Rapid molecular diagnostics of severe primary immunodeficiency determined by using targeted next-generation sequencing

Hui Yu, PhD,[a]* Victor Wei Zhang, MD, PhD,[b]* Asbjørg Stray-Pedersen, MD, PhD,[c,d,e,f] Imelda Celine Hanson, MD,[c,d] Lisa R. Forbes, MD,[c,d] M. Teresa de la Morena, MD,[g] Ivan K. Chinn, MD,[c,d] Elizabeth Gorman, PhD,[a] Nancy J. Mendelsohn, MD,[h] Tamara Pozos, MD, PhD,[i] Wojciech Wiszniewski, MD, PhD,[e] Sarah K. Nicholas, MD,[c,d] Anne B. Yates, MD,[j] Lindsey E. Moore, DO,[j] Knut Erik Berge, MD, PhD,[k] Hanne Sorte, MS,[k] Diana K. Bayer, DO,[l] Daifulah ALZahrani, MD,[m] Raif S. Geha, MD,[n] Yanming Feng, PhD,[a] Guoli Wang, PhD,[a] Jordan S. Orange, MD, PhD,[c,d] James R. Lupski, MD, PhD, DSc (hon),[b,e,o] Jing Wang, MD,[b] and Lee-Jun Wong, PhD[b] *Houston and Dallas, Tex, Oslo, Norway, Minneapolis and St Paul, Minn, Jackson, Miss, Iowa City, Iowa, Jeddah, Saudi Arabia, and Boston, Mass*

Background: Primary immunodeficiency diseases (PIDDs) are inherited disorders of the immune system. The most severe form, severe combined immunodeficiency (SCID), presents with profound deficiencies of T cells, B cells, or both at birth. If not treated promptly, affected patients usually do not live beyond infancy because of infections. Genetic heterogeneity of SCID frequently delays the diagnosis; a specific diagnosis is crucial for life-saving treatment and optimal management.

Objective: We developed a next-generation sequencing (NGS)–based multigene-targeted panel for SCID and other severe PIDDs requiring rapid therapeutic actions in a clinical laboratory setting.

Methods: The target gene capture/NGS assay provides an average read depth of approximately 1000×. The deep coverage facilitates simultaneous detection of single nucleotide variants and exonic copy number variants in one comprehensive assessment. Exons with insufficient coverage (<20× read depth) or high sequence homology (pseudogenes) are complemented by amplicon-based sequencing with specific primers to ensure 100% coverage of all targeted regions.

Results: Analysis of 20 patient samples with low T-cell receptor excision circle numbers on newborn screening or a positive family history or clinical suspicion of SCID or other severe PIDD identified deleterious mutations in 14 of them. Identified pathogenic variants included both single nucleotide variants and exonic copy number variants, such as hemizygous nonsense, frameshift, and missense changes in *IL2RG*; compound heterozygous changes in *ATM*, *RAG1*, and *CIITA*; homozygous changes in *DCLRE1C* and *IL7R*; and a heterozygous nonsense mutation in *CHD7*.

Conclusion: High-throughput deep sequencing analysis with complete clinical validation greatly increases the diagnostic yield of severe primary immunodeficiency. Establishing a molecular diagnosis enables early immune reconstitution through prompt therapeutic intervention and guides management for improved long-term quality of life. (J Allergy Clin Immunol 2016;■■■:■■■-■■■.)

Key words: *Severe combined immunodeficiency, severe combined immunodeficiency newborn screening, next-generation sequencing, molecular diagnostics*

From [a]Baylor Genetics, Houston; [b]the Department of Molecular and Human Genetics, Baylor College of Medicine, Houston; [c]the Department of Pediatrics, Section of Immunology, Allergy, and Rheumatology, Baylor College of Medicine and Texas Children's Hospital, Houston; [d]the Center for Human Immunobiology, Immunology, Allergy and Rheumatology, Texas Children's Hospital, Feigin Center, Houston; [e]Baylor-Hopkins Center for Mendelian Genomics of the Department of Molecular and Human Genetics, Baylor College of Medicine, Houston; [f]the Norwegian National Unit for Newborn Screening and [k]the Department of Medical Genetics, Oslo University Hospital, Oslo; [g]the Division of Allergy and Immunology, University of Texas Southwestern Medical Center, Children's Medical Center, Dallas; [h]Children's Hospital and Clinics of Minnesota, Minneapolis; [i]Pediatric Infectious Diseases and Immunology, Children's Hospitals and Clinics of Minnesota, St Paul; [j]the University of Mississippi Medical Center, Jackson; [l]the Department of Pediatrics, Division of Pediatric Allergy/Immunology and Pulmonology, University of Iowa Carver College of Medicine, Iowa City; [m]the Department of Pediatrics, King Saud Bin Abdulaziz University for Health Sciences, Jeddah; [n]the Division of Immunology, Boston Children's Hospital and Department of Pediatrics, Harvard Medical School, Boston; and [o]the Department of Pediatrics, Baylor College of Medicine and Texas Children's Hospital, Houston.

*These authors contributed equally to this work.

Disclosure of potential conflict of interest: H. Yu is an employee of Baylor Genetics (Baylor College of Medicine and Miraca Holdings Inc have formed a joint venture with shared ownership and governance of the Baylor Genetics). L. R. Forbes serves on the board for Baxaltal, receives grant support from Baylor College of Medicine and Horizon Investigator initiated restricted Award, receives payments for lectures from Horizon, and received travel support from the Jeffery Model Foundation and the Clinical Immunology Society. M. T. de la Morena serves on the board for the Atlantic Research group. I. K. Chinn receives grants from the National Institutes of Health and the Jeffrey Modell Foundation. E. Gorman is an employee of Baylor Genetics. J. S. Orange serves as a consultant for Baxalta, CSL Behring, and Grifols; holds patents with the Children's Hospital of Philadelphia; and receives royalties from UpToDate. J. R. Lupski is an employee of Baylor College of Medicine and derives support through a professional services agreement with Baylor Genetics; serves on the Scientific Advisory Board of Baylor Genetics; has stock ownership in 23andMe; is a paid consultant for Regeneron Pharmaceuticals; has stock options in Lasergen, Inc and is a co-inventor on multiple United States and European patents related to molecular diagnostics for inherited neuropathies, eye diseases and bacterial genomic fingerprinting; and receives funding in part by grants R01 NS058529 (JRL) from the National Institute of Neurological Disorders and Stroke and U54 HG006542 (JRL) from the National Human Genome Research Institute/National Heart Lung and Blood Institute to the Baylor Hopkins Center for Mendelian Genomics. L.-J. Wong is an employee of Baylor Genetics. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication February 3, 2016; revised May 4, 2016; accepted for publication May 31, 2016.

Corresponding author: Lee-Jun Wong, PhD, Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, NAB 2015, Houston, TX 77030. E-mail: ljwong@bcm.edu.

0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2016.05.035

Abbreviations used
AT: Ataxia telangiectasia
CGH: Comparative genomic hybridization
CHARGE: Coloboma, heart defects, choanal atresia, growth retardation, genital and/or urinary defects, ear anomalies and/or deafness
CNV: Copy number variant
DGS: DiGeorge syndrome
NK: Natural killer
PIDD: Primary immunodeficiency disease
SCID: Severe combined immunodeficiency
SNV: Single nucleotide variant
TREC: T-cell receptor excision circle

Severe combined immunodeficiency (SCID) is one of the most severe primary immunodeficiencies and characterized by profound impairment of cellular and humoral immune responses.[1] Children with unrecognized and untreated SCID usually cannot live beyond infancy because of recurrent or life-threatening infections. However, if given a diagnosis within the first 3.5 months of life before infections develop, prompt treatment with hematopoietic stem cell transplantation or gene therapy can result in a greater than 90% survival rate. In addition to improved clinical outcomes, overall morbidity, mortality, and treatment costs are significantly reduced.[2-5] Therefore the diagnosis of SCID is a medical emergency.

SCID is defined by the absence or significant deficiency of T lymphocytes. B cells can also be absent or nonfunctional in patients with SCID. During T-cell maturation in the thymus, small circular DNA episomes are generated as byproducts of T-cell receptor gene rearrangement. Therefore a low number of these T-cell receptor excision circles (TRECs) measured by using DNA isolated from dried blood spots in Guthrie card newborn screening specimens can serve as a surrogate biomarker for a potential diagnosis of SCID.[6,7]

Since the Department of Health and Human Services recommended adding SCID to uniform newborn screening in May 2010, more than 30 states have begun screening for SCID, and additional states plan to offer the test shortly.[8-11] Pilot TREC screening programs have been assessed in European countries, such as France and the United Kingdom,[12,13] and most recently, The Netherlands became the first nation in Europe to officially include SCID in its newborn screening program (http://www.isns-neoscreening.org/news/123/2788/2015/04/25).

The occurrence of SCID is estimated to be 1 in 58,000 births based on data from SCID newborn screening programs in 11 US states.[14] This incidence rate approximately doubles the previous estimates of 1 in 100,000.[15] More than 40 genes have been implicated to cause SCID and other severe primary immunodeficiency diseases (PIDDs; eg, familial hemophagocytic lymphohistiocytosis syndromes, zeta chain–associated protein of 70 kDa deficiency, MHC class II deficiency, and dedicator of cytokinesis 8 deficiency), and the list continues to expand.[16-18] The most common type of SCID occurs because of deleterious mutations in the X-linked interleukin receptor common gamma chain gene (*IL2RG*) and accounts for 20% to 40% of cases.[14,19-21] Defects in the IL-7 receptor α chain gene (*IL7R*) are the second most common cause of SCID.[21] Molecular defects in the recombination-activating genes *RAG1* and *RAG2* affect the V(D)J recombination necessary for antigen receptor gene assembly and represent the third most common form of SCID.[21] Other causes of SCID include deficiency of the *ADA* gene, which encodes the enzyme adenosine deaminase in the purine catabolic pathway; the intracellular tyrosine kinase Janus kinase 3 gene (*JAK3*); and the Artemis gene *DCLRE1C* (DNA cross-link repair protein 1C) involved in V(D)J recombination/DNA repair.[21] In addition, genetic disorders that can present with severe T lymphopenia at birth, such as coloboma, heart defects, choanal atresia, growth retardation, genital and/or urinary defects, ear anomalies and/or deafness (CHARGE) syndrome, DiGeorge syndrome (DGS), or trisomy 21, are also identified by using the TREC newborn screening assay.[14,22-24]

The genetic heterogeneity of PIDDs makes a timely and accurate diagnosis challenging, hindering the prompt treatment of patients with SCID. The use of high-throughput next-generation sequencing (NGS) technologies to interrogate multiple genes simultaneously allows a rapid, cost-effective, genotype-based approach to molecular diagnosis.[25-27] Here we report the high diagnostic yield of SCID and other severe PIDDs using targeted capture NGS-based comprehensive analyses.

METHODS

Specimens and sample preparation

Patient specimens were submitted to Baylor Genetics, previously Molecular Genetics Laboratories at Baylor College of Medicine, for NGS-based panel analyses. Samples with known mutations selected through the Center for Mendelian Genomics at Baylor College of Medicine were used for validation. The study was conducted under protocols approved for human subjects by the Baylor College of Medicine institutional review board. Additional DNA samples from cultured cells (Coriell Institute for Medical Research, Camden, NJ) and 1 normal saliva sample were also included for test development and validation.

Target capture and sequencing

Custom-designed NimbleGen SeqCap probes (Roche NimbleGen, Madison, Wis) were used for in-solution hybridization to enrich target sequences. Enriched DNA samples were indexed and sequenced on a HiSeq2000 sequencer (Illumina, San Diego, Calif) with 100 cycles of single end reads, according to the manufacturer's protocols. The insufficiently covered (<20×) target exons were analyzed by using gene-specific amplicon-based Sanger sequencing.

Variant annotation and interpretation

Nucleotide changes observed in more than 5% of aligned reads were called and reviewed by using NextGENe software (SoftGenetics, State College, Pa). An RNA-oriented variant report setting and filter was used to examine variants in the noncoding RNA gene *RMRP*. Deleterious mutations and novel variants detected by using NGS were confirmed by means of Sanger sequencing.[28,29] A coverage-based algorithm developed in-house, eCNVscan, was used to detect large exonic deletions and duplications.[30] The normalized coverage depth of each exon of a test sample is compared with the mean coverage of the same exon in the reference file, which is generated from 8 to 15 samples from the same or consecutive runs, to detect copy number variants (CNVs). Based on the validation results of array comparative genomic hybridization (CGH) experiments, ratios of less than 0.65 and greater than 1.35 are scored as deletions and duplications, respectively. All positive calls were further investigated and confirmed by using a second method.

RESULTS

Target enrichment and coverage

Coding regions and flanking intronic sequences of 191 genes associated with various primary immunodeficiency phenotypes