News Release

Title
Integrated Proteogenomic Analysis for Inherited Bone Marrow Failure Syndrome

Key Points
○ Inherited bone marrow failure syndrome (IBMFS) is a heterogeneous group of disorders characterized by cytopenia in at least one hematopoietic cell lineage.

○ In-depth proteome analysis for IBMFS revealed distinct protein alterations and biological characteristics of each disease.

○ Targeted proteome analysis may be a useful diagnostic test for IBMFS, such as the Shwachman–Diamond syndrome and ADH5/ALDH2 deficiency.

Summary
Prof. Yoshiyuki Takahashi, Dr. Hideki Muramatsu, and Dr. Manabu Wakamatsu at the Department of Pediatrics, Nagoya University Graduate School of Medicine; Dr Osamu Ohara, deputy director at the Kazusa DNA Research Institute, and Dr Yusuke Kawashima, the director of the Applied Proteome Group, Kazusa DNA Research Institute, successfully established a rapid and simple screening system for inherited bone marrow failure syndrome (IBMFS) based on protein expression.

IBMFS is a heterogeneous group of disorders characterized by cytopenia in at least one hematopoietic cell lineage, which may progress to pancytopenia. Its genetic etiology consists of germline variants in >30 distinct disorders, including Shwachman–Diamond syndrome (SDS), Fanconi anemia, dyskeratosis congenita, and Diamond–Blackfan anemia, and recently identified alcohol dehydrogenase 5/aldehyde dehydrogenase 2 (ADH5/ALDH2) deficiency. These diseases are associated with systemic complications and tumorigenesis predisposition and require precise diagnosis, appropriate treatment, and follow-up. Although genetic analysis by next-generation sequencing (NGS) has greatly improved the convenience of genetic diagnosis, some patients are still incorrectly diagnosed due to technical problems, and some cases are overlooked. Therefore, a new approach to diagnoses other than genetic analysis by NGS should be established.

Using a high-performance mass spectrometer, proteome analysis can identify and quantify proteins from biological samples, and comprehensive protein

expression analysis was also performed. Although conventional proteome analysis measured only about 3,000 different proteins due to technical limitations, in-depth proteome analysis using the latest mass spectrometer measures >10,000 proteins, including kinases and transcription factors.
In this study, proteome analysis identified eight different proteome clusters, each with distinctive protein expression profiling. In particular, patients with SDS and ADH5/ALDH2 deficiency had significantly decreased SBDS and ADH5 protein expressions, respectively, which was very useful for definitive diagnosis. Next, a simple and rapid targeted proteome analysis was performed on >400 samples, which confirmed its sufficient performance as a clinical diagnostic test for IBMFS.

Research Background

Inherited bone marrow failure syndrome (IBMFS) is a heterogeneous group of disorders characterized by cytopenia in at least one hematopoietic cell lineage that results in anemia, thrombocytopenia, and neutropenia. IBMFS include Shwachman–Diamond syndrome (SDS), Fanconi anemia (FA), Diamond–Blackfan anemia (DBA), dyskeratosis congenita (DC), and alcohol dehydrogenase 5/aldehyde dehydrogenase 2 (ADH5/ALDH2) deficiency. Each disease is extremely rare, with only approximately 10 new cases diagnosed per year in Japan, and is associated with various systemic complications and malignancy development at a young age. A rapid and accurate diagnosis should be made, and appropriate treatment and follow-up should be provided to patients with IBMFS. Next-generation sequencing (NGS) has been reportedly useful in diagnosing IBMFS. However, more than half of patients with IBMFS are still not genetically diagnosed, and a new diagnostic test system other than NGS analysis has been needed.
SDS is an autosomal recessive form of IBMFS with abnormal pancreatic exocrine secretion, anemia, and skeletal abnormalities. Approximately 15–30% of SDS progress to myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML). A previous study reported that approximately 4% of patients with MDS aged 18–40 years were genetically diagnosed with SDS, and the majority of these patients were overlooked during childhood and were not diagnosed with SDS until MDS development.
ADH5/ALDH2 deficiency is caused by two genetic variants of *ADH5* and *ALDH2* genes, which prevent the breakdown of endogenous formaldehyde and produce symptoms similar to those of patients with FA, including short stature, mental retardation, pancytopenia, and MDS. This *ALDH2* gene variant is a single nucleotide polymorphism carried by one of four East Asians, and then, the prevalence of ADH5/ALDH2 deficiency is predicted to be higher in Asian

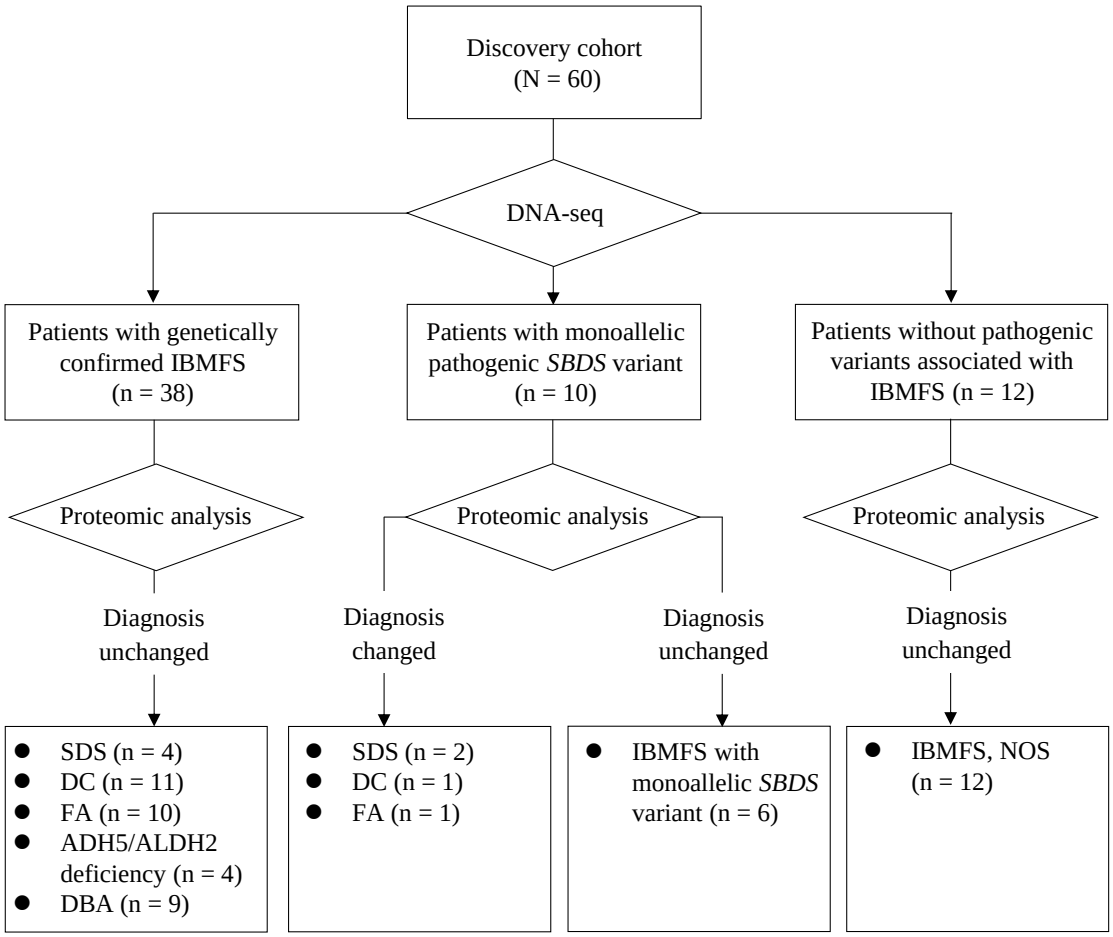
countries, which should be differentiated in patients with suspected IBMFS in Japan.
Recently, dramatic technological innovations in protein measurement systems and in-depth proteome analysis have comprehensively identified and quantified >10,000 proteins, including kinases and transcription factors, which were previously considered difficult to detect. This study conducted in-depth proteome analysis on patients with IBMFS to clarify the biological properties of each IBMFS and thereby developed a new diagnostic test system using proteome analysis.

Research Results

Proteogenomic analysis for IBMFS

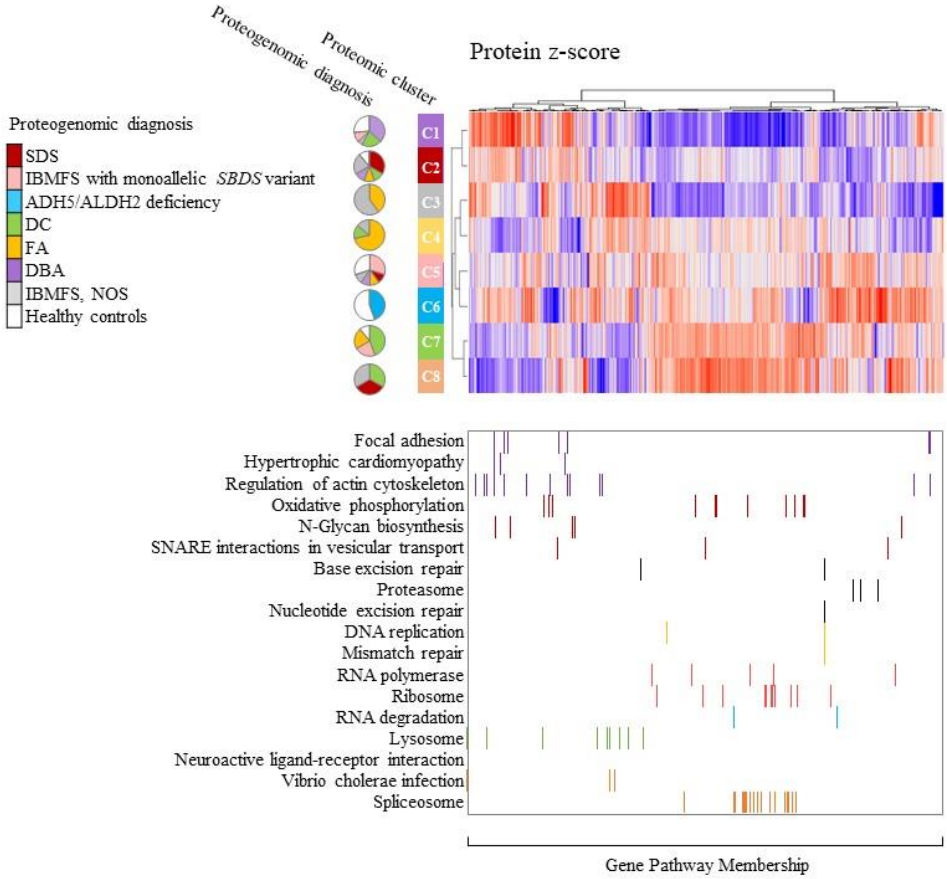
As a discovery cohort, 60 patients with clinically diagnosed IBMFS were analyzed. All patients in the discovery cohort underwent targeted NGS analysis and in-depth proteome analysis. An in-depth proteome analysis with a mass spectrometer (Q-Exactive HF-X [Thermo Fisher Scientific]) identified and quantified proteins using peripheral blood mononuclear cells. Targeted gene analysis identified 38 patients with genetically confirmed IBMFS, 10 with only one allele pathological mutation of the *SBDS* gene, and 12 without genetic mutations associated with IBMFS. Two of 10 patients were identified with only one allele pathological mutation in the *SBDS* gene; however, proteome analysis revealed significantly decreased SBDS protein expression, and RNA sequencing reads identified pathogenic variants in the compound heterozygous *SBDS* gene in both cases, finally diagnosing them with SDS (**Figure 1**).

Figure 1. Diagnostic flowchart based on proteogenomic analysis



Unsupervised clustering analysis of the proteome data from 74 samples, including 14 normal controls in the discovery cohort, revealed eight independent proteome clusters (C1–C8): the C1 cluster for DBA, C2 and C8 clusters for SDS, C3 and C4 clusters for FA, the C6 cluster for ADH5/ALDH2 deficiency, and the C7 cluster for DC. Furthermore, significant downregulation of ribosome-associated proteins was observed, especially in C1 and C2 clusters, a consistent result with DBA and SDS pathogenesis caused by ribosome dysfunction (**Figure 2**).

Figure 2. Unsupervised proteomic-based cluster for IBMFS subtypes

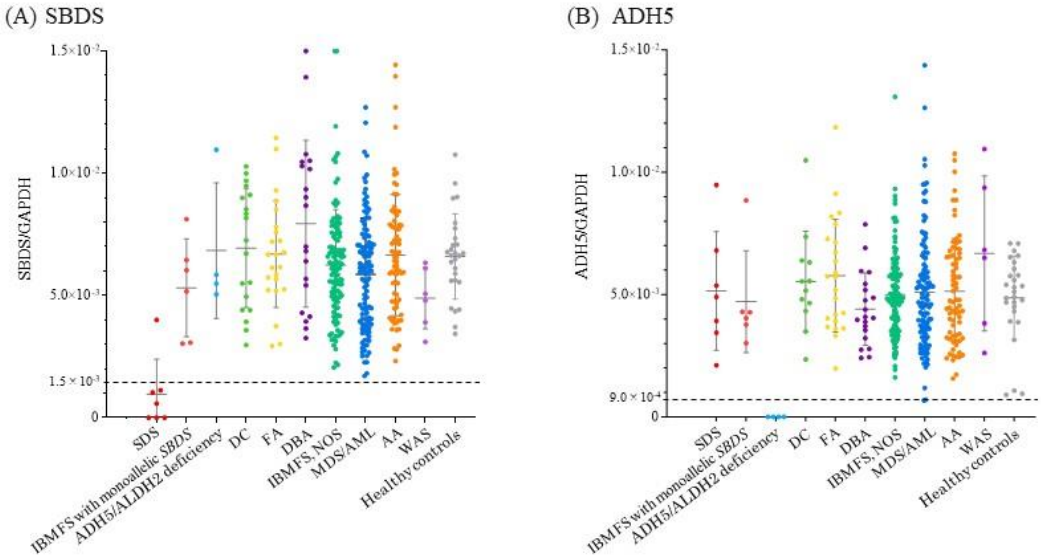


Development of a rapid and simple diagnostic test system by targeted proteome analysis

Next, proteins significantly differentially expressed in each IBMFS were used. In the discovery cohort, SBDS protein expression was significantly decreased in six of 60 patients with IBMFS. Similarly, ADH5 protein expression was significantly decreased in four patients with ADH5/ALDH2 deficiency, which were both consistent with a genetic diagnosis.

To apply a rapid and convenient screening system for IBMFS in clinical practice, a small panel of ADH5 and SBDS proteins was created for targeted proteome analysis. A mass spectrometer (Orbitrap Exploris 480 [Thermo Fisher Scientific]) was used to evaluate protein expression for an expanded cohort of 417 samples: 390 with hematologic tumors associated with IBMFS and 27 normal controls. Results revealed that SBDS protein expression was decreased in SDS and ADH5 protein expression was significantly decreased in ADH5/ALDH2 deficiency. The sensitivity and specificity of diagnostic tests were 85.7% and 93.4% for SDS and 100.0% and 97.5% for ADH5/ALDH2 deficiency, respectively, indicating that performing diagnostic tests was considered adequate for identifying patients requiring early diagnosis and therapeutic intervention (**Figure 3**).

Figure 3. Targeted proteomic analysis using IBMFS-related small panels



Research Summary and Future Perspective

The first in-depth proteome analysis of IBMFS was performed and succeeded in constructing a system for IBMFS diagnosis. Although NGS analysis has been previously used, it still fails to identify the causative gene in more than half of patients with IBMFS. In addition, some patients with IBMFS not diagnosed during childhood have not received appropriate follow-up. The diagnostic testing approach based on proteome analysis developed in this study can accurately diagnose IBMFS during childhood and can potentially be a clinical test that complements conventional NGS analysis. In the future, screening testing for MDS/AML should be performed in patients who develop the disease in young adults in clinical practice.

Publication

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