News Release

Title

Detection of Subclonal *SETBP1* and *JAK3* Mutations in Juvenile Myelomonocytic Leukemia Using Droplet Digital PCR

Key Points

- **ddPCR is a useful tool for the detection of subclonal *SETBP1* and *JAK3* mutations with low variant allele frequencies (VAF).**
- **The detection of subclonal *SETBP1* and *JAK3* mutations, including those with low VAF, help stratify patients with JMML at initial diagnosis.**

Summary

Prof. Yoshiyuki Takahashi, Dr. Hideki Muramatsu, Dr. Manabu Wakamatsu in Department of Pediatrics, Nagoya University Graduate School of Medicine, and Dr. Yusuke Okuno in Medical Genomics Center, Nagoya University Hospital, and their colleagues assessed subclonal *SETBP1* and *JAK3* hotspot mutations that identify patients with poor prognosis in juvenile myelomonocytic leukemia using droplet digital PCR.

Juvenile myelomonocytic leukemia (JMML), a rare and aggressive myelodysplastic/myeloproliferative neoplasm occurring in infants and early childhood, is characterized by excessive myelomonocytic cell proliferation. More than 90% of patients harbor germline and somatic mutations in RAS pathway genes, such as *PTPN11, NF1, NRAS, KRAS,* and *CBL*. Recent mutational analyses identified secondary mutations, such as *SETBP1*, *JAK3*, and several other mutations that occur in addition to the RAS pathway mutations. Among the secondary mutations, most frequent hotspot mutations are *SETBP1* p.D868N and *JAK3* p.R657Q.

Droplet digital PCR (ddPCR) is a highly sensitive form of PCR that provides absolute quantification of PCR products. Using ddPCR, we assessed both *SETBP1* p.D868N and *JAK3* p.R657Q hotspot mutations in 128 patients with JMML. We identified 9 (7.0%) and 15 (11.7%) patients with *SETBP1* and *JAK3* mutations, respectively; among them, 9 mutations with <1% VAF (9/24; 37.5%) were not identified by conventional targeted deep sequencing. The presence of subclonal *SETBP1* and/or *JAK3* mutations was significantly associated with poor transplantation-free survival (P = 0.017). Interestingly, we identified five patients carrying both mutations; colony forming assay revealed the presence of both mutations within the same clone.

ddPCR is a useful tool for the assessment of subclonal *SETBP1* and *JAK3* mutations. In clinical practice, the detection of subclonal *SETBP1* and *JAK3* mutations, including those with extreme low VAF, will help stratify patients with JMML at initial diagnosis.

Research Background

Juvenile myelomonocytic leukemia (JMML) is a rare myelodysplastic/myeloproliferative neoplasm that occurs in infants and during early childhood, characterized by excessive myelomonocytic cell proliferation and granulocyte-macrophage colony-stimulating factor hypersensitivity. Hematopoietic stem cell transplantation (HSCT) is the only curative treatment for JMML.

More than 90% of JMML patients harbor somatic and/or germline mutations in canonical RAS pathway genes; *PTPN11*, *NF1*, *NRAS*, *KRAS*, and *CBL*. Preceding studies identified secondary mutations, such as *SETBP1*, *JAK3*, and several other mutations that occur in addition to the RAS pathway mutations. Patients with secondary mutations had shorter survival compared to those without mutations. Among the secondary mutations, most frequent hotspot mutations are *SETBP1* p.D868N and *JAK3* p.R657Q.

Droplet digital polymerase chain reaction (ddPCR) is a novel molecular biology technique providing absolute quantification of target PCR products. In the previous study, ddPCR identified subclonal *SETBP1* hotspot mutations, including those with <0.1% variant allele frequency (VAF), among patients with JMML at initial diagnosis. These rare *SETBP1* mutations were independently correlated with a dismal prognosis. However, *JAK3* hotspot mutations with low VAF have not been assessed.

Research Results

In this study, we enrolled 128 patients with JMML. ddPCR was performed to detect subclonal *SETBP1* p.D868N and *JAK3* p.R657Q hotspot mutations. We defined the detected threshold in the *SETBP1* and *JAK3* mutations as 0.05% VAF (**Figure 1**; dotted line). Among the 128 patients with JMML, *SETBP1* p.D868N and *JAK3* p.R657Q mutations were detected in 9 (7.0%) and 15 (11.7%) patients, respectively (**Figure 1**). Nine out of the total of 24 mutations (37.5%) showed <1% VAF of *SETBP1* or *JAK3* mutation, which had not been identified by conventional targeted deep sequencing, suggesting the superior sensitivity of ddPCR assay.

To assess the clinical outcomes, patients with JMML were categorized into three groups according to the VAF in the *SETBP1* and *JAK3* mutations (**Figure 2**). JMML with *CBL* mutation (n = 24) were not included in this survival analysis. Patients carrying mutations with ≥1% VAF in at least one gene were categorized as the Major group (n = 14), those carrying only mutations with <1% VAF were defined as the Minor group (n = 5), and those who did not carry the mutations were categorized as the Wildtype group (n = 85). The presence of subclonal *SETBP1* and/or *JAK3* mutations were significantly associated with poor transplantation-free survival (P = 0.017).

Next, we found that among the 128 patients with JMML, 19 (14.8%) harbored *SETBP1* and/or *JAK3* mutations, including five patients (26.3%) who carried both the mutations. We evaluated whether *SETBP1* and *JAK3* mutations were present within the same clone in patients with both mutations. Bone marrow mononuclear cells of the case with both mutations were cultured with granulocyte-colony stimulating factor. Of the 93 colonies analyzed

individually by Sanger sequencing, two colonies (2.1%) showed *SETBP1*, *JAK3*, and *PTPN11* mutations.

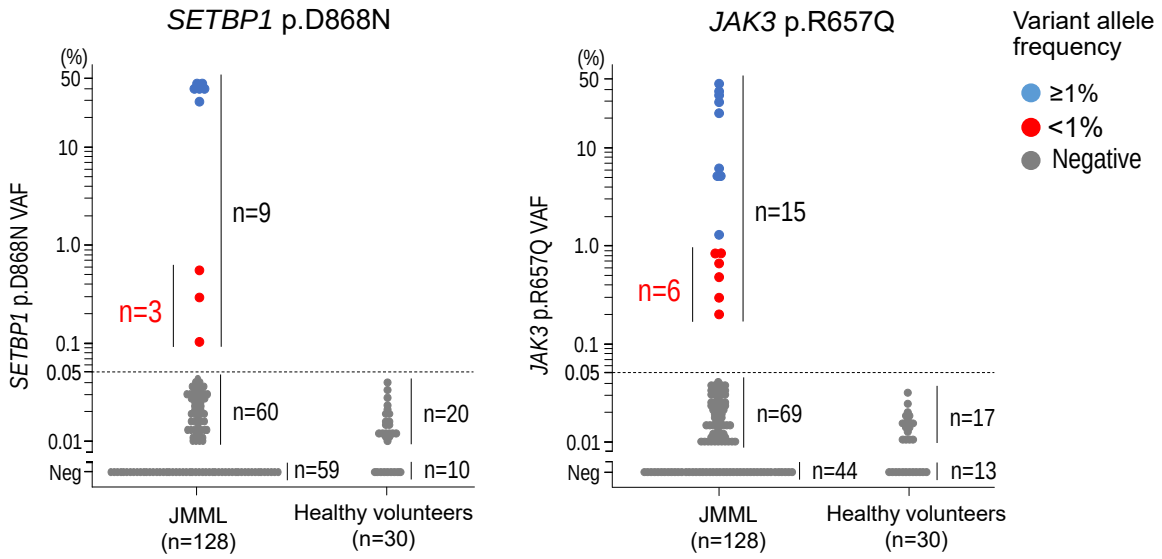

Figure 1. Identification of *SETBP1* and *JAK3* mutations in JMML using ddPCR

We identified *SETBP1* (n = 9) and *JAK3* (n = 15) mutations in JMML. Dotted lines (0.05%) indicate the detection threshold.

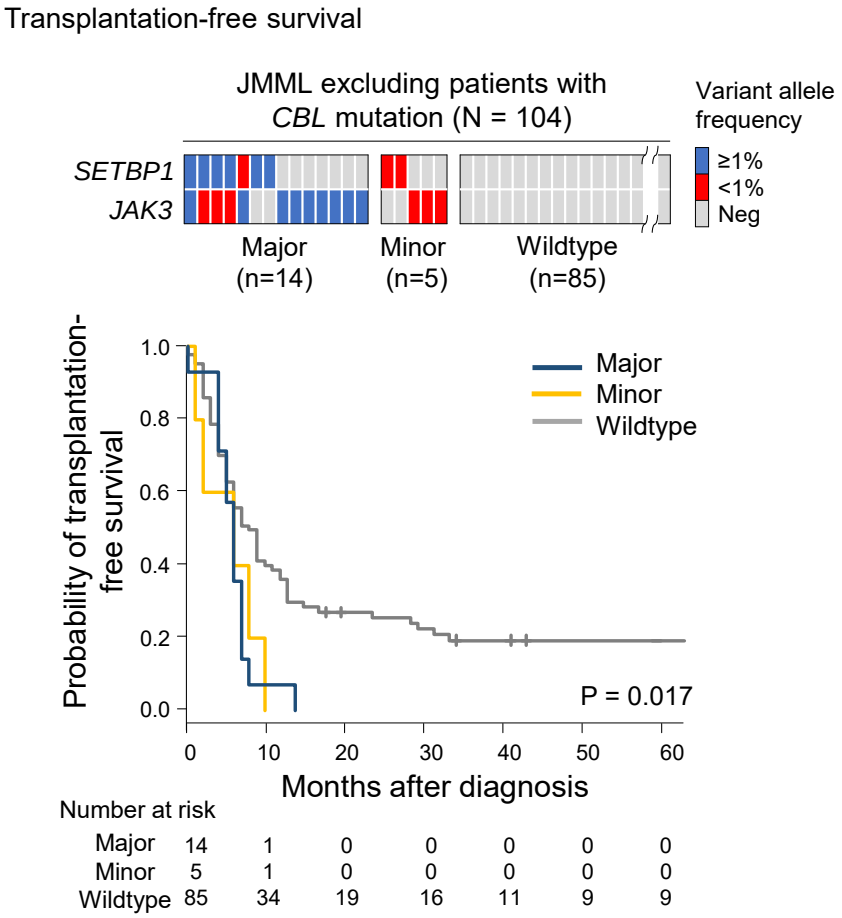

Figure 2. Overall and transplantation-free survival according to VAF in *SETBP1* and *JAK3*

mutations

The presence of subclonal *SETBP1* and/or *JAK3* mutations (Major and Minor groups) was significantly associated with poor transplantation-free survival (P = 0.017).

Research Summary and Future Perspective

This is the first study to evaluate subclonal *JAK3* hotspot mutations with extremely low VAF in JMML patients, which might be associated with poor clinical outcome. In the future, it may be possible to modify the treatment based on these mutations; for example, preparations for HSCT can be initiated in patients with JMML harboring subclonal *SETBP1* and/or *JAK3* mutations at initial diagnosis.

Despite the extremely small number of somatic mutations in JMML, the patients carrying both *SETBP1* and *JAK3* mutations were recurrently observed. These mutations were suggested to be acquired within a same clone by a colony formation assay. Recently, the order of somatic mutations in *JAK2* and *TET2* has been reported to be associated with the clinical features, response to treatment, biology of disease, and clonal evolution among patients with MPN. In JMML, the correlations between the mutation order in *SETBP1* and *JAK3* and clinical outcome are not well understood. Further studies that are using larger sample sizes are required to clarify these correlations.

Publication

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