News Release

Title

Clinical Utility of Next-Generation Sequencing for Inherited Bone Marrow Failure Syndromes

Key points

- **This study developed a next-generation sequencing-based comprehensive diagnostic system for inherited bone marrow failure syndromes (IBMFS).**
- **A successful genetic diagnosis was achieved for 121 of 371 patients (33%).**
- **The approach used targeted sequencing and whole-exome sequencing, achieving satisfactory diagnostic rates and supporting the efficacy of massive parallel sequencing as a diagnostic tool for IBMFS.**

Summary

Prof. Seiji Kojima, M.D., Ph.D. (corresponding author) and Hideki Muramatsu, M.D., Ph.D. [Department of Pediatrics, Nagoya University Graduate School of Medicine (Dean: Masahide Takahashi, M.D., Ph.D.)]; Yusuke Okuno, M.D., Ph.D. (Center for Advanced Medicine and Clinical Research, Nagoya University Hospital); Prof. Seishi Ogawa and Kenichi Yoshida, M.D., Ph.D. (Department of Pathology and Tumor Biology, Graduate School of Medicine, Kyoto University); and Satoru Miyano and Yuichi Shiraishi, Ph.D. (DNA Information Analysis Laboratory, Human Genome Center, Institute of Medical Science, The University of Tokyo) developed a new diagnostic test for inherited bone marrow failure syndromes (IBMFS) using the next-generation sequencing technology.

IBMFS belong to a heterogeneous inherited disease category in which at least one hematopoietic cell lineage is decreased in the bone marrow. IBMFS comprise many rare disease entities. Even Fanconi anemia, which typically affects a large number of patients, affects only approximately 10 patients per year in Japan. Although a precise genetic diagnosis is very important for appropriate clinical decision making, achieving an accurate diagnosis of an IBMFS using conventional Sanger sequencing is very difficult because of the large number of genes associated with IBMFS.

We developed a novel next-generation sequencing analysis pipeline for IBMFS, covering more than 100 genes associated with IBMFS. We genetically diagnosed 121 of 371 patients (33%) with an IBMFS. Moreover, clinical diagnoses were incompatible with genetic diagnoses in 10% of patients, clearly demonstrating the clinical value of next-generation sequencing.

Background

Inherited bone marrow failure syndromes (IBMFS) belong to a heterogeneous inherited disease category in which at least one hematopoietic cell lineage is decreased in the bone marrow. IBMFS comprise many rare disease entities. Even Fanconi anemia (FA), which typically affects

a large number of patients, affects only approximately 10 patients per year in Japan. Combined with other rare IBMFS such as Diamond–Blackfan anemia (DBA), dyskeratosis congenita, and severe congenital neutropenia, the total number of affected patients in Japan is within 100 per year.

A decline in the number of blood cells is a clinical finding common among IBMFSs. However, the responsible molecular mechanism differs for each type. For example, FA patients develop anemia due to a defect in the DNA repair pathway, whereas DBA patients develop it due to a defect in the function of ribosome, a molecular machine which generate protein based on genetic information.

For patients with an IBMFS, a precise genetic diagnosis is critical for the selection of appropriate treatment modalities. However, obtaining an accurate genetic diagnosis of an IBMFS using conventional Sanger sequencing is very difficult because of the large number (>100) and relatively larger size of the genes associated with IBMFS. For example, the FA genetic test that uses Sanger sequencing requires skilled technicians and takes more than 1 week. Testing genes associated with multiple diseases is very difficult. In this context, molecular genetic testing appeared impossible for patients without distinct clinical diagnosis.

Recent advances in next-generation sequencing have achieved an improvement of 100 million times in sequencing efficiency. We developed a molecular IBMFS diagnostic pipeline using next-generation sequencing and assessed its clinical utility.

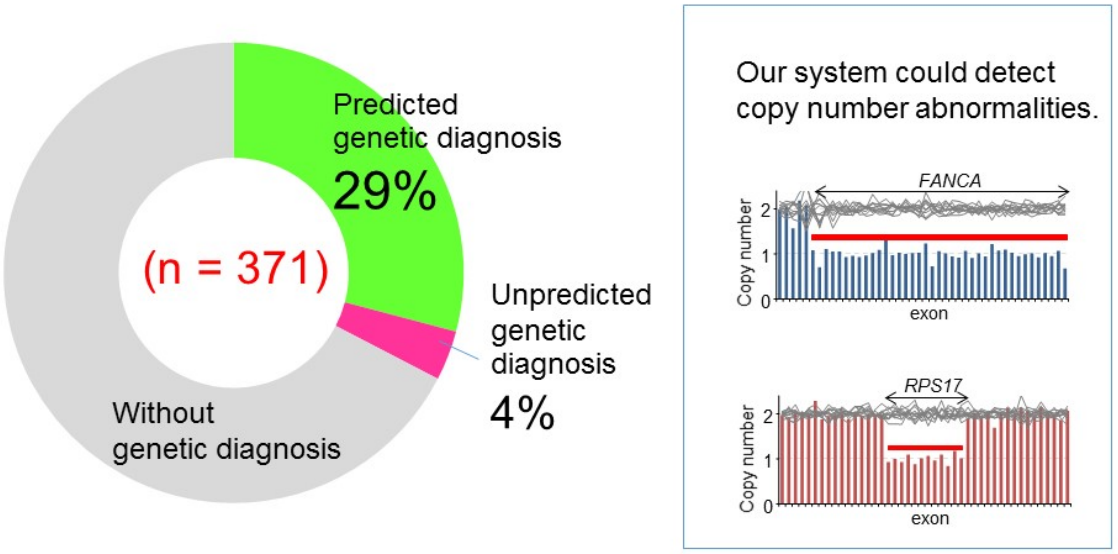
Results

We selected 184 genes associated with IBMFS and designed a molecular diagnostic pipeline using a next-generation sequencer (target sequencing). From the raw sequencing data, our analysis pipeline could identify the pathognomonic gene mutation causing IBMFS.

We analyzed 121 IBMFS patients using targeted sequencing and 250 IBMFS patients using whole-exome sequencing (WES). We made successful genetic diagnoses for 53 of 121 patients (44%) using targeted sequencing and 68 of 250 patients (27%) using WES. In majority of the cases, the detected variants were concordant with the clinical diagnoses. However, in 13 cases, the clinical diagnoses were incompatible with the detected variants. For example, in a patient with initial diagnosis of idiopathic thrombocytopenia, a benign hematological disease, the clinical diagnosis was changed to familial platelet disorder with propensity to myeloid malignancy after the identification of *RUNX1* gene mutation.

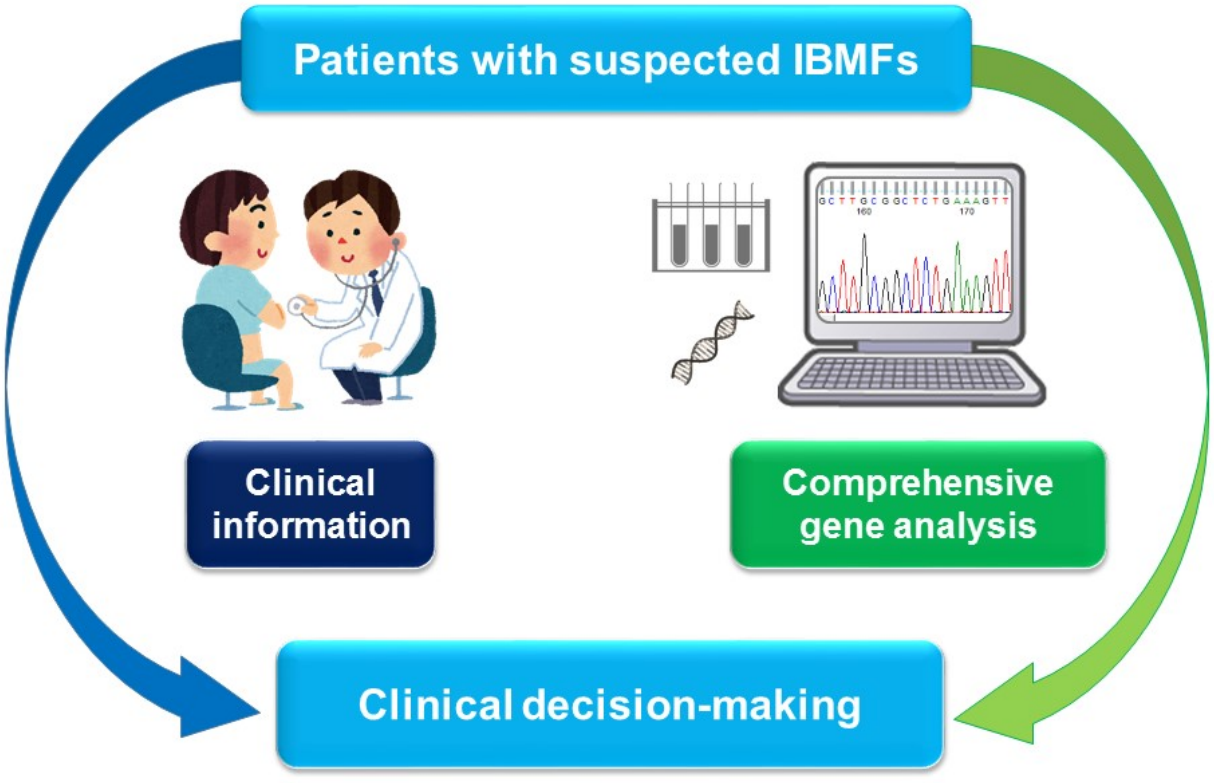
Our approach utilizing targeted sequencing and WES achieved satisfactory diagnostic rates and supported the efficacy of massive parallel sequencing as a diagnostic tool for IBMFS.

（Fig.1）Results of targeted next-generation sequencing



Our system is effective in establishing genetic diagnoses in patients with IBMFs.

(Fig.2）New diagnostic approach for IBMFs



Comprehensive gene analysis enables accurate genetic diagnosis and appropriate treatment decision-making.

Publication

Hideki Muramatsu, Yusuke Okuno, Kenichi Yoshida, Yuichi Shiraishi, Sayoko Doisaki, Atsushi Narita, Hirotoshi Sakaguchi, Nozomu Kawashima, Xinan Wang, Yinyan Xu, Kenichi Chiba, Hiroko Tanaka, Asahito Hama, Masashi Sanada, Yoshiyuki Takahashi, Hitoshi Kanno, Hiroki Yamaguchi, Shouichi Ohga, Atsushi Manabe, Hideo Harigae, Shinji Kunishima, Eiichi Ishii, Masao Kobayashi, Kenichi Koike, Kenichiro Watanabe, Etsuro Ito, Minoru Takata, Miharu Yabe, Seishi Ogawa, Satoru Miyano, and Seiji Kojima. Clinical Utility of Next-generation Sequencing for Inherited Bone Marrow Failure Syndromes. *Genetics in Medicine: official journal of the American College of Medical Genetics.* , published online on Jan.19, 2017.

Japanese ver.

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