

Clinical Communications

Linking newborn severe combined immunodeficiency screening with targeted exome sequencing: A case report

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Clinical Implications

- Newborn screening (NBS) is a clinically useful tool in severe combined immunodeficiency detection.
- Linking NBS with targeted genetic testing for early intervention is feasible.¹
- Selection of proper genetic testing and experienced genetic counseling is critical for diagnosing primary immune deficiency disorders.

TO THE EDITOR:

Severe combined immunodeficiency (SCID) is a “pediatric emergency” with increased survival seen following early identification and intervention.² Newborn screening (NBS) is a validated tool for identifying most infants with SCID, allowing for definitive diagnosis according to published standards.³ Family history (FH) of affected relatives can be equally useful for prompting analysis of a known genetic variant. Prenatal genetic testing has become more routine in identifying common chromosomal defects, including Trisomy 21 syndrome and cystic fibrosis, and can be used in SCID.⁴ Here, we describe a male neonate with FH of X-linked SCID and undetectable T-cell receptor excision circle findings on the NBS where prenatal maternal genetic carrier testing confounded diagnosis.

A 17-year-old Hispanic pregnant female presented for obstetric care with an FH of SCID: an affected living full brother with stem cell transplant at age 9 months and another earlier delivery full brother with infant demise due to infection (Figure 1). Neither boy had a definitive genetic diagnosis despite commercial and National Institutes of Health testing for X-linked SCID (1999 and 2006), and there was no history of consanguinity. The patient underwent “Pan-Ethnic Carrier Testing” through a commercial laboratory offering X-linked SCID evaluation. No SCID-associated genetic variant was identified (Table 1), and the mother was counseled that she was not a carrier and her baby would have “no SCID risk.”

Her male infant was born at term via C-section for cephalopelvic disproportion with no postnatal complications. Routine NBS performed at age 2 days surprisingly showed undetectable T-cell receptor excision circles, and the infant was referred to an immunologist. Subsequent analysis at age 13 days showed an absolute lymphocyte count of 1262 cells/mm³

(normal, 3400-7600 cells/mm³). Both the patient (13 days old) and the affected maternal uncle (7 months old) had severely decreased T-cell number (cells/mm³) and function with respective CD3 of 13 (normal, 2500-5500) and 25 (normal, 1900-5900), CD4 of 3 (normal, 1600-4000) and 10 (normal, 1400-4300), CD4+CD45RA+ of 0 (normal, 1200-3700) and 3 (normal, 1100-3700), CD19 of 119 (normal, 300 to 2000) and 512 (normal, 610-2600), and CD3-16/56+ of 50 (normal, 170-1100) and 320 (normal, 160-950). Most striking was that thymic emigrant cells representative of naive T cells were nonexistent in the infant. PHA stimulation response was less than 10% of predicted. He was identified clinically as having SCID.

Because previous targeted IL2RG gene analysis did not identify a variant on 2 separate occasions before 2006, a next-generation sequencing-based multigene panel testing targeting multiple SCID variants was performed in the infant, and identified a novel hemizygous mutation in *IL2RG*, c173C>G (p.P58R), later confirmed in his mother and living maternal uncle (Figure 1, Table 1). With the recent history of “negative” genetic testing for maternal SCID carrier status, the family expressed concern regarding the accuracy of their baby’s diagnosis. Fortunately, intervention was not delayed, and the infant underwent transplant (cord blood donor) at age 2 months with engraftment at age 4 months and is presently well.

Early SCID diagnosis by immune parameters is dependent on predictive FH and abnormal NBS, the latter with more widespread full implementation reported now in 42 states and Puerto Rico as of the end of 2016.⁵ The utility and importance of SCID NBS is also gaining international attention with implementation in part of the Middle East and Canada, and pilot programs in countries throughout Europe, Asia, the Middle East, and South America. Appropriate genetic testing can confirm patterns of inheritance and assist with early intervention. In our case, NBS identified an affected infant in the first week of life, but incomplete genetic testing and suboptimal genetic counseling impeded the family’s understanding of their child’s disorder.

Given the genetic diversity of X-linked SCID and other primary immune deficiency disorders (PIDDs), targeted single specific variant testing is unlikely to be informative. In turn, next-generation sequencing has revolutionized genetic testing in recent years.^{6,7} Current genetic testing with next-generation multigene panel and whole-exome sequencing offer expanded opportunities to identify novel or previously reported variants by assessing multiple genes and in some laboratories extending assessment to include more than 10⁵ base pairs or even all coding exons of the human genome.^{1,7,8}

The commercial product initially used for our patient assessed only the *IL2RG* gene but only 3 specific variants (Table 1), and the test description did not specify fully their limited approach for SCID carrier identification or diagnosis. In a review of carrier status testing available commercially in the United States, we note that not all commercial vendors offering prenatal carrier testing for SCID identify the specific genetic variants being tested. In a review of 10 such vendors, only 50% of vendors provided specific information on which genes would be assessed

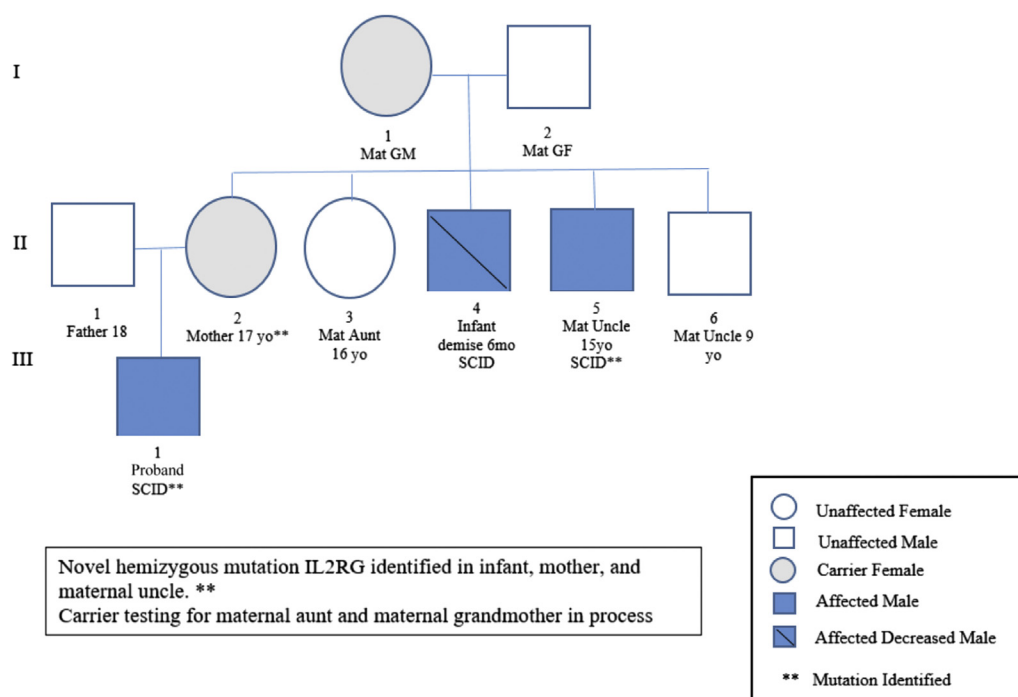


FIGURE 1. Family pedigree: 3 generation pedigree demonstrating an X-linked inheritance pattern. Maternal grandmother is an obligate carrier. Mom is a confirmed carrier with 2 affected brothers, one who passed away from infection as an infant.

TABLE I. Comparison of Baylor Genetics comprehensive exomic SCID panel and commercial pan-ethnic carrier screen

Test name	Baylor Genetics comprehensive exomic SCID panel	Commercial pan-ethnic carrier screen
Genes included	T–B+ SCID: <i>CD3D</i> , <i>CD3E</i> , <i>CORO1A</i> , <i>IL2RG</i> , <i>IL7R</i> , <i>JAK3</i> , <i>PTPRCT</i> , B– SCID: <i>ADA</i> , <i>AK2</i> , <i>DCLRE1C</i> , <i>LIG4</i> , <i>NHEJ1</i> , <i>PRKDC</i> , <i>RAG1</i> , <i>RAG2</i> . OTHERS (LESS COMMON)*	<i>IL2RG</i> gene: Arg226Cys, Arg285Gln, Arg289X (3 specific point mutations)
Target size	135,941 bp	3 bp

Comprehensive SCID panel with 46 specific genes included as well as target size highlighted.¹

Targeted coding regions and flanking intronic sequences within 20 bp were evaluated by the listed Baylor sequencing assay. Positions harboring known intronic mutations up to 350 bp upstream or downstream were also examined. Deletions and duplications of 50-5000 nucleotides are detectable in the assay and 100% coverage of all targeted regions included. Insufficiently covered (<20X) target exons and exons with highly homologous sequences were analyzed by gene-specific amplicon-based Sanger sequencing. Single nucleotide variants and small insertions and deletions were detected through alignment of sequences reads to the reference sequences using NextGENe software (SoftGenetics, State College, Pa). Large exonic copy number variants were included on the basis of read depth. Mutations in the promoter region or deep intronic mutations, complex structural variants such as translocation, inversion, and gene conversion might be missed in this Baylor sequencing array.

*Others: *AP3B1*, *CASP8*, *CD3G*, *CD8A*, *CHD7*, *CIITA*, *DOCK8*, *FOXN1*, *FOXP3*, *IKZF1*, *ITK*, *LCK*, *LYST*, *ORAI1*, *PNP*, *PRF1*, *RFX5*, *RFXANK*, *RFXAP*, *RMRP*, *SH2D1A*, *STAT5B*, *STIM1*, *STX11*, *STXB2*, *TAP1*, *TBX1*, *TTC7A*, *UNC13D*, *XIAP*, *ZAP70*.

and what analysis would be used, for example, targeted gene analysis, exome, or whole-genome sequencing.

Genetic testing is a most important adjunctive confirmation for PIDDs, but equally critical is the interpretation of any test result to the patient/caregiver and the ordering provider. In clinical medicine, adjunctive testing is often required to completely eliminate a diagnosis. Our case highlights 3 specific issues in PIDD diagnosis and counseling that were either missed or inappropriately reported and discusses options to avoid such errors for future patients.

- The paramount importance of the ordering physician's familiarity with genetic testing for prenatal carrier status. It is imperative that genetic testing be augmented by careful discussions with experts in view of the rarity of these conditions and the need for relevant expertise. NBS programs across the

United States typically have established relationships with such geneticists and/or immunologists who can provide insight into current "best practice" testing for PIDD to obstetric or pediatric providers. Testing for this pregnant teenager could have been correctly selected with a collaborative approach.

- Commercial vendors of genetic carrier testing may not consistently provide transparent and specific information regarding the extent of their testing for PIDD carrier status. It is critical that ordering providers fully understand the limitations of genetic testing for any disorder and ALL commercial vendors MUST be transparent in clearly describing the assay used for testing and the breadth of exploration for genetic variants for any disorder.
- Genetic counselors should be knowledgeable of the disorder being addressed and the limitations of the performed assay when sharing genetic test findings with providers or families.

The term “negative” should be used with great caution in the discussion of genetic testing results. The extraordinary utility of genetic testing can be markedly reduced or even misunderstood (as here) without assay familiarity or accurate interpretation by an expert immunologist, given the rarity of SCID and other PIDDs.

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Conflicts of interest: H. Yu is employed by Baylor Genetics. J. R. Lupski has received research support from the National Human Genome Research Institute, the National Cancer Institute, the National Institute of Neurological Disorders and Stroke, and the National Heart Lung and Blood Institute; is employed by Baylor Miraca Genetics Laboratories; has stock in 23 and Me; has stock options in Lasergen Inc; and received consultancy fees from Regeneron. N. L. Rider is employed by Baylor College of Medicine and receives royalties from UpToDate. J. S. Orange is on the ADMA Biologics board; has received consultancy fees from CSL Behring, Grifols, Baxalta, Walgreens/Optioncare, and ASD HealthCare; has received research support from CSL Behring; has received lecture fees from Baxalta; and receives royalties from UpToDate. The rest of the authors declare that they have no relevant conflicts of interest.

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