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Abstract

Background and aims: Recently, genome-wide analyses have revealed mutations in spliceosome machinery associated with myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML). Single-nucleotide polymorphisms (SNPs) of serine/arginine-rich splicing factor 2 (SRSF2) and splicing factor 3a subunit 1 (SF3A1) were investigated in a Japanese population of patients and healthy control group. We aimed to find associations with prognosis and pathology.

Methods: We obtained genomic DNA from 99 patients with MDS, 92 patients with AML, and 172 healthy controls and detected SRSF2 (rs237057) and SF3A1 (rs2074733) genotypes using polymerase chain reaction–restriction fragment length polymorphism.

Results: There was no statistical significance to associate these polymorphisms with susceptibility to MDS/AML. However, the SF3A1 rs2074733 TC was significantly associated with higher hemoglobin level, compared to the TT genotype (mean±standard deviation, 10.6±1.63 vs 9.09±2.19 g/dL; P=0.022). In addition, patients with rs2074733 TC showed a significantly lower frequency of chromosomal abnormality [2 (18.2%) vs. 46 (53.5%), P=0.027]. We observed no statistical significance between these polymorphisms and clinical variables for AML, or the prognosis of MDS and AML.

Conclusions: Our study indicates that the SF3A1 rs2074733 TC genotype is associated with some clinical features of MDS.

Article Information

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Introduction

Myelodysplastic syndromes (MDS) are characterized by clonal disorders of hematopoietic stem cells, and present as refractory cytopenia, and unilineage to multilineage dysplasia. Patients with MDS show highly variable outcomes and a risk of progression to acute myeloid leukemia (AML).[1] AML is characterized by abnormally differentiated myeloid progenitor cells, gradually replacing normal hematopoiesis. Key genetic alterations, including those in *TET2*, *DNMT3A*, *WT1*, *FLT3-ITD*, *CEBPA* and *NPM1*, have been identified as prognostic factors and for therapeutic response in patients with MDS and AML. Recently, genome-wide analyses have revealed genetic mutations in spliceosome machinery genes associated with MDS and AML.[2]

The spliceosome catalyzes precursor mRNA (pre-mRNA) in the course of splicing. The structured spliceosome consists of five, small nuclear ribonucleoprotein particles (snRNPs), each containing a single, small nuclear RNA (snRNA; U1, U2, U4, U5, or U6), together with a number of other snRNPs. Serine/arginine-rich splicing factor 2 (SRSF2) and splicing factor 3a subunit 1 (SF3A1) are also components of the spliceosome and are involved in mRNA processing. SRSF2 is required for ATP-dependent interactions of both U1 and U2 snRNPs with pre-mRNA. SF3A1 is necessary for the conversion of 15S U2 snRNP into an active 17S particle that carries

out pre-mRNA splicing.

Single-nucleotide polymorphisms (SNPs) of the *SRSF2* and *SF3A1* genes have previously been investigated in some cancers, such as colorectal cancer and pancreatic cancer.[3-5] No previous studies have found any association between *SRSF2* polymorphism and cancer. Recently, mutations in splicing factor 3B subunit 1 (*SF3B1*), U2 small nuclear RNA auxiliary factor 35 (*U2AF35*), zinc finger *CCCH*-Type, RNA binding motif, serine/arginine rich 2 (*ZRSR2*), and SRSF2 have been frequently observed among spliceosome machinery gene mutations in patients with MDS and AML.[2,6-7] However, *SF3B1, U2AF1* and *ZRSR2* gene polymorphisms have not shown appreciable distribution in Japanese populations. Therefore, we selected *SRSF2* and *SF3A1* gene polymorphisms for our analysis. We investigated the role of *SRSF2* and *SF3A1* SNPs in MDS and AML pathogenesis, including susceptibility to the diseases and clinical features. To our knowledge, there has been no study reporting the association among the SNPs in spliceosome genes and adult MDS/AML.

Materials and Methods

Patient characteristics

The present study included 99 patients diagnosed with MDS, 92 with AML, and 172 healthy, race-matched controls. It was carried out at Gunma University Hospital and Saiseikai Maebashi Hospital, both in Gunma, Japan. The characteristics of patients with MDS are summarized in Table 1. MDS was defined according to the World Health Organization (WHO) classification (2016). The revised International Prognostic Scoring System (IPSS-R) was used to assess prognostic scoring.[8] The characteristics of patients with AML are summarized in Table 2. They were classified according to the French-American-British (FAB) classification (1976), and the UK Medical Research Council (MRC) classification (2010). This study was approved by the Institutional Review Board of Gunma University Hospital (Approval #160007).

SRSF2 and *SF3A1* genotyping

To determine *SRSF2* SNP (rs237057 G/A/C) and *SF3A1* SNP (rs2074733 T/C), we used the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Genomic DNA was isolated from whole blood, using a DNA extraction kit (Qiagen GmbH, Hilden, Germany). The following primers were used for analysis of *SRSF2* polymorphism: upstream 5'-CAAGGTGGACAACCTGACCTAC-3', and downstream 5'-ATGGCATCCATAGCGTCCT-3'. For analysis of *SF3A1* polymorphism, we used upstream primer 5'-CCTCCTTCGGAACAGAATGGAA-3' and downstream 5'-CAAAGGCCAAAGAAACCTGGAG-3'. The PCR products of the *SRSF2* rs237057 A/G allele were digested with restriction enzyme *Sau3AI* (New England BioLabs, Massachusetts, USA), *Ava*II (New England Biolabs) was used to digest the *SRSF2* rs237057 C allele, and *Fai*I (SibEnzyme, Novosibirsk, Russia) was used for *SF3A1* rs2074733. All SNP digestion products were separated by electrophoresis in a 3% agarose gel.

Statistical analysis

All statistical analyses were performed using the IBM SPSS software package ver. 26 (IBM, Armonk, NY, USA). The genotype and allele frequency of *SRSF2* and *SF3A1* SNPs in patients with MDS or AML were compared to healthy controls using the chi-square test. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated for each analysis. The characteristics and laboratory values of the MDS and AML patients with *SF3A1* polymorphisms were compared using an independent t-test for continuous variables, and the chi-square test for categorical variables. Overall survival (OS), leukemia-free survival (LFS), and progression-free survival (PFS) of MDS patients were estimated using the Kaplan-Meier method and compared using the log-rank test. LFS was defined as the time from the date of diagnosis of MDS to the time of transformation to leukemia; PFS was defined as the time from the date of diagnosis of MDS to the time of death or transformation to leukemia. OS and relapse-free survival (RFS) of AML patients were also calculated using the Kaplan-Meier method and compared using the log-rank test. RFS was defined as the time from complete remission to relapse. $P<0.05$ was considered statistically significant.

Results

Clinical characteristics of MDS and AML patients (Table 1 and 2)

Of the 99 MDS patients, 64 were men (64.6%) and 35 were women (35.4%). Their median age at diagnosis was 65 years (range, 18-86 years). Thirty-six patients (36.4%) were classified as MDS with single lineage dysplasia (SLD), 20 (20.2%) as MDS with multilineage dysplasia (MLD), 6 (6.1%) as MDS with ring sideroblasts (RS), 12 (12.1%) as MDS with excess blasts-1 (EB-1), 11 (11.1%) as MDS with excess blasts-2 (EB-2), 13 (13.1%) as MDS-unclassifiable (U), and 1 (1.0%) as 5q-. The IPSS-R risk at diagnosis was very low for 14 patients (14.3%), low for 42 patients (42.9%), intermediate for 27 patients (27.6%), high for 8 patients (8.2%), very high for 7 (7.1%), and undetermined for 1 patient.

Of the 92 AML patients, 54 were men (58.7%) and 38 were women (41.3%). Their median age at diagnosis was 59 years (range 15-86 years). According to the FAB classification, 6 patients were classified as M0 (6.5%), 14 (15.2%) as M1, 32 (34.8%) as M2, 21 (22.8%) as M3, 12 (13.0%) as M4, 4 (4.3%) as M5, 2 (2.2%) as M6, and 1 (1.1%) as M7. According to the MRC classification, 36 patients (39.1%) had a favorable karyotype, 49 (53.3%) had an intermediate karyotype, and 7 (7.6%) had an adverse karyotype.

Genotype and allele frequencies among healthy controls, MDS patients, and AML patients

The distributions of genotype and allele frequencies are shown in Table 3. The *SRSF2* rs237057 C allele was

not found in this population. No significant differences in genotype or allele frequencies were observed between the MDS patients and healthy controls, for *SRSF2* and *SF3A1* SNPs. Neither were significant differences found between AML patients and the controls, for *SRSF2* and *SF3A1* SNPs.

Table 1 The clinical characteristics of the patients with MDS.

Number	99
Male/Female	64/35
Age (median)	18–86 (65)
WHO classification	
MDS-SLD	36 (36.4%)
MDS-MLD	20 (20.2%)
MDS-RS	6 (6.1%)
MDS-EB-1	12 (12.1%)
MDS-EB-2	11 (11.1%)
MDS-U	13 (13.1%)
5q-	1 (1.0%)
IPSS-R	(N=98)
Very low	14 (14.3%)
Low	42 (42.9%)
Intermediate	27 (27.6%)
High	8 (8.2%)
Very high	7 (7.1%)
Treatment	
Azacitidine	14 (14.1%)
Chemotherapy	7 (7.1%)
Cyclosporin A	14 (14.1%)
Transfusion	58 (58.6%)
Stem cell transplantation	6 (6.1%)
Abnormal Karyotype	48 (N=98)
IPSS-R Karyotype	(N=98)
Very good	3 (3.1%)
Good	61 (62.2%)
Intermediate	23 (23.5%)
Poor	2 (2.0%)
Very poor	9 (9.2%)

IPSS-R: revised International Prognostic Scoring System

Associations of *SRSF2* polymorphism with clinical variables and prognosis of MDS patients

The association of *SRSF2* polymorphism with the clinical variables of patients with MDS is summarized in Table 4. We divided into two groups which were the *SRSF2* rs237057 major homozygous genotype in Japanese population (AA) and the others (AG/GG). There were no significant differences between the *SRSF2* rs237057 and clinical variables. Subsequently, we examined the effect of *SRSF2* polymorphism on the OS, LFS and PFS in patients with MDS (Figure 1A, B, C). There were no significant differences for the SNP in the prognosis of MDS patients.

Table 2 The clinical characteristics of the patients with AML.

Number	92
Male / Female	54/38
Age (median)	15–86 (59)
FAB classfication	
M0	6 (6.5%)
M1	14 (15.2%)
M2	32 (34.8%)
M3	21 (22.8%)
M4	12 (13.0%)
M5	4 (4.3%)
M6	2 (2.2%)
M7	1 (1.1%)
MRC classfication	
Favorable	36 (39.1%)
Intermadiate	49 (53.3%)
Adverse	7 (7.6%)
Stem cell transplantation	11 (12.0%)
Complete response	87 (94.6%)

MRC classification: UK Medical Research Council classification

Table 3 Genotype and allele distributions of *SRSF2* and *SF3A1* polymorphisms.

	Control		MDS (vs. Control)					AML (vs. Control)				
	Number	%	N	%	OR	95%CI	*p* value	N	%	OR	95%CI	*p* value
SRSF2 rs237057												
AA	129	75.0	79	79.8	1.32	0.72–2.40	0.45	72	77.4	1.20	0.66–2.20	0.55
AG	42	24.4	17	17.2	0.64	0.34–1.20	0.16	18	19.4	0.75	0.40–1.40	0.37
GG	1	0.6	3	3.0	5.34	0.55–52.1	0.14	2	2.2	3.8	0.34–42.5	0.28
	172		99					92				
A allele	300	87.2	175	88.4				162	88.0			
G allele	44	12.8	23	11.6	0.9	0.52–1.53	0.69	22	12.0	0.92	0.54–1.60	0.78
C allele	0	0.0	0	0.0				0	0.0			
	344		198					184				
SF3A1 rs2074733												
TT	159	92.4	87	87.9	0.59	0.26–1.36		84	91.3	0.86	0.34–2.15	
TC	13	7.6	12	12.1	1.69	0.74–3.86	0.21	8	8.7	1.17	0.46–2.92	0.74
CC	0	0.0	0	0.0				0	0.0			
	172		99					92				
T allele	331	96.2	186	93.9	0.61	0.27–1.36		176	95.7	0.86	0.35–2.12	
C allele	13	3.8	12	6.1	1.64	0.73–3.67	0.22	8	4.3	1.16	0.47–2.85	0.75
	344		198					184				

Table 4 Clinical characteristics of MDS patients according to the *SRSF2* rs237057 genotypes.

Number	AA genotype 79	AG and GG genotype 20	*p* value
Male / Female	52/27	12/8	0.62
Age (median)	18-86 (65)	31-85 (68)	0.80
WHO classification			
MDS-SLD	27 (34.2%)	9 (45.0%)	0.37
MDS-MLD	15 (19.0%)	5 (25.0%)	0.54
MDS-RS	5 (6.3%)	1 (5.0%)	1.00
MDS-EB-1	11 (13.9%)	1 (5.0%)	0.64
MDS-EB-2	9 (11.4%)	2 (10.0%)	1.00
MDS-U	11 (13.9%)	2 (10.0%)	1.00
5q-	1 (1.3%)	0 (0%)	1.00
IPSS-R		(N=86)	
Very low	10 (12.7%)	4 (21.1%)	0.46
Low	32 (40.5%)	10 (52.6%)	0.34
Intermediate	24 (30.4%)	3 (15.8%)	0.20
High	7 (8.9%)	1 (5.3%)	1.00
Very high	6 (7.6%)	1 (5.3%)	1.00
Treatment			
Azacitidine	10 (12.7%)	4 (20.0%)	0.47
Chemotherapy	7 (8.9%)	0 (0%)	0.34
Cyclosporin A	13 (16.5%)	1 (5.3%)	0.29
Transfusion	47 (59.5%)	11 (55.0%)	0.72
Stem cell transplantation (SCT)	6 (7.6%)	0 (0%)	0.34
		(N=86)	
Abnormal Karyotype	40 (51.3%)	8 (42.1%)	0.47
IPSS-R Karyotype			
Very good	2 (2.5%)	1 (5.3%)	0.48
Good	48 (60.8%)	13 (68.4%)	0.61
Intermediate	20 (25.3%)	3 (15.8%)	0.55
Poor	2 (2.5%)	0 (0%)	1.00
Very poor	7 (8.9%)	2 (10.5%)	1.00
Very good & good	50 (63.3%)	14 (73.7%)	0.39
Others	29 (36.7%)	5 (26.3%)	
WBC ($\times 10^9$/L)	3.47±1.88	2.56±0.94	0.05
Hb (g/dL)	9.30±2.26	9.18±1.87	0.84
Plt ($\times 10^9$/L)	122±105	127±88.4	0.86
LDH (IU/L)	219±69.6	190±44.7	0.086

Table 5 Clinical characteristics of AML patients according to the *SRSF2* rs237057 genotypes.

				non-M3 patients		
	AA genotype 72	AG & GG genotype 20	*p* value	AA genotype 55	AG & GG genotype 16	*p* value
Male / Female	44/28	10/10	0.37	37/18	8/8	0.21
Age (median)	15–80 (58)	21–86 (63)	0.81	15–77 (60)	21–86 (65)	0.18
Disease progression	30 (41.7%)	11 (55.0%)	0.29	26 (47.3%)	10 (62.5%)	0.28
Stem cell transplantation	9 (12.5%)	2 (10.0%)	1.00	7 (12.7%)	1 (6.3%)	0.67
Complete response	70 (97.2%)	17 (85.0%)	0.07	53 (96.4%)	13 (81.3%)	0.07
FAB classfication						
M0	4 (5.6%)	2 (10.0%)	0.61	4 (17.3%)	2 (12.5%)	0.61
M1	9 (12.5%)	5 (25.0%)	0.18	9 (16.4%)	5 (31.3%)	0.28
M2	28 (38.9%)	4 (120.%)	0.12	28 (50.9%)	4 (25.0%)	0.07
M3	17 (23.6%)	4 (20.0%)	1.00			
M4	11 (15.3%)	1 (15.0%)	0.45	11 (20.0%)	1 (16.3%)	0.28
M5	2 (2.8%)	2 (10.0%)	0.21	2 (13.6%)	2 (12.5%)	0.22
M6	1 (1.4%)	1 (15.0%)	0.39	1 (11.8%)	1 (16.3%)	0.40
M7	0 (0%)	1 (15.0%)	0.22	0 (0%)	1 (16.3%)	0.23
MRC classfication						
Favorable	31 (43.1%)	5 (25.0%)	0.14	14 (25.5%)	1 (16.3%)	0.16
Intermadiate	35 (48.6%)	14 (70.0%)	0.09	35 (63.6%)	14 (87.5%)	0.12
Adverse	6 (8.3%)	1 (5.0%)	1.00	6 (10.9%)	1 (16.3%)	1.00

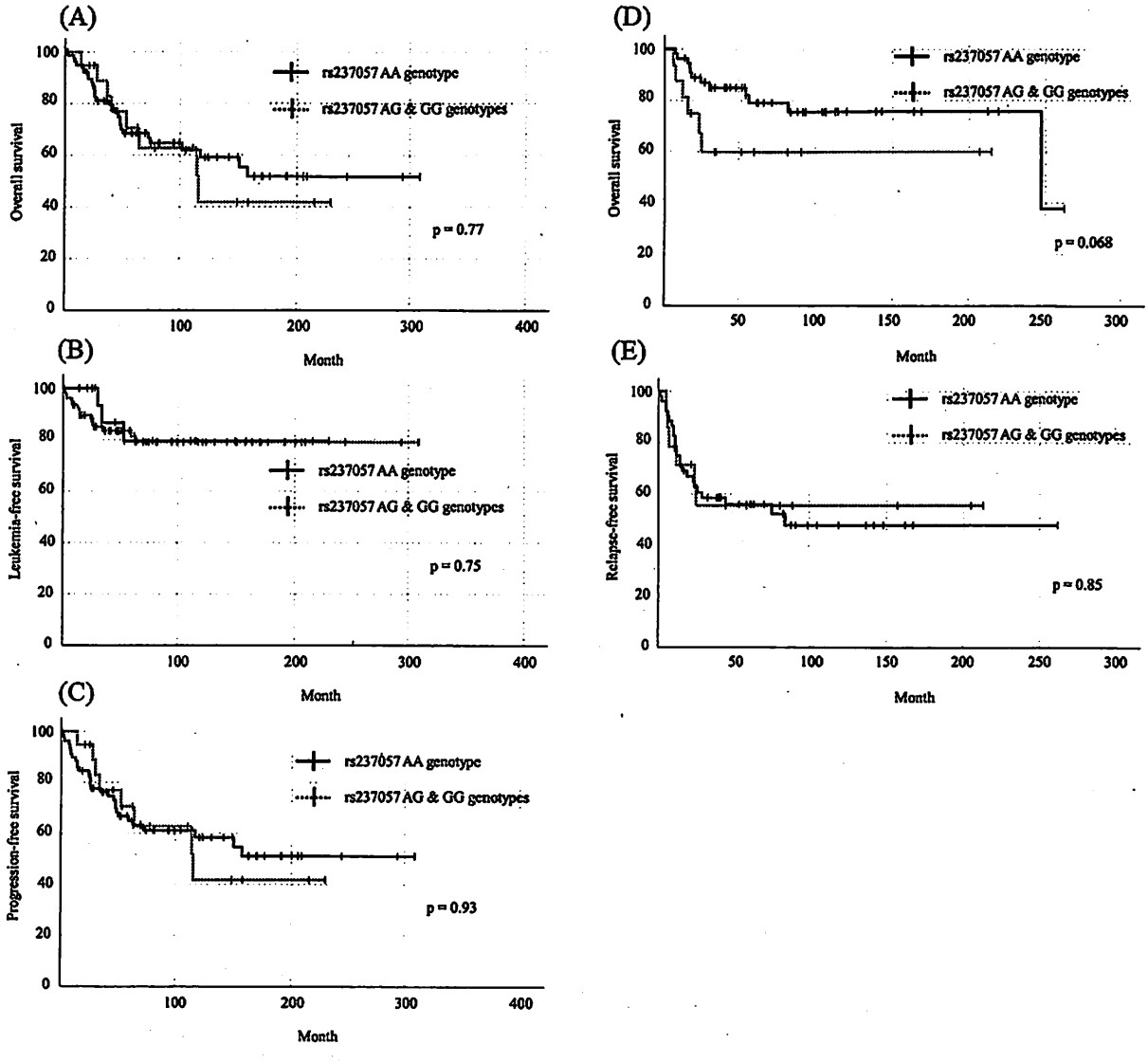

Fig. 1 (A) Overall survival (OS) of MDS patients according to the *SRSF2* rs237057 genotypes. The median survival times of patients with the AA and AG & GG genotypes were "not reached" and 115.9 months, respectively (P=0.77). (B) Leukemia-free survival (LFS) of MDS patients according to the *SRSF2* rs237057 genotypes. The median survival times of patients were "not reached", for both the AA and AG & GG genotypes (P=0.75). (C) Progression-free survival (PFS) of MDS patients according to the *SRSF2* rs237057 genotypes. The median survival time of patients with the AA and AG & GG genotypes were "not reached" and 116.0 months, respectively (P=0.93). (D) OS of non-M3 AML patients, according to their *SRSF2* rs237057 genotype. The median survival time of patients with the AA and AG & GG genotypes were 247.3 months and "not reached", respectively (P=0.068). (E) Relapse-free survival (RFS) of non-M3 AML patients, according to their *SRSF2* rs237057 genotypes. The median survival times of patients with the AA and AG & GG genotypes were 84 months and "not reached", respectively (P=0.85).

Associations of *SRSF2* polymorphism with clinical variables and prognosis of AML patients

The association of *SRSF2* polymorphism with the clinical variables of patients with AML is summarized in Table 5. There were no significant differences between the *SRSF2* rs237057 and clinical variables. Subsequently, we examined the effect of *SF3A1* polymorphism on the OS and RFS in patients with non-M3 AML (Figure 1D, E), because the M3 AML patients clearly had a better prognosis than the non-M3 AML patients. There was also no significant difference in non-M3 AML patients.

Associations of *SF3A1* polymorphism with clinical variables and prognosis of MDS patients

The association of *SF3A1* polymorphism with the clinical variables of patients with MDS are summarized in Table 6. Hemoglobin level of patients with the *SF3A1* rs2074733 TC genotype was significantly higher than the TT genotype (mean±standard deviation, 10.6±1.63 vs 9.09±2.19 g/dL; P=0.022). In addition, patients with the rs2074733 TC genotype showed a significantly lower frequency of chromosomal abnormality [2 (18.2%) vs. 46 (53.5%), P=0.027]. We divided the IPSS-R karyotypes into two groups as "very good & good" and "the others". However, no significant difference was observed between the *SF3A1* rs2074733 TC genotype and TT genotype in

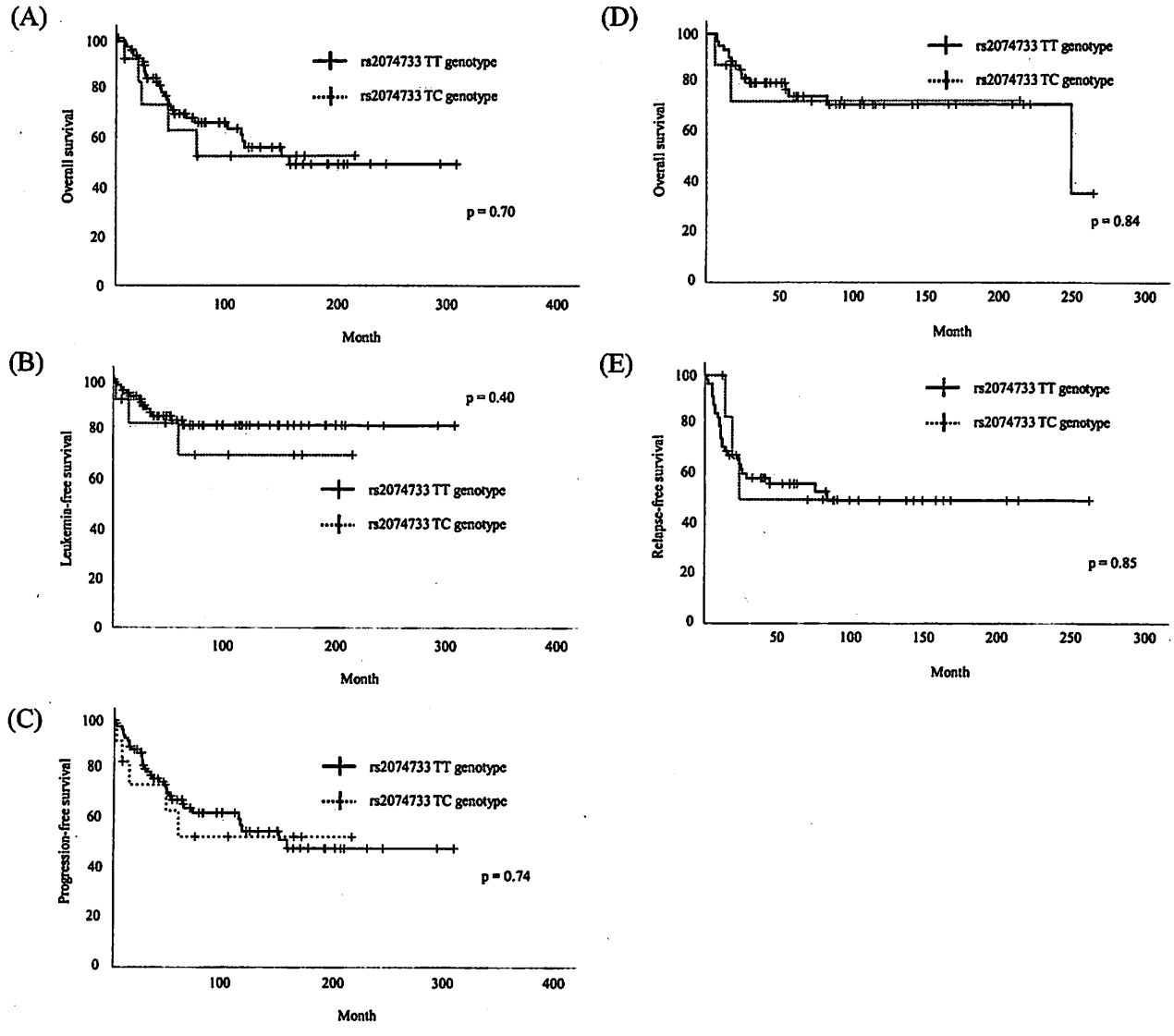

Fig. 2 (A) Overall survival (OS) of MDS patients according to the *SF3A1* rs2074733 genotypes. The median survival times of patients with the TT and TC genotypes were 150.7 months and "not reached", respectively (P=0.70). (B) Leukemia-free survival (LFS) of MDS patients according to the *SF3A1* rs2074733 genotypes. The median survival times of patients were "not reached", for both the TT and TC genotypes (P=0.40). (C) Progression-free survival (PFS) of MDS patients according to the *SF3A1* rs2074733 genotypes. The median survival time of patients with the TT and TC genotypes were 157.0 months and "not reached", respectively (P=0.74). (D) OS of non-M3 AML patients, according to their *SF3A1* rs2074733 genotype. The median survival time of patients with the TT and TC genotypes were 247.3 months and "not reached", respectively (P=0.84). (E) Relapse-free survival (RFS) of non-M3 AML patients, according to their *SF3A1* rs2074733 genotypes. The median survival times of patients were "not reached" for both TT and TC genotypes (P=0.85).

clinical variables.

Subsequently, we also examined the effect of *SF3A1* polymorphism on the OS, LFS and PFS in patients with MDS (Figure 2A, B, C). There were no significant differences for the SNP in the prognosis of MDS patients.

Associations of *SF3A1* polymorphism with clinical variables and prognosis of AML patients

The association of *SF3A1* polymorphisms with the clinical variables of patients with AML is shown in Table 7. There was no statistically significant difference for *SF3A1* polymorphism and clinical variables of patients with AML. Interestingly, the patients with TC genotype had no M3 subtype, using FAB classification; however, there was no statistically significant difference in the incidence of M3 between TC and TT genotypes. There were no significant differences for *SF3A1* polymorphism and the OS/LFS of AML patients. In addition, we examined the effect of *SF3A1* polymorphism on the OS and LFS in patients with non-M3 AML (Figure 2D, E), because the M3 AML patients clearly had a better prognosis than the non-M3 AML patients. There was also no significant difference in non-M3 AML patients.

Table 6 Clinical characteristics of MDS patients according to the *SF3A1* rs2074733 genotypes.

Number	TC genotype 12	AG and GG genotype 20	*p* value
Male / Female	6/6	58/29	0.34
Age (median)	48-78 (64)	18-86 (66)	
WHO classification			
MDS-SLD	6 (50.0%)	30 (34.5%)	0.35
MDS-MLD	2 (16.7%)	18 (20.7%)	1.00
MDS-RS	0 (0%)	6 (6.9%)	1.00
MDS-EB-1	2 (16.7%)	10 (11.5%)	0.64
MDS-EB-2	2 (16.7%)	9 (10.3%)	0.62
MDS-U	0 (0%)	13 (14.9%)	0.36
5q-	0 (0%)	1 (1.1%)	1.00
IPSS-R		(N=86)	
Very low	3 (25.0%)	11 (12.6%)	0.37
Low	3 (25.0%)	39 (44.8%)	0.18
Intermediate	5 (41.7%)	22 (25.3%)	0.30
High	1 (8.3%)	7 (8.0%)	1.00
Very high	0 (0%)	7 (8.0%)	0.59
Treatment			
Azacitidine	2 (16.7%)	12 (13.8%)	0.68
Chemotherapy	2 (16.7%)	5 (5.7%)	0.20
Cyclosporin A	4 (33.3%)	10 (11.5%)	0.064
Transfusion	6 (50.0%)	52 (59.8%)	0.55
Stem cell transplantation (SCT)	1 (8.3%)	5 (5.7%)	0.55
		(N=86)	
Abnormal Karyotype	2 (18.2%)	46 (53.5%)	0.027
IPSS-R Karyotype			
Very good	1 (8.3%)	2 (2.3%)	0.33
Good	10 (83.3%)	51 (59.3%)	0.13
Intermediate	1 (8.3%)	22 (25.6%)	0.28
Poor	0 (0%)	2 (2.3%)	1.00
Very poor	0 (0%)	9 (10.5%)	0.60
Very good & good	11 (91.7%)	53 (61.6%)	0.052
Others	1 (8.3%)	33 (38.4%)	
WBC ($\times 10^9$/L)	3.01±1.07	3.40±1.82	0.48
Hb (g/dL)	10.6±1.63	9.09±2.19	0.022
Plt ($\times 10^9$/L)	84.5±53.8	129±106	0.16
LDH (IU/L)	218±66.6	213±66.5	0.81

Table 7 Clinical characteristics of AML patients according to the *SF3A1* rs2074733 genotypes.

	TC genotype 8	TT genoptype 84	*p* value	non-M3 patients		
				TC genotype 8	TT genotype 63	*p* value
	5/3	49/35	1.00	5/3	40/23	1.00
	27-74 (59)	15-86 (58)	0.81	27-74 (59)	15-86 (62)	0.80
Disease progression	4 (50.0%)	37 (40.0%)	1.00	4 (50.0%)	32 (50.8%)	1.00
Stem cell transplantation	0 (0%)	11 (13.1%)	0.59	0 (0%)	8 (12.7%)	0.58
Complete response	7 (87.5%)	80 (95.2%)	0.37	7 (87.5%)	59 (93.7%)	0.46
FAB classfication						
M0	1 (12.5%)	5 (6.0%)	0.43	1 (12.5%)	5 (7.9%)	0.53
M1	2 (25.0%)	12 (14.3%)	0.35	2 (25.0%)	12 (19.0%)	0.65
M2	4 (50.0%)	28 (33.3%)	0.44	4 (50.0%)	28 (44.4%)	1.00
M3	0 (0%)	21 (25.0%)	0.19			
M4	1 (12.5%)	11 (13.1%)	1.00	1 (12.5%)	11 (17.5%)	1.00
M5	0 (0%)	4 (4.8%)	1.00	0 (0%)	4 (6.3%)	1.00
M6	0 (0%)	2 (2.4%)	1.00	0 (0%)	2 (3.2%)	1.00
M7	0 (0%)	1 (1.1%)	1.00	0 (0%)	1 (1.4%)	1.00
MRC classfication						
Favorable	2 (25.0%)	34 (40.5%)	0.48	2 (25.0%)	13 (20.6%)	0.67
Intermadiate	5 (62.5%)	44 (52.4%)	0.72	5 (62.5%)	44 (69.8%)	0.70
Adverse	1 (12.5%)	6 (7.1%)	0.48	1 (12.5%)	6 (9.5%)	0.5

Discussion

SRSF2 promotes exon recognition by binding to exonic splicing enhancer motifs in pre-mRNA, through its RNA binding domain, and binding between U2AF heterodimer and U1 snRNP to the upstream 3′ splice site.[9] The *SRSF2* rs237057 G/A/C genetic variant results in an amino acid substitution, from aspartic acid (G allele or A allele) to glutamic acid (C allele), in exon 1. Choi *et al.* investigated the link between *SRSF2* rs237057 with childhood AML in a Korean population,[3] and found no significant association.

SF3A1 facilitates branch site recognition by U2 snRNA with *SF3B1*, and tethering of U2 snRNP to the pre-mRNA.[10] *SF3A1* is necessary for pre-mRNA splicing by U2 snRNA. *SF3A1* rs2074733 is located in intron 5, without amino acid substitution. It shows complete linkage disequilibrium with *SF3A1* SNPs rs5753071, rs10376, and rs10427610, which are found to be located at the site of transcription factor binding, histone modification and open chromatin, by bioinformatic analysis.[4,11] Tian et al. reported that, in a Chinese population, the *SF3A1* rs2074733 T allele was related to susceptibility to pancreatic cancer.[4]

Recent studies have revealed that RNA splicing pathway mutations were detected in MDS, MDS-related disorders and AML.[2,6-7] Yoshida *et al.* found the *SRSF2* mutation at position 95, proline residue (P95H, L), in 11.6% of MDS cases without RS, in 5.5% of MDS-RS and in 0.7% of AML.[2] The *SRSF2* rs237057 C allele induces an amino acid substitution. However, almost the entire Asian population was reported to have only the G or A allele, according to data from the International Haplotype Map (HapMap) Project. In this study, we did not find the C allele in healthy control subjects, or in the MDS and AML groups. Choi *et al.* also reported that there were no C allele patients with childhood AML in Korea.[3] On the other hand, the *SF3A1* mutation was observed in 1.3% MDS without RS and in 0.7% of AML cases.[2] However, there were no significant differences in *SF3A1* polymorphism among MDS patients, AML patients and healthy individuals in our study. Our results suggest that the *SRSF2* rs237057 and *SF3A1* rs2074733 have no association with the susceptibility to MDS and AML in a Japanese population.

The *SF3A1* rs2074733 TC genotype was associated with higher hemoglobin level and lower frequency of chromosomal abnormality, compared with the TT genotype, in MDS patients. This polymorphism may affect the production of *SF3A1*, but its relative effect is unclear. The abnormal expression of splicing factors, including *SF3A1*, is known to modify splice site selection and induce the skipped exon and/or retained intron.[12] However, O'Connor et al. reported that *SF3A1* inhibition by siRNA leads to intron retention in several TLR signaling pathway transcripts, such as interleukin 1 receptor associated kinase 1 (*IRAK1*) and I κ B kinase (*IKK-2*).[13] Although the effects of change in *SF3A1* expression are still controversial, the aberrant balance in expression of *SF3A1* might induce abnormal function of the spliceosome. Yoshida *et al.* did not mention the relationship between the spliceosome gene mutation and clinical characteristics of MDS, such as IPSS or clinical laboratory values.[2] To the best of our knowledge, no previous studies have examined the possible association between *SF3A1* and clinical characteristics of MDS patients. A high prevalence of somatic mutation of *SF3B1*, which helps the U2 snRNP bind to the 3′ SS with *SF3A1*, was reported in MDS with ring sideroblasts.[14] Damm *et al.* showed that the MDS patients with *SF3B1* mutation presented with lower hemoglobin levels, increased WBC and platelet counts.[15] Our results suggested that the *SF3A1* rs2074733 TT might be a risk factor for disease severity of MDS, by affecting *SF3A1* protein production.

Several studies have demonstrated an association between mutations in the spliceosome machinery and the prognosis of MDS and AML.[14,16-18] *SF3B1* mutations were found to be independently associated with better overall survival and lower risk of progression to AML, in MDS patients.[14] In low-risk MDS patients, those with *SF3B1* mutations showed a better prognosis, whereas those with *SRSF2* mutations had worse survival.[16] *SRSF2* mutations predicted poor overall survival and more frequent AML progression, compared with the wild type.[17] In addition, the *SRSF2* mutation was associated with shorter overall survival, in AML patients.[18] However, Damm *et al.* found no prognostic impact of the *SRSF2* mutation in MDS patients.[15] There have been no reports about the relationship between *SF3A1* and prognosis of MDS and AML. In the current study, we could not assess the association between the *SRSF2* polymorphism and prognosis because there were no patients in our cohort with the *SRSF2* rs237057 C allele. In this study, the *SF3A1* rs2074733 TT genotype associated with lower hemoglobin level and higher frequency of chromosomal abnormality. The effects of *SF3A1* polymorphisms and other prognostic factors including hemoglobin level and chromosomal abnormality on survival of MDS patients were also examined using the multivariate Cox proportional hazards model. The Cox proportional hazards model demonstrated that both IPSS-R and age at diagnosis over 65 were significantly associated with poor OS (data not shown). In this cohort, the lower hemoglobin level and higher frequency of chromosomal abnormality had no significant impact on MDS prognosis. Moreover, our study suggests that the *SF3A1* rs2074733 is not implicated in prognosis of MDS and AML in a Japanese population.

In conclusion, our study indicates that the polymorphisms of *SRSF2* and *SF3A1* are not associated with a susceptibility to MDS and AML, but *SF3A1* rs2074733 does affect the clinical features of MDS patients. However, there are limitations to the interpretation of the results in this study because the sample size was relatively small. Therefore, further investigations with larger sample sizes are needed to corroborate our results.

Conflicts of interest

The authors declare no conflicts of interest.

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