

## News Release

### Title

Application of extensively targeted next-generation sequencing for the diagnosis of primary immunodeficiencies

### Key Points

- This study developed a targeted next-generation sequencing-based comprehensive diagnostic system covering 349 genetic regions relevant to primary immunodeficiencies.
- It was successful in the genetic diagnosis of 8/59 (14%) patients who lacked initial molecular diagnosis.
- Our NGS-based method is effective in establishing genetic diagnoses in patients with primary immunodeficiencies.

### Summary

Prof. Seiji Kojima (corresponding author), Hideki Muramatsu, and Daiei Kojima (first author) at Department of Pediatrics, Nagoya University Graduate School of Medicine (Dean: Masahide Takahashi, M.D., Ph.D.), and Yusuke Okuno at Center for Advanced Medicine and Clinical Research Nagoya University Hospital developed a new diagnostic test for Primary immunodeficiency (PID) using next-generation sequencing technology.

PIDs are inherited disorders associated with more than 300 causative genes. Obtaining molecular diagnosis for PID using phenotype-based approach is often difficult in clinical practices. Next-generation sequencing (NGS) has a potential to offer exhaustive analysis of gene mutations, which can facilitate rapid molecular diagnosis. We designed a target enrichment assay covering 349 genes associated with PID and congenital bone marrow failure syndrome using next-generation sequencer. We performed gene analysis of 97 PID or PID suspected patients. Our NGS-based method accurately detected point mutations and deletions in all PID patients with known mutations. Also, it was successful in the genetic diagnosis of 8 in 59 patients who lacked initial molecular diagnosis. Our result indicated that targeted NGS may become a cost-effective, first-line genetic test for the evaluation of PID suspected patients. Early diagnosis enables a rapid start of treatment and improves patient outcomes.

### Research Background

Primary immunodeficiencies (PIDs) represent a diverse group of inherited disorders caused by congenital defects of the immune system. The latest International Union of Immunological Societies (IUIS) classification has identified as many as 300 causative genes and large

chromosomal segment deletions such as 22q11.2 deletion. Patients with severe combined immunodeficiency (SCID) which is considered to be the most serious PID, are susceptible to recurrent infections such as pneumonia, and can die before the first year of life.

Treatments for PIDs involve preventing and treating infections as well as boosting the immune system. Hematopoietic stem cell transplantation (HSCT) offers a permanent cure for several forms of life-threatening immunodeficiency. Early diagnosis of SCID, followed by HSCT, significantly improves affected children's chance of survival compared with children with SCID diagnosed later in life.

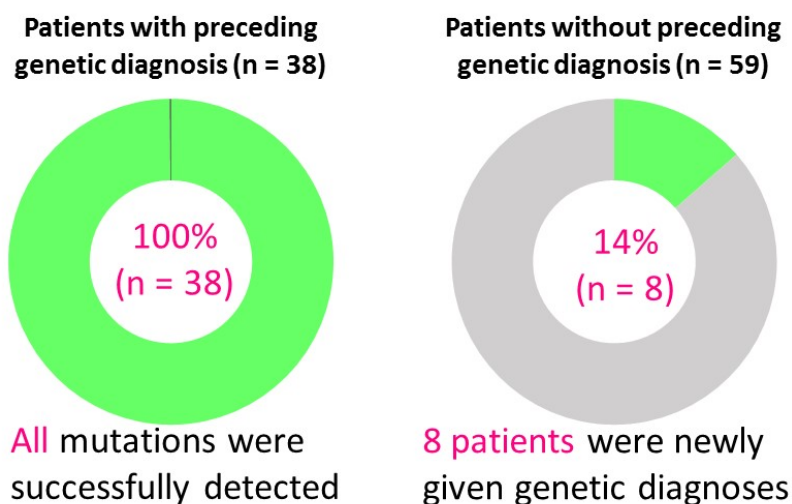
Precise molecular diagnosis for patients with PID is critical for an appropriate management of patients, but a timely and accurate genetic diagnosis in daily clinical practice is difficult. The clinical phenotype may vary in patients with identical genotypes, and more than one genotype could produce similar clinical phenotypes. Furthermore, several congenital bone marrow failure syndromes (CBMFSs) may mimic PIDs. Recent progress in next-generation sequencing (NGS) enables simultaneous sequencing of a massive amount of nucleic acids.

## **Research Results**

We designed a comprehensively targeted sequencing platform that covers causal genes associated with PID, CBMFS, or 22q11.2 region. A total of 349 genes associated with PIDs, CBMFSs, and 22q11.2 region were subjected to DNA capture designed by SureDesign (Agilent, Santa Clara, CA). PID-related genes were selected on the basis of the 2014 IUIS classification, 2014 European Society for Immunodeficiencies (ESID) meeting (<http://esid.org/Working-Parties/Registry/New-ESID-Registry/List-of-diseases-and-genes>), and Resource of Asian Primary Immunodeficiency Diseases (<http://web16.kazusa.or.jp/rapid/>). The target region included the coding exon plus 10 flanking bases. As a result, 55,877 probes (2 Mb), covering 99.3% of the target region, were prepared.

We studied 97 patients, which included 38 patients with known PID mutations and 59 patients without any genetic diagnoses. Our targeted sequencing covered 99.1% of the target-coding region with 20 times of read coverage. Our system successfully detected all mutations and CNVs in 38 patients with preceding genetic diagnoses. Moreover, in 59 patients without genetic diagnoses, we detected 9 diagnostic variants in 8 patients (14%), which includes 22q11.2 deletion syndrome (FIG1).

(FIG1) Results of targeted next-generation sequencing

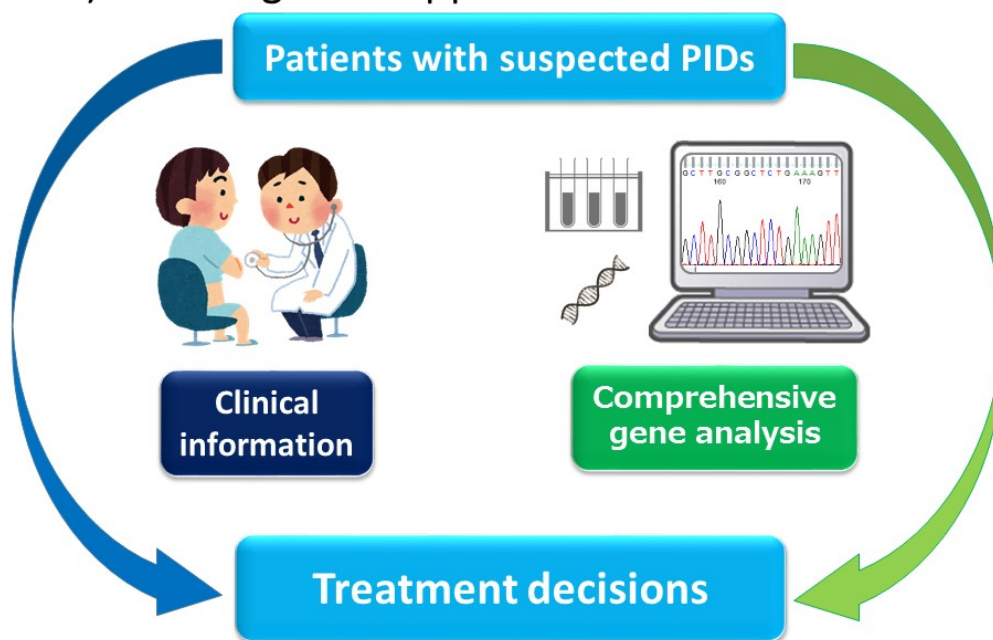


Our system is **effective** in establishing genetic diagnoses in patients with PID.

### Research Summary and Future Perspective

Our NGS-based comprehensive, rapid, and efficient PID diagnostic system could become a first-line genetic analysis of PID-suspected patients (FIG2). Early diagnosis enables a rapid start of treatment and improves patient outcomes.

(FIG2) New diagnostic approach for PIDs



Comprehensive gene analysis enables accurate genetic diagnosis and appropriate treatment decisions.

**Publication**

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