**SF3A1 Gene Polymorphism Affects Clinical Features, but not Susceptibility to Myelodysplastic Syndromes**

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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.

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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.\textsuperscript{19} 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.\textsuperscript{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.\textsuperscript{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'-CAAG-GTGGACAACCTGACCT-3' \textsuperscript{9}, and downstream 5'-ATGGCATCCATAGCGTCCT-3'. For analysis of SF3A1 polymorphism, we used upstream primer 5'-CCTCTTTCGGAACAGAATGGAA-3' \textsuperscript{10} and downstream 5'-CAAGGCGCAGAAGGCCTTGG-3'. The PCR products of the SRSF2 rs237057 A/G allele were digested with restriction enzyme Sau3AI (New England Biolabs, Massachusetts, USA), AvaI (New England Biolabs) was used to digest the SRSF2 rs237057 C allele, and FaiI (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

(Tables 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 (MDL), 6 (6.1%) as MDS with ring sideroblasts (RS), 1 (1.0%) as MDS with excess blasts (EB), and 1 (1.0%) as MDS-unclassifiable (U), respectively. The IPSS-R score in 20 patients was very low for 1 patient, low for 4 patients, intermediate for 2 patients, high for 4 patients, 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.

| WHO classification       | MDS-SLD | MDS-MLD | MDS-RS | MDS-EB-1 | MDS-EB-2 | MDS-U | 5q- |
|--------------------------|---------|---------|--------|----------|----------|-------|-----|
| Number                   | 99      |         |        |          |          |       |     |
| Male/Female              | 64/35   |         |        |          |          |       |     |
| Age (median)             | 18–86 (65) |       |        |          |          |       |     |

| 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 | Chemotherapy | Cyclosporin A |
|--------------------------|-------------|--------------|---------------|
| Number                   | 14 (14.1%)  | 7 (7.1%)     | 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%)     |

Table 2 The clinical characteristics of the patients with AML.

| FAB classification       | M0 | M1 | M2 | M3 | M4 | M5 | M6 | M7 |
|--------------------------|----|----|----|----|----|----|----|----|
| Number                   | 6  | 14 | 32 | 21 | 12 | 4  | 2  | 1  |
| Male / Female            | 14 | 14 | 14 | 14 | 14 | 14 | 14 | 14 |
| Age (median)             | 15–86 (59) |       |    |    |    |    |    |    |

| MRC classification       | Favorable | Intermediate | Adverse |
|--------------------------|-----------|--------------|---------|
| Number                   | 36 (39.1%)| 49 (53.3%)   | 7 (7.6%) |
| Stem cell transplantation| 11 (12.0%)|              |         |
| Complete response         | 87 (94.6%)|              |         |

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 |
| 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  |       |         |     |
| SF3A1 rs2074733 |       |       |    |       |       |         |     |       |       |         |     |
| TT     | 159    | 92.4 | 87 | 87.9 | 0.59 | 0.26–1.36 | 0.21 | 84 | 91.3 | 0.86 | 0.34–2.15 | 0.74 |
| 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  |       |         |     |
| T allele | 331   | 96.2 | 186 | 93.9 | 0.61 | 0.27–1.36 | 0.22 | 176 | 95.7 | 0.86 | 0.35–2.12 | 0.75 |
| 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 |

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 4 The clinical characteristics of the patients with MDS.

| R     | Number | %    | N   | %    | OR 95%CI | p value | N   | %    | OR 95%CI | p value |
|-------|--------|------|-----|------|---------|---------|-----|------|---------|---------|
| MDS  | 11      | 11.0 | 85  | 9.5  | 0.26–1.36 | 0.21   | 84  | 91.3 | 0.86 | 0.34–2.15 | 0.74 |
| AML  | 12      | 12.0 | 87  | 9.5  | 0.26–1.36 | 0.21   | 84  | 91.3 | 0.86 | 0.34–2.15 | 0.74 |
| SF3A1 | 13      | 13.0 | 12  | 1.3  | 0.4–1.8  | 0.21   | 84  | 91.3 | 0.86 | 0.34–2.15 | 0.74 |

IPSS-R: revised International Prognostic Scoring System

MRC classification: UK Medical Research Council classification
**Table 4** Clinical characteristics of MDS patients according to the *SRSF2* rs237057 genotypes.

| Number | AA genotype | AG and GG genotype | p value |
|--------|-------------|---------------------|---------|
| Male / Female | 52/27 | 12/8 | 0.62 |
| Age (median) | 18-86 (65) | 31-85 (68) | 0.80 |

**WHO classification**

| | MDS-SLD | MDS-MLD | MDS-RS | MDS-EB-1 | MDS-EB-2 | MDS-U | 5q- |
|---|---|---|---|---|---|---|---|
| Age (median) | 15 | 19 | 5 | 6 | 1 | 1 | 1 |
| Stem cell transplantation | 9 | 5 | 1 | 1 | 2 | 2 | 0 |

**IPSS–R**

| | Very low | Low | Intermediate | High | Very high |
|---|---|---|---|---|---|
| LDH (IU/L) | 10 | 32 | 24 | 7 | 6 |
| Hb (g/L) | 10 | 52 | 15 | 5 | 5 |
| IPSS –R | (N=86) | (N=86) | (N=86) | (N=86) | (N=86) |

**Treatment**

| | Azacitidine | Chemotherapy | Cyclosporin A | Transfusion | Stem cell transplantation (SCT) |
|---|---|---|---|---|---|
| Type | 10 | 7 | 13 | 47 | 6 |
| N | (12.7%) | (8.9%) | (16.5%) | (59.4%) | (7.6%) |
| p value | 0.47 | 0.34 | 0.29 | 0.72 | 0.34 |

**Abnormal Karyotype**

| | Very good | Good | Intermediate | Poor | Very poor | Very good & good | Others |
|---|---|---|---|---|---|---|---|
| Abnormal karyotype | 2 | 48 | 20 | 2 | 7 | 50 | 29 |
| N | (2.5%) | (60.8%) | (25.3%) | (2.5%) | (8.9%) | (63.3%) | (36.7%) |
| p value | 0.48 | 0.61 | 0.55 | 1.00 | 0.39 | 0.98 |

**IPSS–R Karyotype**

| | WBC (×10^9/L) | Hb (g/dL) | Plt (×10^9/L) | LDH (IU/L) |
|---|---|---|---|---|
| Abnormal karyotype | 3.47±1.88 | 9.30±2.26 | 122±105 | 219±69.6 |
| Normal karyotype | 2.56±0.94 | 9.18±1.87 | 127±88.4 | 190±44.7 |

**FAB classification**

| | M0 | M1 | M2 | M3 | M4 | M5 | M6 | M7 |
|---|---|---|---|---|---|---|---|---|
| Complete response | 70 | 17 | 4 | 2 | 1 | 1 | 1 | 1 |
| Disease progression | 30 | 11 | 2 | 1 | 0 | 0 | 0 | 0 |
| Stem cell transplantation | 9 | 2 | 1 | 0 | 0 | 0 | 0 | 0 |

**MRC classification**

| | Favorable | Intermediate | Adverse |
|---|---|---|---|
| Complete response | 31 | 35 | 6 |
| Disease progression | 43 | 48 | 8 |

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

| Number | AA genotype | AG & GG genotype | p value | non-M3 patients |
|--------|-------------|-------------------|---------|-----------------|
| 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 |

**FAB classification**

| | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| Complete response | 70 | 17 | 4 | 2 | 1 | 1 | 1 | 1 |
| Disease progression | 30 | 11 | 2 | 1 | 0 | 0 | 0 | 0 |
| Stem cell transplantation | 9 | 2 | 1 | 0 | 0 | 0 | 0 | 0 |

**MRC classification**

| | Favorable | Intermediate | Adverse |
|---|---|---|---|
| Complete response | 31 | 35 | 6 |
| Disease progression | 43 | 48 | 8 |
The association of \textit{SRSF2} polymorphism with the clinical variables of patients with AML is summarized in Table 5. There were no significant differences between the \textit{SRSF2} rs237057 genotype and clinical variables. Subsequently, we examined the effect of \textit{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.

The association of \textit{SRSF2} polymorphism with clinical variables and prognosis of MDS patients

There were no significant differences between the \textit{SRSF2} rs237057 and clinical variables. Subsequently, we examined the effect of \textit{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.

Fig. 1 (A) Overall survival (OS) of MDS patients according to the \textit{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 \textit{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 \textit{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 \textit{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 \textit{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).
SF3A1 SNP affects clinical features of MDS

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.

| Variable                          | TC genotype | AG and GG genotype | p value |
|-----------------------------------|-------------|--------------------|---------|
|                                   | 12          | 20                 |         |
| 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    |
| Stem cell transplantation (SCT)   | 0 (0%)      | 7 (8.0%)           | 0.59    |
| 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 (×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 (×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.

| Variable                          | TC genotype 8 | TT genotype 84 | p value | TC genotype 63 | TT genotype 84 | 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 classification                |               |                |         |                |                |         |
| 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 classification                |               |                |         |                |                |         |
| Favorable                         | 2 (25.0%)     | 34 (40.5%)     | 0.48    | 2 (25.0%)      | 13 (20.6%)    | 0.67    |
| Intermediate                      | 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. 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, and found no significant association.

**SF3A1** facilitates branch site recognition by U2 snRNA with **SF3B1**, and tethering of U2 snRNP to the pre-mRNA. **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. Tian et al. reported that, in a Chinese population, the **SF3A1 rs2074733 T allele** was related to susceptibility to pancreatic cancer.

Recent studies have revealed that RNA splicing pathway mutations were detected in MDS, MDS-related disorders and AML. 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. 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. On the other hand, the **SF3A1 mutation** was observed in 1.3% MDS without RS and in 0.7% of AML cases. 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 induced the skipped exon and/or retained intron. 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 1 κ B kinase (IKK-2). 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. 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. Damm et al. showed that the MDS patients with **SF3B1** mutation presented with lower hemoglobin levels, increased WBC and platelet counts. 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. **SF3B1 mutations** were found to be independently associated with better overall survival and lower risk of progression to AML, in MDS patients. In low-risk MDS patients, those with **SF3B1 mutations** showed a better prognosis, whereas those with **SRSF2 mutations** had worse survival. **SRSF2 mutations** predicted poor overall survival and more frequent AML progression, compared with the wild type. In addition, the **SRSF2 mutation** was associated with shorter overall survival, in AML patients. However, Damm et al. found no prognostic impact of the **SRSF2 mutation** in MDS patients. 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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