Low-level somatic *JAK2* V617F mutation in myelodysplastic/myeloproliferative neoplasm with ring sideroblasts, and thrombocytosis with co-mutated *SF3B1* and *CALR*

TO THE EDITOR: Myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T) is a unique, overlapping neoplasm. This neoplasm is characterized by features that include *SF3B1*-mutant MDS with ring sideroblasts and development of thrombocytosis secondary to the acquisition of signaling mutations, most commonly *JAK2* V617F [1-3]. Here, we report a rare case of late-onset MDS/MPN-RS-T with triple driver mutations in *SF3B1*, *CALR*, and *JAK2* V617F, at the age of 95. *SF3B1* and *CALR* were co-dominant mutations, whereas *JAK2* V617F was a low-level somatic mutation that was missed in next-generation sequencing (NGS) analysis.

A 95-year-old woman visited Soonchunhyang University Bucheon Hospital for the evaluation of dyspnea. She had suffered from anemia and heart failure over the past three months. A complete blood count revealed macrocytic anemia (hemoglobin, 6.1 g/dL) with a mean corpuscular volume as high as 99.4 fL, leukopenia (white blood cell, 3.7×10^9/L), and thrombocytosis (platelet count, 490×10^9/L). Serum biochemical analysis revealed normal folate and cobalamin levels. Haptoglobin and unconjugated bilirubin levels were also normal, and the direct Coombs test result was negative. There was no history of inflammatory or infectious disorders or any other causes of reactive thrombocytosis. Bone marrow analysis revealed dyserythropoiesis with multinuclear and megaloblastoid changes (Fig. 1A). Iron staining showed normal iron levels (grade 2), but ring sideroblasts in up to 20% of all erythroblasts (Fig. 1B). Blasts accounted for <5% of the total nucleated cells. A bone marrow aspirate smear showed the proliferation of large, atypical megakaryocytes (Fig. 1C). The bone marrow cellularity was normal, and reticulin and trichrome staining revealed no myelofibrosis.

The karyotype of the patient was normal. For thrombocytosis, an allele-specific, real-time polymerase chain reaction (PCR, BioSewoom, Seoul, Korea) for the *JAK2* V617F mutation was performed using bone marrow aspirate, and the result was positive. The results from the NGS panel for MDS/MPN detected a Tier 1 *SF3B1* R625H mutation and a Tier 1 *CALR* D373TfsTer57 mutation with variant allele frequencies of 36% and 32%, respectively (Fig. 1D, E). However, unlike the previous PCR result for the *JAK2* V617F study, the mutation was negative in the targeted NGS panel test and in the repeat NGS test. The genomic data visualization tool Integrative Genomics Viewer (IGV) [4] identified a *JAK2* V617F mutation with a low allele burden of

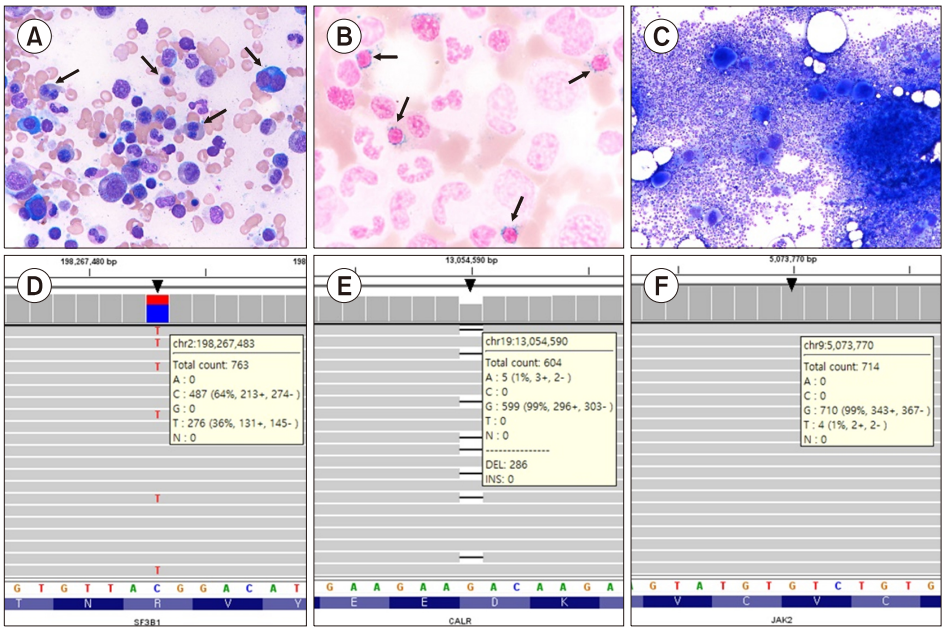

Fig. 1. Hematological and molecular characteristics of the present MDS/MPN-RS-T patient with triple *SF3B1*, *CALR*, and *JAK2* driver mutations. **(A)** Wright–Giemsa stain (×400) of bone marrow aspirate showing dyserythropoiesis with multinuclearity and megaloblastoid changes (arrows). **(B)** Iron stain (×1,000) showing ring sideroblasts (arrows). **(C)** Wright–Giemsa stain (×200) showing increased cellularity and proliferation of large pleomorphic megakaryocytes. **(D–F)** Integrative Genomics Viewer snapshot of *SF3B1* missense mutation (NC_000002.11:g.198267483C>T, R625H) **(D)**, *CALR* frameshift mutation (NC_000019.9:g.13054590del, D373TfsTer57) **(E)**, and *JAK2* missense mutation (NC_000009.11:g.5073770G>T, V617F) **(F)** identified by next-generation sequencing (arrowheads).

less than 1% (Fig. 1F), lower than the variant calling cut-off (3%) used for NGS analysis in the laboratory. Based on these results, the patient was diagnosed with *SF3B1/CALR/JAK2* triple-mutated MDS/MPN-RS-T. The patient is currently receiving aspirin for thrombocytosis and a darbepoetin injection every 2 weeks for anemia. Although the requirement for red blood cells has decreased after the darbepoetin treatment, approximately 2 units of blood transfusion are still required per month.

Reporting of this case without informed consent was approved by the Institutional Review Board of Soonchunhyang University Bucheon Hospital (SCHBC 2022-02-023).

We present a rare case of MDS/MPN-RS-T with *SF3B1*, *CALR*, and *JAK2* mutations in this study. This is an important finding given that the disease is defined by the specific presence of *SF3B1* and *JAK2* V617F mutations [2]. Other signaling mutations, such as *MPL* and *CALR* are infrequent (<5%) in MDS/MPN-RS-T [2]. Cases of MDS/MPN-RS-T with triple mutations have rarely been reported (Table 1) [5, 6]. Triple *SF3B1/CARL/JAK2* mutations, as in the current patient, have previously only been described in one other case [5]. The affected individual had a triple mutation in the MDS/MPN-RS-T disease phase, but the *JAK2* and *CALR* mutations weakened while the SF3B1 mutant clone strengthened as the patient developed myelofibrosis and acute myeloid leukemia [5]. Compared to the previous case [5], the current patient experienced late disease onset accompanied by leukopenia and mild thrombocytosis at the time of diagnosis, showing a tendency towards MDS features. To date, little is known about the prognosis of MDS/MPN-RS-T with triple mutations, making long-term follow-ups with our patient essential.

The *JAK2* V617F allele burden in our case was very low (0.6%) compared to that of *SF3B1* (36%) and *CALR* (32%) mutations. The antecedent relationship between these mutations was unclear at the time of the initial diagnosis. The low *JAK2* mutant burden might be explained by pre-existing clonal hematopoiesis before overt signs of disease [7], followed by the acquisition of second oncogenic mutations. Meanwhile, in most patients with MDS/MPN-RS-T, it is believed that *SF3B1*-mutant MDS clonally evolves into MDS/MPN-RS-T with the acquisition of signaling mutations [1, 2, 5]. The *JAK2* V617F mutation might have been acquired at the latest stage of the disease.

NGS plays an important role in the investigation of somatic mutations in hematological malignancies. Mutation frequency is a major problem in tumor mutation detection, and, as in the present case, detection of low-level (<1%) somatic mutations is a challenge for conventional NGS [8]. In addition to improving mutation calling performance by increasing sequencing depth, it is also important to verify the mutation using visualization tools, such as IGV, for important known driver mutations, regardless of the defined cut-off value for analysis.

Table 1. Summary of patients with MDS/MPN-RS-T carrying triple driver mutations.

	This study	Yasuda *et al.* [5]	Ye *et al.* [6]
Age at diagnosis (yr)	95	60	68
Sex	Female	Male	Female
Splenomegaly	No	ND	No
Complete blood count			
Hb (g/dL)	6.1	9.4	9.0
WBC ($\times 10^9$/L)	3.7	6.5	4.4
Platelets ($\times 10^9$/L)	490	775	658
Bone marrow features			
Ring sideroblasts (%)	20	>15	70
Megakaryocytic hyperplasia	Yes	Yes	Yes
Fibrosis	No	ND	Yes
Cytogenetics	Normal	Normal	Normal
Mutation (allele burden)			
SF3B1 mutation	R625H (36%)	R625C (40%)	K700E (36%)
MPN-associated driver mutation			
JAK2	V617F (1%)	V617F (5–10%)	V617F (20%)
CALR	D373TfsTer57 (32%)	E378RfsTer45 (5–10%)	Wild type
MPL	Wild type	Wild type	W515L (4%)
Treatment	Aspirin	Hydroxyurea, anagrelide	Darbepoetin alpha, hydroxyurea, and aspirin
Transfusion dependency	Yes	ND	ND
Follow-up/outcome	Alive	Dead Myelofibrosis/AML progression	ND

Abbreviations: AML, acute myeloid leukemia; Hb, hemoglobin; MDS/MPN-RS-T, myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis; MPN, myeloproliferative neoplasm; ND, no data; WBC, white blood cell.

In conclusion, we identified a rare case of MDS/MPN-RS-T with a triple *SF3B1/CALR/JAK2* mutation. The presence of multiple mutations is rarely observed, and the molecular mechanisms causing molecular complexity and their consequent clinical impact is unclear. We believe that this report contributes to a better understanding of the clinical features and molecular basis of this rare type of MDS/MPN-RS-T.

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Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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A rare case of pediatric immune thrombocytopenia with secondary antiphospholipid syndrome in Korea

TO THE EDITOR: Chronic immune thrombocytopenia (ITP) is defined as thrombocytopenia that persists for more than 12 months [1, 2]. In most cases, childhood ITP resolves spontaneously within 6 months [2]. However, approximately 20–30% of the children diagnosed with ITP develop chronic ITP [2]. Although ITP is a relatively common disease in childhood, chronic ITP in children must be carefully evaluated for its underlying cause. Risk factors for chronic ITP in children include older age at the time of ITP diagnosis, less severe thrombocytopenia, slow onset of symptoms, gradual improvement in platelet count at 4 weeks, and insufficient evidence of prior infection or vaccination [2].

Approximately 20% of ITPs are related to an underlying disorder and are classified as secondary ITPs [2]. In such cases, physicians should carefully reanalyze the patient's history. Furthermore, bone marrow aspiration should be performed, and peripheral blood smears should be retested [1]. Secondary causes of ITP in children include systemic autoimmune diseases, infections, lymphoproliferative disorders, or immunodeficiency [3]. Systemic autoimmune diseases include systemic lupus erythematosus (SLE), antiphospholipid syndrome (APS), rheumatoid arthritis, and autoimmune thrombocytopenia (e.g., Evans syndrome) [3]. Since APS is one of the causes of secondary ITP, patients with chronic ITP should be tested for APS [3]. However, there is little data on APS as an underlying cause of chronic ITP in pediatric patients. Herein, we report the case of an 11-year-old female Korean patient with chronic ITP, who was eventually diagnosed with APS at 15 years of age. This study was approved by the Institutional Review Board of Keimyung University Dongsan Hospital (approval no. 2022-03-028) and was conducted in accordance with the Declaration of Helsinki.