News Release

Error-Corrected Next-Generation Sequencing for Profiling the Subclonal Genetic Architecture of Juvenile Myelomonocytic Leukemia

Key Points

- **Juvenile myelomonocytic leukemia is a rare and aggressive leukemia of early childhood.**
- **Ultra-sensitive error-corrected next-generation sequencing enabled comprehensive detection of low-frequency genetic mutations that are difficult to identify with conventional sequencing methods.**
- **Minor subclonal secondary mutations present at diagnosis were strongly associated with disease progression and poor clinical outcomes.**
- **These findings support more precise risk stratification and may inform optimization of treatment strategies in juvenile myelomonocytic leukemia.**

Summary

Prof. Yoshiyuki Takahashi, Dr. Hideki Muramatsu, and Dr. Daichi Sajiki of the Department of Pediatrics, Nagoya University Graduate School of Medicine, conducted a comprehensive genetic analysis of juvenile myelomonocytic leukemia (JMML) using error-corrected next-generation sequencing (NGS), an ultra-sensitive genomic profiling technology. Their study demonstrated that even small subpopulations of leukemia cells harboring additional genetic mutations are closely associated with disease progression and poor clinical outcomes.

JMML is a rare and aggressive leukemia that primarily affects infants and young children and remains difficult to treat. In approximately 90% of patients, mutations are detected in genes involved in the RAS signaling pathway—*PTPN11*, *NF1*, *NRAS*, *KRAS*, or *CBL*—which play critical roles in regulating cell differentiation and proliferation. In addition, some patients harbor secondary genetic abnormalities, such as mutations in *SETBP1* and *JAK3*, which have been implicated in enhanced leukemia cell proliferation and disease progression.

Error-corrected NGS is an advanced sequencing technology that corrects errors generated during the sequencing process at the molecular level, enabling highly accurate detection of low-frequency genetic mutations that are difficult to distinguish using conventional

NGS approaches. This method allows comprehensive and quantitative analysis of genetic abnormalities present only in a subset of leukemia cells within the tumor population.

In this study, error-corrected NGS was applied to samples from 104 patients with JMML, identifying a total of 159 genetic mutations. Of these, 30 mutations (19%) were low-frequency mutations detected only in a subset of JMML cells, representing minor subclonal populations.

Survival analyses revealed that patients harboring secondary mutations had poor treatment outcomes, even when these mutations were present only as minor subclones in a small fraction of leukemia cells. Notably, in some patients who later experienced relapse, these low-level genetic mutations were detectable several months before clinical relapse was diagnosed.

Collectively, these findings demonstrate that error-corrected NGS enables more precise and comprehensive evaluation of the genetic landscape of JMML. This approach is expected to improve risk stratification at diagnosis and contribute to the optimization of treatment strategies for patients with JMML.

Research Background

Juvenile myelomonocytic leukemia (JMML) is a leukemia with a poor prognosis that primarily affects infants and young children under five years of age. In Japan, approximately 10–20 cases are diagnosed annually, and hematopoietic cell transplantation is required to achieve a cure.

In approximately 90% of patients, mutations are detected in genes involved in the RAS signaling pathway—*PTPN11*, *NF1*, *NRAS*, *KRAS*, or *CBL*—which are considered the major drivers of JMML pathogenesis. In addition, some patients harbor secondary genetic abnormalities, such as mutations in *SETBP1* and *JAK3*, which have been associated with an unfavorable prognosis.

Previously, the most frequently observed secondary mutations, *SETBP1* p.D868N and *JAK3* p.R657Q, were analyzed using a specialized PCR-based method known as droplet digital PCR (ddPCR). This approach enabled the detection of low-frequency mutations present only in a subset of JMML cells within the leukemia cell population, referred to as minor clones, which had been overlooked by conventional genetic analysis methods. These mutations were found to be associated with poor prognosis.

Based on these findings, the present study employed error-corrected next-generation sequencing (NGS), a technology that corrects sequencing errors at the molecular level during analysis and enables highly accurate detection of low-frequency genetic mutations that are difficult to distinguish using conventional NGS. Using this approach, minor clones in patients with JMML were comprehensively and quantitatively analyzed.

Research Results

In this study, diagnostic samples from 104 patients with JMML were analyzed using error-corrected NGS. A total of 159 mutations were identified, of which 30 (19%) were classified as minor clone mutations (**Fig. 1**). These mutations were detected in several genes implicated in JMML pathogenesis, including *SETBP1*, *JAK3*, *NF1*, and *NRAS*.

Survival analysis revealed that patients harboring secondary mutations had poor outcomes, even when these mutations were present only as minor subclonal populations (**Fig. 2**). Notably, secondary mutations affecting genes involved in the RAS signaling pathway were strongly associated with disease progression and an unfavorable prognosis.

To further characterize the clonal architecture, colony assays were performed using diagnostic samples from patients with secondary mutations. Leukemia cells were isolated and cultured as colonies derived from single cells, and each colony was analyzed individually. While major driver mutations were detected in all colonies, secondary mutations were present only in specific colonies, demonstrating the coexistence of multiple distinct minor clones. In addition, these minor clone mutations were mutually exclusive within individual colonies, indicating that JMML exhibits a complex and heterogeneous clonal architecture.

Longitudinal analysis using pre- and post-treatment samples was also conducted with error-corrected NGS. In patients who relapsed after hematopoietic cell transplantation, minute genetic mutations indicative of relapse were detectable several months before clinical relapse was diagnosed (**Fig. 3**).

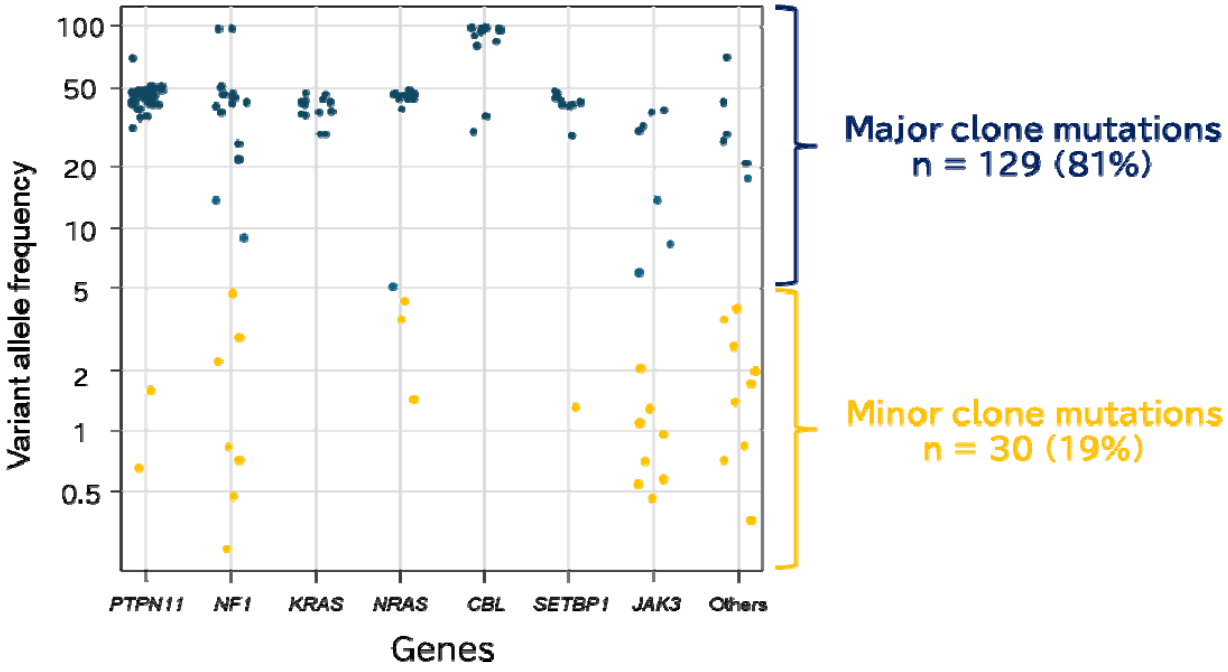

Figure 1. Genetic mutations detected by error-corrected next-generation sequencing

A total of 159 mutations were identified in diagnostic samples from 104 patients with JMML. Of these, 30 mutations (19%) were classified as minor clone mutations, which are difficult to accurately detect using conventional NGS.

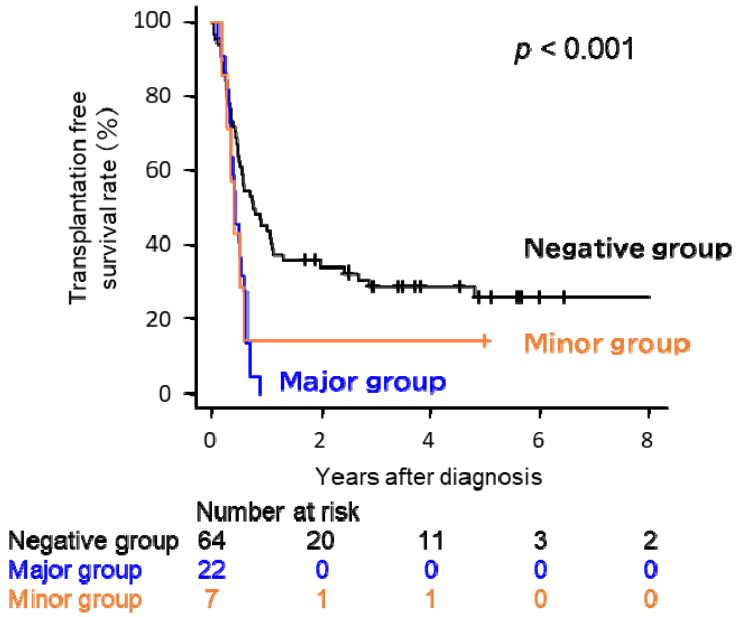

Figure 2. Survival according to the presence of secondary mutations

Survival analysis was performed in 93 patients with JMML, excluding 11 patients with Noonan syndrome. Patients harboring secondary mutations exhibited poorer clinical outcomes, even when these mutations were present only as minor subclonal populations.

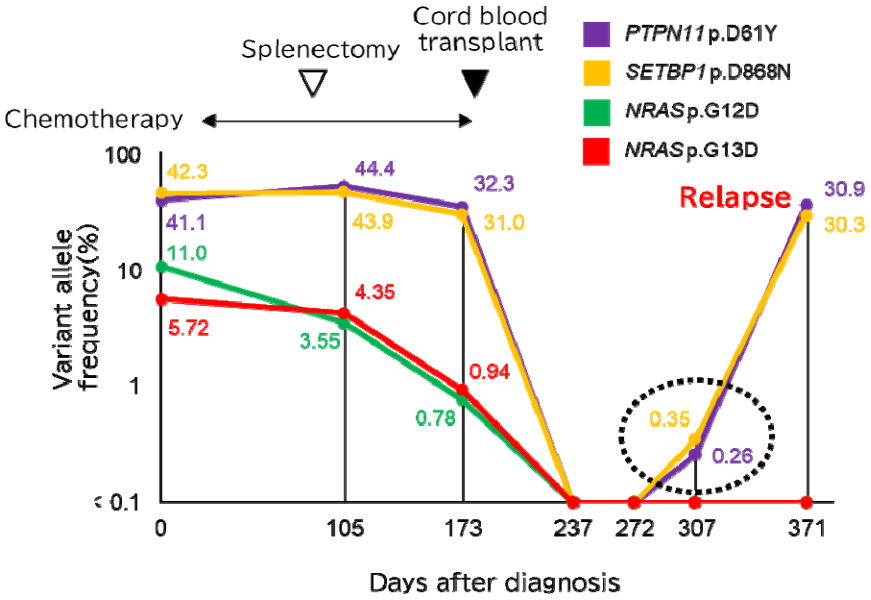

Figure 3. Longitudinal analysis of a patient with multiple mutations

Error-corrected NGS was performed on pre- and post-treatment samples from a patient harboring a *PTPN11* mutation, along with a *SETBP1* mutation and two *NRAS* mutations at diagnosis. Following cord blood transplantation, all four mutations became undetectable. Notably, two months prior to clinical relapse, the *PTPN11* and *SETBP1* mutations were detected at allele frequencies of 0.26% and 0.36%, respectively.

Research Summary and Future Perspective

This study demonstrates that secondary mutations in JMML can be evaluated with high precision, including detailed characterization of their clonal architecture. The application of error-corrected NGS in clinical practice is expected to enable more precise risk assessment at diagnosis and provide a robust foundation for clinical decision-making. Furthermore, during post-treatment follow-up, this approach has the potential to serve as a novel method for early molecular detection of relapse, allowing earlier therapeutic intervention.

Publication

Sajiki D, Muramatsu H, Wakamatsu M, Tsumura Y, Yamashita D, Yamamori A, Narita K, Kataoka S, and Takahashi Y. Error-corrected Next-generation Sequencing for Profiling the Subclonal Genetic Architecture of Juvenile Myelomonocytic Leukemia. *Leukemia* (2026).

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