanding of the association between MM and immune checkpoint molecules is essential.

The production of PD-1 and CTLA-4 is strongly influenced by genetic factors. Single-nucleotide polymorphisms (SNPs) of the PDCD-1, PD-L1, and CTLA-4 genes (Fig. 1) are implicated in the pathogenesis of some cancers such as colon cancer, breast cancer, non-small cell lung cancer, and esophageal squamous cell carcinoma [11–14]. However, to our knowledge, there has been no study reporting the association among the SNPs in the immune checkpoint genes and MM. We investigated the role of *PDCD1*, *PDCD1LG1*, and *CTLA4* SNPs in MM pathogenesis and the susceptibility to and clinical features of MM.

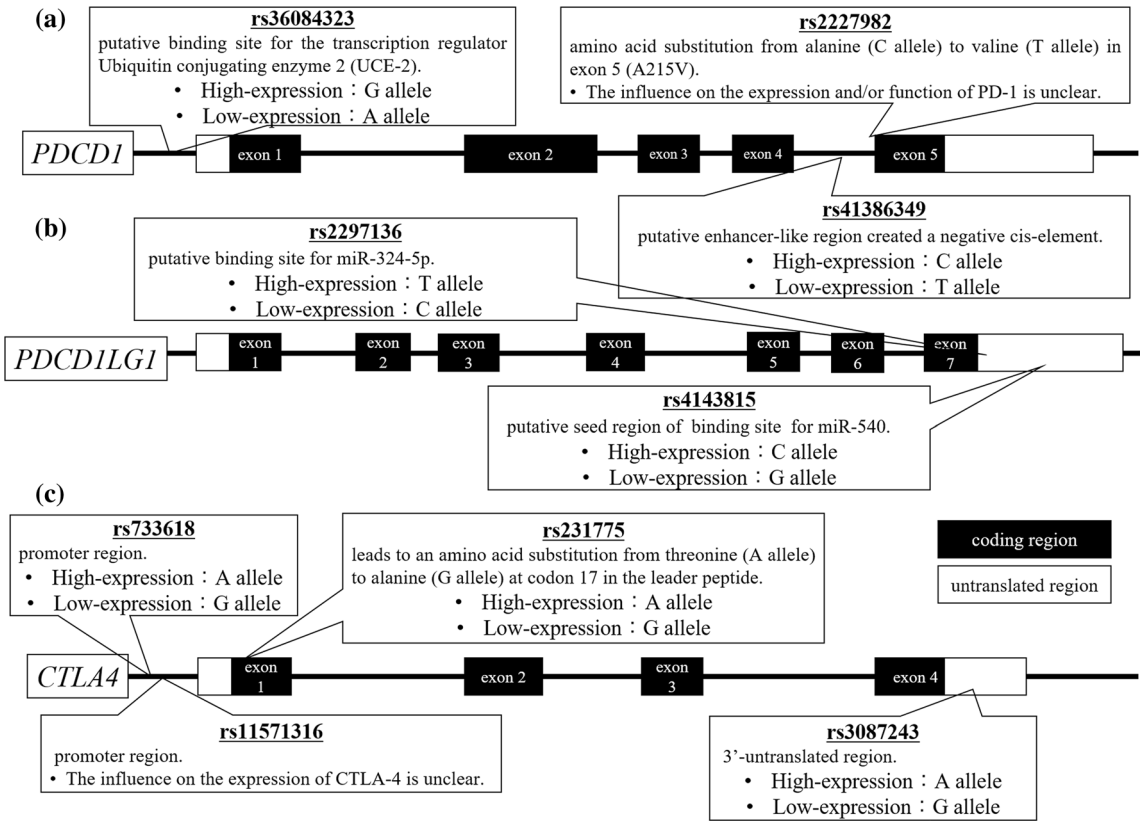


Fig. 1 The *PDCD1*, *PDCD1LG1,* and *CTLA4* single-nucleotide polymorphisms and their interactions. The coding region is represented by a filled black box. **a** Single-nucleotide polymorphisms in *PDCD1*. **b** Single-nucleotide polymorphisms in *PDCD1LG1*. **c** Single-nucleotide polymorphisms in *CTLA4*

Materials and methods

Patient characteristics

A total of 124 patients with MM and 211 healthy controls (both groups from Japan) were enrolled in this study. Patients with MM were diagnosed according to the International Myeloma Working Group Classification (2003) between 1994 and 2010. This study was approved by the Institutional Review Board of Gunma University Hospital, Japan (Approval #160007). Various clinical characteristics of all the patients were recorded (Table 1).

PDCD1, PDCD1LG1, and CTLA4 genotyping

To detect *PDCD1* SNPs (rs36084323, rs41386349, and rs2227982), *PDCD1LG1* SNPs (rs2297136), and *CTLA4* SNPs (rs733618, rs11571316, rs231775, and rs3087243), we used the PCR–restriction fragment length polymorphism method (PCR–RFLP). *PDCD1LG1* SNP (rs4143815) was genotyped using the TaqMan allelic discrimination real-time PCR method. Genomic DNA was isolated from whole blood using a DNA extraction kit (Qiagen GmbH, Hilden, Germany).

For analyzing the *PDCD1* variants, the forward primer 5′-GGGAAGAAGGTCAAGGCTGG-3′ and the reverse primer 5′-CCACTCCCATTCTGTCGGAG-3′ for rs36084323, the forward primer 5′-CAGCAACCTCAATCCCTAAAGC-3′ and the reverse primer 5′-GAAATCCAGCTCCCCATAGTCC-3′ for rs41386349, and the forward primer 5′-GGACAGCTCAGGGTAAGCAG-3′ and the reverse primer 5′-GAAATCCAGCTCCCCATAGTCC-3′ for rs2227982 were used. For analyzing the *PDCD1LG1* variants, the forward primer 5′-TGAAAGTATCAAGGTCTCCCTCC-3′ and the reverse primer 5′-GGGTTTTCCAGGATATCATGTAAGG-3′ for rs2297136 were used. For analyzing the *CTLA4* variants, the forward primer 5′-CAAGCTTTGTCCTGTGACCA-3′ and the reverse primer 5′-AAGCGCCAACAAGCAATAAC-3′ for rs733618, the forward primer 5′-GTCCTGTGACCATAATGAACTCTTC-3′ and the reverse primer 5′-TTTCTGACCTGCCTGTTTTCTATAC-3′ for rs11571316, the forward primer 5′-CTCTACTTCCTGAAGACCTGAACAC-3′ and the reverse primer 5′-ATTCATGAAGCCCCTACTAAATACC-3′ for rs231775, and the forward primer 5′-TTTCTGAAAATTAACACTGCTTGTG-3′ and the reverse primer 5′-ACTGTAATGCCTGTGATAGTTGAGC-3′ for rs3087243 were used. The PCR products were digested with the restriction enzyme NciI (for *PDCD1* rs36084323), BstUI (for *PDCD1* rs41386349), DrdI (for *PDCD1* rs2227982), PspOMI (for *PDCD1LG1* rs2297136), ApeKI (for *CTLA4* rs733618), HpyAV (for *CTLA4* rs11571316), BbvI (for *CTLA4* rs231775), and HpyCH4 (for *CTLA4* rs3087243). The products were analyzed on 2% agarose gels by electrophoresis. To confirm the accuracy of PCR–RFLP, the amplification products of several individuals were sequenced using an ABI Prism Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

Table 1 Characteristics of patients with multiple myeloma

Number of patients	124
Male/female	66/58
Age (years), median (range)	
Immunoglobulin subtype, *n* (%)	
IgG	70 (56.4%)
IgA	24 (19.4%)
IgD	3 (2.4%)
Bence Jones protein	25 (20.2%)
Non-secretory	2 (1.6%)
Stage according to the International Staging System (ISS), *n* (%)	
I	40 (32.3%)
II	41 (33.1%)
III	43 (34.7%)
Bone lesions, *n* (%)	93 (75.0%)
MGUS transformed, *n* (%)[a]	22 (17.7%)
Stem cell transplantation (SCT), *n* (%)[b]	35 (28.5%)
Treatment with thalidomide and/or bortezomib, *n* (%)[b]	46 (37.4%)

[a]Total 120 patients

[b]Total 123 patients

Statistical analysis

All statistical analyses were performed with the IBM SPSS software package ver. 24 (IBM, Armonk, NY, USA). Genotype and allele frequencies of *PDCD1*, *PDCD1LG1*, and *CTLA4* were compared between the healthy controls and patients with MM using the Chi-square test. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated for each analysis. The clinical parameters of the myeloma patients were compared with *PDCD1*, *PDCD1LG1*, and *CTLA4* polymorphisms using the independent t-test for continuous variables and the Chi-square test for categorical variables. Overall survival (OS) was defined as the interval from the date of diagnosis to the date of death or the last clinical appointment. OS was estimated by the Kaplan–Meier method and was compared using the log-rank test. $P < 0.05$ was considered statistically significant.

Results

Clinical characteristics of patients with MM

The clinical characteristics of patients with MM are shown in Table 1. Of the 124 MM patients, 66 were men (53.2%)

and 58 were women (46.8%). Their median age at diagnosis was 65.9 years (range 34.2–83.3 years). The immunoglobulin subtype was IgG for 70 patients (56.4%), IgA for 24 patients (19.4%), IgD for 3 patients (2.4%), Bence Jones protein for 25 patients (20.2%), and non-secretory type for 2 patients (1.6%). According to the International Staging System (ISS), 40 patients (32.3%) were classified as stage I, 41 (33.1%) were classified as stage II, and 43 (34.7%) were classified as stage III. Forty-six patients (37.9%) were treated with thalidomide and/or bortezomib.

Distribution of genotypes and allele frequencies among the healthy controls and patients with MM

The distributions of genotype and allele frequencies of the SNPs are summarized in Tables 2 and 3. There were no significant differences between the controls and the MM patients in *PDCD1*, *PDCD1LG1*, and *CTLA4* SNPs. To analyze the haplotypes of *PDCD1*, *PDCD1LG1,* and *CTLA-4* polymorphisms, we used Haploview software (BROAD institute, Cambridge, MA, UK). The patients with MM had a significantly higher frequency of the *PDCD1* GCC/GCC haplotype (rs36084323/rs41386349/rs2227982) compared with the healthy controls (10.5% vs. 4.3%, odds ratio = 2.63, 95% confidence interval = 1.09–6.34, $P = 0.027$) (Table 2). When we compared the distribution of each genotype according to sex and age among the control group and MM patients as a whole, no significant differences were observed in the genotype or in the allele frequencies.

Association of *PDCD1*, *PDCD1LG1*, and *CTLA4* polymorphisms with clinical variables and prognosis of patients with MM

We summarized the association of *PDCD1*, *PDCD1LG1*, and *CTLA4* polymorphisms with the clinical variables of patients with MM in Tables 4, 5, and 6. Compared with the CT and TT types, the *PDCD1* rs2227982 CC genotype was associated significantly with a higher frequency of bone lesions (37 [86.0%] vs. 56 [69.1%]; $P = 0.038$) (Table 4). Patients with *PDCD1LG1* rs2297136 TT and TC types (high-expression types) showed lower albumin level than those with CC genotype (mean ± standard deviation (SD), 3.84 ± 0.65 vs. 4.36 ± 0.54 mg/dL; $P = 0.040$) (Table 5). In addition, the *PDCD1LG1* rs4143815 CC and CG types (high-expression types) were associated significantly with higher frequency of patients who were treated with thalidomide and/or bortezomib (41 [41.8%] vs. 5 [20.0%]; $P = 0.044$) (Table 5). However, there was no statistical significance between *CTLA4* polymorphisms and clinical variables of patients with MM. In addition, the rate of ISS high-risk patients was not significantly different for all *PDCD1*, *PDCD1LG1*, and *CTLA4* SNPs. However, the R-ISS could not be evaluated because our patients were diagnosed between 1994 and 2010 and the chromosome analysis/FISH was done in a small number of patients. Moreover, no significant differences in frequencies of patients with the *PDCD1*, *PDCD1LG1,* and *CTLA4* haplotypes were observed in clinical variables.

Subsequently, we also examined the effect of *PDCD1*, *PDCD1LG1*, and *CTLA4* polymorphisms on the OS in patients with MM (Fig. 2). There were no significant differences between all the polymorphisms and OS.

Discussion

Both *PDCD1* and *CTLA4* are located on chromosome 2, and *PDCD1LG1* is located on chromosome 9. Previous studies have shown that the SNPs in these genes influenced the expression of PD-1, PD-L1, or CTLA-4 (Fig. 1) [15–20]. A luciferase assay of the human embryonic kidney 293 (HEK293) cells showed that the promoter activity of the *PDCD1* rs36084323 A allele was lower than that of the *PDCD1* rs36084323 G allele [16]. Using the same method, Zheng et al. showed that the *PDCD1* rs41386349 C/T in the putative enhancer-like region created a negative element in *PDCD1* and that the *PDCD1* rs41386349 T allele had lower transcriptional activity of PD-1 in human T cells than the *PDCD1* rs41386349 C allele (Fig. 1a) [17]. The *PDCD1* rs41386349 C/T results in an amino acid substitution from alanine (C allele) to valine (T allele) in exon 5 (A215V). Exon 5 in *PDCD1* encodes the cytoplasmic domain of PD-1, including the immunoreceptor tyrosine-based inhibitory motif (ITIM) and the immunoreceptor tyrosine-switch motif (ITSM). The *PDCD1LG1* rs2297136 and rs4143815 were located in the binding sites of miR-324-5p and miR-570, respectively. The *PDCD1LG1* rs2297136 C allele and rs4143815 G allele are complementary sequence to the bound micro-RNA and may affect the PD-L1 expression [19, 20]. The *CTLA4* rs733618 A/G and rs11571316 A/G alleles are located in the promoter region. The *CTLA4* rs733618 A allele showed an increased transcriptional activity in the luciferase assay performed on the JURKAT cell line (CD4+ T cell line) [18]. The *CTLA4* rs231775 A/G leads to an amino acid substitution from threonine (A allele) to alanine (G allele) in the leader peptide. Anjos et al. reported a higher expression of the *CTLA4* rs231775 A allele cell surface compared with the *CTLA4* rs231775 G allele by quantitative confocal microscopy [15]. The *CTLA4* rs3087243 A/G is located in the 3′-untranslated region of the gene. In addition, activated human T cells containing the *CTLA4* rs3087243 GG genotype had lower expression levels of soluble *CTLA4* mRNA, a transcript variant that lacks the transmembrane domain.

Table 2 Genotype and haplotype distributions of *PDCD1* and *PDCD1LG1* polymorphisms

	Control *n* (%)	MM *n* (%)	OR	95% CI	*P* value
PDCD1					
rs36084323					
GG	51 (24.2%)	37 (29.8%)	1.33	0.81–2.19	0.26
GA	110 (52.1%)	54 (43.6%)	0.71	0.45–1.11	0.13
AA	50 (23.7%)	33 (26.6%)	1.17	0.70–1.94	0.55
rs41386349					
CC	122 (57.8%)	74 (59.7%)	1.08	0.69–1.70	0.74
CT	79 (37.4%)	40 (32.2%)	0.80	0.50–1.27	0.34
TT	10 (4.8%)	10 (8.1%)	1.76	0.71–4.36	0.22
rs2227982					
CC	55 (26.1%)	43 (34.7%)	1.51	0.93–2.44	0.094
CT	116 (55.0%)	55 (44.3%)	0.65	0.42–1.02	0.060
TT	40 (18.9%)	26 (21.0%)	1.13	0.65–1.97	0.66
PDCD1 haplotype (rs36084323/rs41386349/rs2227982)					
GCC/GCC	9 (4.3%)	13 (10.5%)	2.63	1.09–6.34	0.027
GCC/GTC	24 (11.4%)	15 (12.1%)	1.25	0.62–2.52	0.54
GCC/GCT	6 (2.8%)	1 (0.8%)	0.28	0.033–2.34	0.27
GTC/GTC	7 (3.3%)	8 (6.5%)	1.99	0.71–5.64	0.19
GTC/GCT	4 (1.9%)	1 (0.8%)	2.01	0.71–5.68	0.18
GCT/GCT	1 (0.5%)	0 (0.0%)	Not calculated		1.00
GCC/ACC	4 (1.9%)	3 (2.4%)	1.28	0.28–5.83	0.71
GCC/ACT	47 (22.3%)	24 (19.4%)	0.84	0.48–1.45	0.53
GTC/ACC	4 (1.9%)	1 (0.8%)	0.42	0.05–3.81	0.66
GTC/ACT	45 (21.3%)	22 (17.7%)	0.80	0.45–1.40	0.43
GTC/ATT	1 (0.5%)	1 (0.8%)	1.71	0.11–27.5	1.00
GTC/ATC	0 (0.0%)	1 (0.8%)	Not calculated		0.37
GCT/ACT	9 (4.3%)	1 (0.8%)	0.18	0.023–1.46	0.098
ACT/ACC	12 (5.6%)	5 (4.0%)	0.70	0.24–2.03	0.51
ACT/ACT	30 (14.2%)	25 (20.2%)	1.52	0.85–2.73	0.16
ACT/ATC	1 (0.5%)	1 (0.8%)	1.71	0.11–27.5	1.00
ACC/ATC	2 (0.9%)	0 (0.0%)	Not calculated		0.53
ACC/ACC	5 (2.4%)	2 (1.6%)	0.68	0.13–3.54	1.00
PDCD1LG1					
rs2297136					
TT	127 (60.2%)	78 (62.9%)	1.12	0.71–1.77	0.62
TC	74 (35.1%)	39 (31.5%)	0.85	0.53–1.36	0.50
CC	10 (4.7%)	7 (5.6%)	1.20	0.45–3.24	0.72
rs4143815					
CC	53 (25.1%)	40 (32.3%)	1.42	0.87–2.31	0.16
CG	116 (55.0%)	59 (47.6%)	0.74	0.48–1.16	0.19
GG	42 (19.9%)	25 (20.2%)	1.02	0.58–1.77	0.96
PDCD1LG1 haplotype (rs2297136/rs4143815)					
TC/TC	52 (24.6%)	38 (30.6%)	1.35	0.83–2.21	0.25
TC/TG	59 (28.0%)	30 (24.2%)	0.82	0.49–1.37	0.45
TC/CG	56 (26.5%)	28 (22.6%)	0.81	0.48–1.36	0.42
TC/CC	1 (0.5%)	2 (1.6%)	3.44	0.31–38.4	0.56
TG/CG	17 (8.1%)	12 (9.7%)	1.22	0.56–2.65	0.61
TG/TG	16 (7.6%)	7 (5.6%)	0.73	0.29–1.83	0.50
CG/CC	1 (0.5%)	1 (0.8%)	1.71	0.11–27.5	1.00
CG/CG	9 (4.2%)	6 (4.8%)	1.14	0.40–3.29	0.81

Table 3 Genotype and haplotype distributions of *CTLA4* polymorphisms

	Control *n* (%)	MM *n* (%)	OR	95%CI	*P* value
CTLA4					
rs733618					
AA	66 (31.3%)	45 (36.3%)	1.25	0.78–2.00	0.35
AG	114 (54.0%)	62 (50.0%)	0.85	0.55–1.33	0.48
GG	31 (14.7%)	17 (13.7%)	0.92	0.49–1.75	0.80
rs11571316					
AA	16 (7.6%)	14 (11.3%)	1.55	0.73–3.30	0.25
AG	86 (40.8%)	47 (37.9%)	0.89	0.56–1.40	0.61
GG	109 (51.6%)	63 (50.8%)	0.97	0.62–1.51	0.88
rs231775					
AA	31 (14.7%)	14 (11.3%)	0.74	0.38–1.45	0.38
AG	105 (49.8%)	60 (48.4%)	0.95	0.61–1.48	0.81
GG	75 (35.5%)	50 (40.3%)	1.23	0.78–1.93	0.38
rs3087243					
AA	16 (7.6%)	7 (5.7%)	0.73	0.29–1.83	0.50
AG	88 (41.7%)	50 (40.3%)	0.94	0.60–1.48	0.80
GG	107 (50.7%)	67 (54.0%)	1.14	0.73–1.78	0.56
CTLA4 haplotype (rs733618/rs11571316/rs231775/rs3087243)					
AAAA/AAAA	16 (7.6%)	7 (5.6%)	0.73	0.29–1.83	0.50
AAAA/AAAG	0 (0.0%)	1 (0.8%)	Not calculated		0.37
AAAA/AAGG	0 (0.0%)	3 (2.4%)	Not calculated		0.050
AAAA/AGAG	11 (5.2%)	5 (4.0%)	0.76	0.26–2.25	0.63
AAAA/AGGG	18 (8.5%)	14 (11.3%)	1.37	0.65–2.85	0.41
AAAA/GAGG	0 (0.0%)	3 (2.4%)	Not calculated		0.050
AGAG/AGAG	4 (1.9%)	0 (0.0%)	Not calculated		0.30
AGAG/AAAG	0 (0.0%)	1 (0.8%)	Not calculated		0.37
AGGG/AGAA	2 (0.9%)	0 (0.0%)	Not calculated		0.53
AGGG/AGAG	10 (4.7%)	8 (6.5%)	1.39	0.53–3.61	0.50
AGGG/AGGG	7 (3.3%)	5 (4.0%)	1.22	0.38–3.94	0.77
AGGG/AAGG	0 (0.0%)	2 (1.6%)	Not calculated		0.14
GGGG/AAAA	52 (24.6%)	23 (18.5%)	0.70	0.40–1.21	0.20
GGGG/AAGA	0 (0.0%)	1 (0.8%)	Not calculated		0.37
GGGG/AGAA	3 (1.4%)	0 (0.0%)	Not calculated		0.30
GGGG/AGAG	17 (8.1%)	9 (7.3%)	0.89	0.39–2.07	0.79
GGGG/AGGA	0 (0.0%)	1 (0.8%)	Not calculated		0.37
GGGG/AAGG	4 (1.9%)	0 (0.0%)	Not calculated		0.30
GGGG/AGGG	36 (17.1%)	24 (19.4%)	1.17	0.66–2.07	0.60
GGGG/GAAG	1 (0.5%)	0 (0.0%)	Not calculated		1.00
GGGG/GGAA	1 (0.5%)	1 (0.8%)	1.71	0.11–27.5	1.00
GGGG/GAGG	0 (0.0%)	2 (1.6%)	Not calculated		0.14
GGGG/GGGG	29 (13.8%)	14 (11.3%)	0.80	0.41–1.58	0.52

A higher frequency of the *PDCD1* GCC/GCC haplotype was observed in patients with MM compared to the healthy controls in this study. The *PDCD1* GCC/GCC haplotype includes the higher expression alleles of rs36084323 (G allele) and rs41386349 (T allele). The expression of PD-1 was observed in the circulating T cells isolated from advanced MM patients, whereas the expression of PD-1 on circulating T cells was reduced in patients who achieve a minimal disease state following high-dose chemotherapy [21]. Moreover, Benson et al. showed that NK cells from MM patients express PD-1 and down-modulate the anti-myeloma cell effect of NK cells [22]. Although binding of myeloma PD-L1 to PD-1 did not directly affect the proliferation of myeloma cells [23], the inhibition of cancer immunity

Table 4 Clinical characteristics of MM patients according to *PDCD1* polymorphisms

Genotype	*PDCD1* rs36084323		*P* value	*PDCD1* rs41386349		*P* value
	High-expression GG ($n=32$)	Low-expression GA and AA ($n=82$)		High-expression CC ($n=74$)	Low-expression CT and TT ($n=50$)	
Male/female	21/16	45/42	0.61	36/38	30/20	0.21
Age (years), median (range)	64.4 (36.6–82.6)	66.3 (34.2–83.3)	0.90	66.3 (36.6–83.3)	64.0 (34.2–82.6)	0.35
Hb (g/dL), mean ± SD	9.74 ± 2.58	10.1 ± 2.37	0.42	10.0 ± 2.39	9.96 ± 2.51	0.86
Albumin (mg/dL), mean ± SD	3.74 ± 0.63	3.93 ± 0.65	0.12	3.90 ± 0.63	3.83 ± 0.68	0.58
Calcium (mg/dL), mean ± SD	9.53 ± 1.62	9.30 ± 1.41	0.46	9.28 ± 1.47	9.52 ± 1.48	0.39
Beta-2-microglobulin (mg/L), mean ± SD	7.38 ± 17.0	5.35 ± 4.23	0.30	5.29 ± 4.34	7.00 ± 15.0	0.36
ISS-3, *n* (%)	12 (32.4%)	31 (35.6%)	0.73	24 (32.4%)	19 (38.0%)	0.52
ISS-2 or -3, *n* (%)	24 (64.9%)	60 (69.0%)	0.66	49 (66.2%)	35 (70.0%)	0.66
Bone lesions, *n* (%)	30 (81.1%)	63 (72.4%)	0.31	55 (74.3%)	38 (76.0%)	0.82
MGUS transformed, *n* (%)	6 (16.7%)	16 (19.0%)	0.76	12 (16.7%)	10 (20.8%)	0.56
Treatment, *n* (%)	36 (97.3%)	80 (92.0%)	0.43	69 (93.2%)	47 (94.0%)	1.00
Stem cell transplantation, *n* (%)	11 (30.6%)	24 (27.6%)	0.74	21 (28.4%)	14 (28.6%)	0.98
Treatment with thalidomide and/or bortezomib, *n* (%)	12 (32.4%)	34 (39.5%)	0.46	29 (49.7%)	17 (34.0%)	0.52
Genotype	*PDCD1* rs2227982		*P* value	*PDCD1* haplotype (rs36084323/rs41386349/rs2227982)		*P* value
	CC ($n=43$)	CT and TT ($n=81$)		GCC/GCC ($n=13$)	Non-GCC/GCC ($n=111$)	
Male/female	25/18	41/40	0.42	7/6	59/52	0.96
Age (years), median (range)	65.6 (36.6–82.6)	66.3 (34.2–83.3)	0.45	65.7 (36.6–81.9)	66.3 (34.2–83.3)	0.93
Hb (g/dL), mean ± SD	9.85 ± 2.51	10.1 ± 2.40	0.60	9.75 ± 2.72	10.0 ± 2.40	0.69
Albumin (mg/dL), mean ± SD	3.76 ± 0.60	3.94 ± 0.67	0.16	3.88 ± 0.69	3.87 ± 0.65	0.99
Calcium (mg/dL), mean ± SD	9.47 ± 1.53	9.32 ± 1.45	0.61	9.77 ± 1.59	9.33 ± 1.46	0.34
Beta-2-microglobulin (mg/L), mean ± SD	7.09 ± 15.9	5.35 ± 4.27	0.36	4.53 ± 3.10	6.13 ± 10.5	0.59
ISS-3, *n* (%)	14 (32.6%)	29 (35.8%)	0.72	4 (30.8%)	39 (35.1%)	1.00
ISS-2 or -3, *n* (%)	27 (62.8%)	57 (70.4%)	0.39	8 (61.5%)	76 (68.5%)	0.76
Bone lesions, *n* (%)	37 (86.0%)	56 (69.1%)	0.038	11 (84.6%)	82 (73.9%)	0.52
MGUS transformed, *n* (%)	9 (21.4%)	13 (16.7%)	0.52	2 (15.4%)	20 (18.7%)	1.00
Treatment, *n* (%)	41 (95.3%)	75 (92.6%)	0.71	13 (100%)	103 (92.8%)	1.00
Stem cell transplantation, *n* (%)	12 (28.6%)	23 (28.4%)	0.98	4 (30.8%)	31 (28.2%)	1.00
Treatment with thalidomide and/or bortezomib, *n* (%)	12 (27.9%)	34 (42.5%)	0.12	5 (38.5%)	41 (37.3%)	1.00

by PD-1/PD-L1 axis may lead to an environment favoring myeloma cells. Our results suggest that the PD-1 high-expression haplotype is implicated in susceptibility to MM. On the other hand, Karabon et al. reported that the *CTLA4* rs231775 G allele and rs3087243 G allele were related to susceptibility to multiple myeloma in Polish population [24]. However, there were no significant differences in the *CTLA4* SNPs between MM patients and healthy individuals in our study. According to 1000 Genomes Project resource (http://www.internationalgenome.org/), the distribution of *PDCD1*, *PDCD1LG1*, and *CTLA4* polymorphisms varies by race. The present study showed that the *PDCD1LG1* and *CTLA4* SNPs have no association with the susceptibility to multiple myeloma in Japanese population.

A previous study showed that the PD-L1 expression on plasma cells from MM patients with ISS stage II and III tended to be higher than that from the MM patients with ISS stage I [9]. In addition, PD-L1 expression levels on myeloma cells from the same patients were often up-regulated when the patients relapsed or became refractory to anti-MM chemotherapy compared to the levels at initial diagnosis [9]. Lee et al. reported that the expression levels of PD-1 on

Table 5 Clinical characteristics of MM patients according to *PDCD1LG1* polymorphisms

Genotype	*PDCD1LG1* rs2297136		*P* value	*PDCD1LG1* rs4143815		*P* value	*PDCD1LG1* haplotype (rs2297136/rs4143815)		*P* value
	High-expression	Low-expression		High-expression	Low-expression		High-expression	Low-expression	
	TT and TC	CC		CC and CG	GG		Non-CG/CG	CG/CG	
	(*n* = 117)	(*n* = 7)		(*n* = 99)	(*n* = 25)		(*n* = 118)	(*n* = 6)	
Male/female	62/55	4/3	1.00	54/45	12/13	0.56	62/56	4/2	0.68
Age (years), median (range)	66.3 (34.2–83.3)	63.1 (42.4–73.7)	0.59	65.8 (34.2–83.3)	68.9 (39.8–80.3)	0.60	66.3 (34.2–83.3)	62.1 (42.4–73.7)	0.36
Hb (g/dL), mean ± SD	9.93 ± 2.41	11.2 ± 2.69	0.19	10.0 ± 2.47	9.95 ± 2.32	0.90	9.96 ± 2.41	10.9 ± 2.80	0.38
Albumin (mg/dL), mean ± SD	3.84 ± 0.65	4.36 ± 0.54	0.040	3.84 ± 0.67	4.01 ± 0.56	0.24	3.85 ± 0.64	4.37 ± 0.59	0.056
Calcium (mg/dL), mean ± SD	9.34 ± 1.47	9.83 ± 1.58	0.41	9.32 ± 1.53	9.58 ± 1.27	0.43	9.34 ± 1.46	9.98 ± 1.68	0.30
Beta-2-microglobulin (mg/L), mean ± SD	6.10 ± 10.2	3.70 ± 3.35	0.54	5.90 ± 10.8	6.21 ± 6.12	0.89	6.07 ± 10.2	3.89 ± 3.63	0.60
ISS-3, *n* (%)	42 (35.9%)	1 (14.3%)	0.42	33 (33.3%)	10 (40.0%)	0.53	42 (35.6%)	1 (16.7%)	0.66
ISS-2 or -3, *n* (%)	81 (69.2%)	3 (42.9%)	0.21	69 (69.7%)	15 (60.0%)	0.35	81 (68.6%)	3 (50.0%)	0.39
Bone lesions, *n* (%)	90 (76.9%)	3 (42.9%)	0.065	77 (77.8%)	16 (64.0%)	0.16	90 (76.3%)	3 (50.0%)	0.16
MGUS transformed, *n* (%)	20 (17.5%)	2 (33.3%)	0.30	17 (17.5%)	5 (21.7%)	0.76	21 (18.3%)	1 (20.0%)	1.00
Treatment, *n* (%)	110 (94.0%)	6 (85.7%)	0.38	93 (93.9%)	23 (92.0%)	0.66	111 (94.1%)	5 (83.3%)	0.34
Stem cell transplantation, *n* (%)	31 (26.7%)	4 (57.1%)	0.10	29 (29.3%)	6 (25.0%)	0.68	31 (26.5%)	4 (66.7%)	0.054
Treatment with thalidomide and/or bortezomib, *n* (%)	43 (37.1%)	3 (42.9%)	1.00	41 (41.8%)	5 (20.0%)	0.044	44 (37.6%)	2 (33.3%)	1.00

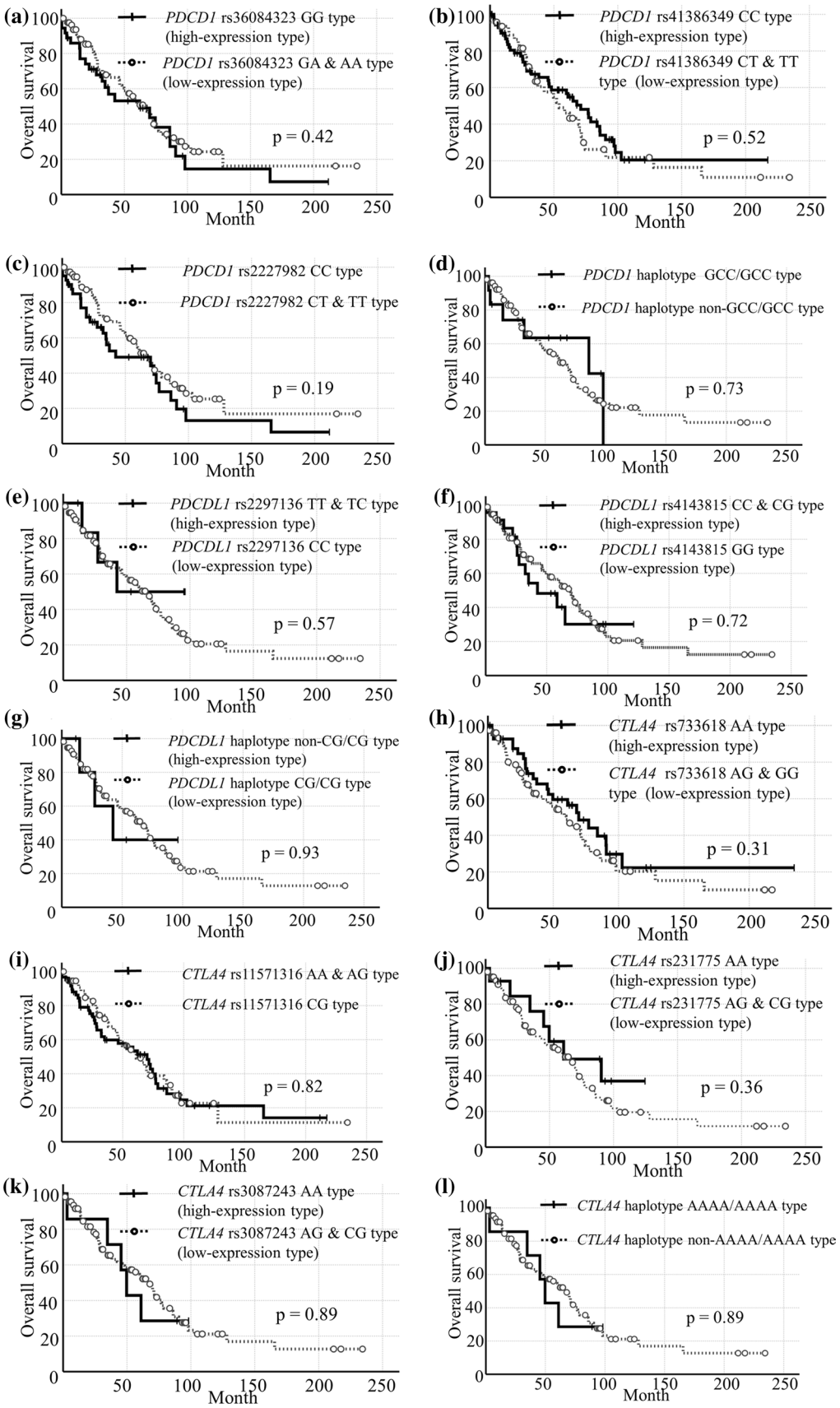
CD4+ T cells from MM patients in refractory state after chemotherapy were significantly higher than those in both at diagnosis and at complete remission after chemotherapy [25]. PD-L1+ human myeloma cell lines had higher proliferative potential compared with PD-L1− myeloma cell lines [26]. The present study showed that *PDCD1LG1* polymorphism was not associated with ISS high risk; however, the *PDCD1LG1* rs2297136 TT and TC types (high-expression type) showed low serum albumin levels. In addition, the PDCD1 rs2227982 CC genotype showed a higher frequency of bone lesion in this study. Nagahama et al. reported the association between PD-1 and osteoclastogenesis using PD-1-deficient mice [27]. Furthermore, the expression levels of CTLA-4 on CD4+ T cells were higher in MM patients with R-ISS stage III than those with R-ISS stage II disease [25]. However, the *CTLA4* polymorphisms had no association with ISS stages of MM in our study. Our results suggested that *PDCD1* and *PDCD1LG1* polymorphisms might contribute to the poor prognosis due to decreased albumin levels and bone lesions.

Previous studies showed the high expression of PD-L1 was associated with an increased risk of disease progression and short overall survival in MM patients [28–30]. Binding of myeloma PD-L1 to PD-1 induced resistance to apoptosis that was induced by melphalan and the proteasome inhibitor bortezomib via the PI3K/AKT pathway [23]. Qin et al.

Table 6 Clinical characteristics of MM patients according to *CTLA4* polymorphisms

Genotype	*CTLA4* rs733618		*P* value	*CTLA4* rs11571316		*P* value	*CTLA4* rs231775		*P* value
	High-expression	Low-expression		AA and AG	GG		High-expression	Low-expression	
	AA	AG and GG					AA	AG and GG	
	(*n* = 45)	(*n* = 79)		(*n* = 61)	(*n* = 63)		(*n* = 14)	(*n* = 110)	
Male/female	26/19	40/39	0.44	34/27	32/31	0.58	7/7	59/51	0.80
Age (years), median (range)	66.5 (34.2–82.7)	65.7 (36.6–83.3)	0.90	64.8 (34.2–83.3)	66.5 (36.6–82.6)	0.54	73.7 (51.8–82.7)	65.7 (34.2–83.3)	0.093
Hb (g/dL), mean ± SD	10.3 ± 2.15	9.82 ± 2.56	0.26	10.4 ± 2.28	9.63 ± 2.52	0.075	10.1 ± 1.96	10.0 ± 2.49	0.90
Albumin (mg/dL), mean ± SD	3.90 ± 0.66	3.86 ± 0.65	0.76	3.92 ± 0.66	3.82 ± 0.64	0.40	3.92 ± 0.58	3.87 ± 0.66	0.76
Calcium (mg/dL), mean ± SD	9.13 ± 1.01	9.51 ± 1.67	0.13	9.27 ± 1.26	9.48 ± 1.66	0.45	9.68 ± 0.93	9.34 ± 1.53	0.42
Beta-2-microglobulin (mg/L), mean ± SD	4.30 ± 2.96	6.87 ± 12.2	0.18	4.43 ± 2.92	7.44 ± 13.6	0.096	5.26 ± 4.00	6.05 ± 10.5	0.78
ISS-3, *n* (%)	11 (24.4%)	32 (40.5%)	0.071	16 (26.2%)	27 (42.9%)	0.052	3 (21.4%)	40 (36.4%)	0.38
ISS-2 or -3, *n* (%)	27 (60.0%)	57 (72.2%)	0.16	40 (65.6%)	44 (69.8%)	0.61	11 (78.6%)	73 (66.4%)	0.55
Bone lesions, *n* (%)	30 (66.7%)	63 (79.7%)	0.11	46 (75.4%)	47 (74.6%)	0.92	10 (71.4%)	83 (75.5%)	0.75
MGUS transformed, *n* (%)	6 (13.6%)	16 (21.1%)	0.31	9 (15.3%)	13 (21.3%)	0.39	1 (7.7%)	21 (19.6%)	0.46
Treatment, *n* (%)	41 (91.1%)	75 (94.9%)	0.46	57 (93.4%)	59 (93.7%)	1.00	13 (92.9%)	103 (93.6%)	1.00
Stem cell transplantation, *n* (%)	9 (20.0%)	26 (33.3%)	0.11	21 (34.4%)	14 (22.6%)	0.15	3 (21.4%)	32 (29.4%)	0.76
Treatment with thalidomide and/or bortezomib, *n* (%)	20 (44.4%)	26 (33.3%)	0.22	27 (44.3%)	20 (30.6%)	0.12	7 (50.0%)	39 (35.8%)	0.30

Genotype	*CTLA4* rs3087243		*P* value	CTLA4 haplotype (rs733618/rs11571316/rs231775/rs3087243)		
	High-expression	Low-expression		AAAA/AAAA	Non-AAAA/AAAA	
	AA	AG and GG				
	(*n* = 7)	(*n* = 117)		(*n* = 7)	(*n* = 117)	
Male/female	4/3	62/55	1.00	4/3	62/55	1.00
Age (years), median (range)	70.1 (58.1–82.7)	65.7 (34.2–83.3)	0.12	70.1 (58.1–82.7)	65.7 (34.2–83.3)	0.12
Hb (g/dL), mean ± SD	9.40 ± 1.78	10.0 ± 2.46	0.50	9.40 ± 1.78	10.0 ± 2.46	0.5
Albumin (mg/dL), mean ± SD	4.00 ± 0.50	3.86 ± 0.66	0.60	4.00 ± 0.50	3.86 ± 0.66	0.6
Calcium (mg/dL), mean ± SD	9.81 ± 0.91	9.35 ± 1.50	0.42	9.81 ± 0.91	9.35 ± 1.50	0.42
Beta-2-microglobulin (mg/L), mean ± SD	7.04 ± 5.11	5.90 ± 10.2	0.77	7.04 ± 5.11	5.90 ± 10.2	0.77
ISS-3, *n* (%)	3 (42.9%)	40 (34.2%)	0.69	3 (42.9%)	40 (34.2%)	0.69
ISS-2 or -3, *n* (%)	6 (85.7%)	78 (66.7%)	0.43	6 (85.7%)	78 (66.7%)	0.43
Bone lesions, *n* (%)	6 (85.7%)	87 (74.4%)	0.68	6 (85.7%)	87 (74.4%)	0.68
MGUS transformed, *n* (%)	0 (0.0%)	22 (19.5%)	0.35	0 (0.0%)	22 (19.5%)	0.35
Treatment, *n* (%)	7 (100%)	109 (93.2%)	1.00	7 (0.0%)	109 (93.2%)	1.00
Stem cell transplantation, *n* (%)	0 (0.0%)	35 (30.2%)	0.19	0 (0.0)	35 (30.2%)	0.19
Treatment with thalidomide and/or bortezomib, *n* (%)	4 (57.1%)	42 (36.2%)	0.42	4 (57.1%)	42 (36.2%)	0.42


(a)
PDCD1 rs36084323 GG type (high-expression type)
PDCD1 rs36084323 GA & AA type (low-expression type)
p = 0.42
(b)
PDCD1 rs41386349 CC type (high-expression type)
PDCD1 rs41386349 CT & TT type (low-expression type)
p = 0.52
(c)
PDCD1 rs2227982 CC type
PDCD1 rs2227982 CT & TT type
p = 0.19
(d)
PDCD1 haplotype GCC/GCC type
PDCD1 haplotype non-GCC/GCC type
p = 0.73
(e)
PDCDL1 rs2297136 TT & TC type (high-expression type)
PDCDL1 rs2297136 CC type (low-expression type)
p = 0.57
(f)
PDCDL1 rs4143815 CC & CG type (high-expression type)
PDCDL1 rs4143815 GG type (low-expression type)
p = 0.72
(g)
PDCDL1 haplotype non-CG/CG type (high-expression type)
PDCDL1 haplotype CG/CG type (low-expression type)
p = 0.93
(h)
CTLA4 rs733618 AA type (high-expression type)
CTLA4 rs733618 AG & GG type (low-expression type)
p = 0.31
(i)
CTLA4 rs11571316 AA & AG type
CTLA4 rs11571316 CG type
p = 0.82
(j)
CTLA4 rs231775 AA type (high-expression type)
CTLA4 rs231775 AG & CG type (low-expression type)
p = 0.36
(k)
CTLA4 rs3087243 AA type (high-expression type)
CTLA4 rs3087243 AG & CG type (low-expression type)
p = 0.89
(l)
CTLA4 haplotype AAAA/AAAA type
CTLA4 haplotype non-AAAA/AAAA type
p = 0.89
Overall survival
Month

◂**Fig. 2** **a** Overall survival (OS) of MM patients according to the *PDCD1* rs36084323 genotypes. The median survival time of patients with the GG genotype and GA and GG genotypes was 62.1 and 65.0 months, respectively ($P=0.42$). **b** The OS of MM patients according to the *PDCD1* rs41386349 genotypes. The median survival time of patients with the CC genotype and CT and TT genotypes was 71.0 and 54.1 months, respectively ($P=0.52$). **c** OS of MM patients according to the *PDCD1* rs2227982 genotypes. The median survival time of patients with the CC genotype and CT and TT genotypes was 42.4 and 65.0 months, respectively ($P=0.18$). **d** OS of MM patients according to the *PDCD1* haplotype. The median survival time of patients with the GCC/GCC haplotype and the other genotypes was 86.1 and 62.1 months, respectively ($P=0.73$). **e** The OS of MM patients according to the *PDCD1LG1* rs2297136 genotypes. The median survival time of patients with the TT and TC genotypes and CC genotype was 65.5 and 42.4 months, respectively ($P=0.57$). **f** The OS of MM patients according to the *PDCD1LG1* rs4143815 genotypes. The median survival time of patients with the CC and CG genotypes and GG genotype was 69.5 and 42.4 months, respectively ($P=0.72$). **g** The OS of MM patients according to the *PDCD1LG1* haplotype. The median survival time of patients with the other genotypes and CG/CG haplotype was 69.5 and 42.4 months, respectively ($P=0.93$). **h** The OS of MM patients according to the *CTLA4* rs733618 genotype. The median survival time of patients with the AA genotype and AG and GG genotypes was 69.5 and 59.8 months, respectively ($P=0.31$). **i** The OS of MM patients according to the *CTLA4* rs11571316 genotypes. The median survival time of patients with the GG genotype and AA and AG genotypes was 70.0 and 61.0 months, respectively ($P=0.82$). **j** The OS of MM patients according to the *CTLA4* rs231775 genotypes. The median survival time of patients with the AA genotype and AG and GG genotypes was 61.0 and 65.0 months, respectively ($P=0.36$). **k** The OS of patients with MM according to the *CTLA4* rs3087243 genotypes. The median survival time of patients with the AA genotype and AG and GG genotypes was 49.7 and 67.9 months, respectively ($P=0.89$). **l** The OS of patients with MM according to the *CTLA4* haplotype. The median survival time of patients with the AAAA/AAAA haplotype and the other haplotypes was 49.7 and 67.9 months, respectively ($P=0.89$)

reported that the *CTLA4* rs733618 GG genotype reduced the progression-free survival and the overall survival of MM patients who received bortezomib-based regimens [31]. However, there were no significant differences between *PDCD1*, *PDCD1LG1*, and *CTLA4* polymorphisms and the prognosis of MM patients in this study. Moreover, previous studies have reported that IMiDs and proteasome inhibitors affect the expression of PD-1, PD-L1, and CTLA-4. Lenalidomide, one of IMiDs, has been shown to affect the PD-1/PD-L1 axis by down-regulating the expression of PD-L1 in MM cells [22, 32] and by decreasing the expression of PD-1 on T cells from MM patients [32, 33]. On the other hand, proteasome inhibitor bortezomib has been shown to increase PD-L1 and PD-L2 levels on MM cells and up-regulate CTLA-4 in normal T cells [34, 35]. The majority of our patients received conventional chemotherapy because the patients were diagnosed between 1994 and 2006. We also examined the effect of *PDCD1*, *PDCD1LG1*, and *CTLA4* polymorphisms on the OS in patients treated with thalidomide and/or bortezomib ($n=47$). However, no significant differences were observed in those polymorphisms (data not shown). In addition, there were also no statistical significance differences between *PDCD1*, *PDCD1LG1*, and *CTLA4* polymorphisms and OS in patients treated with conventional therapy only (data not shown). The present study suggests that the *PDCD1*, *PDCD1LG1*, and *CTLA4* are not implicated in prognosis of MM in the Japanese population.

Conclusion

Our study indicates that the PDCD1 haplotype is associated with a susceptibility to MM. The *PDCD1* rs2227982 and *PDCD1LG1* rs2297136 affect the clinical features of multiple myeloma patients. In addition, there is no effect of SNPs in *CTLA4*. However, there are limitations to the interpretation of the results in this study because the sample size was relatively small with a total of 124 MM patients. Therefore, further investigations with larger sample sizes are needed to corroborate our results.

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Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the Institutional Review Board of Gunma University Hospital (Approval #160007) and with the 1964 Declaration of Helsinki.

Informed consent Informed consent was obtained from all individual participants included in the study.

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