



Published in final edited form as:

*J Allergy Clin Immunol.* 2015 August ; 136(2): 476–9.e6. doi:10.1016/j.jaci.2015.02.040.

## Clinical Application of Whole Genome Sequencing In Patients with Primary Immunodeficiency

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### Summary

This report illustrates the value of whole genome sequencing (WGS) in elucidating the genetic cause of disease in patients with primary immunodeficiency (PID). As sequencing costs decline, we predict that utilization of next generation sequencing (NGS) in the clinical setting will increase.

### Keywords

Next generation sequencing (NGS); Whole genome sequencing (WGS); Primary immunodeficiency (PID); Severe combined immunodeficiency (SCID); Combined immunodeficiency (CID); Chronic granulomatous disease (CGD); Hyper IgM syndrome (HIGM)

### To the Editor

Next-generation sequencing (NGS), including whole-exome and whole-genome sequencing (WES and WGS, respectively), has been successful at identifying causes of Mendelian diseases, even when the condition is seen in a single patient.<sup>1,2,3</sup> Here, we report our

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findings from WGS in six patients with primary immunodeficiency (PID) from five families in whom the molecular defect was unknown.

Patients 1 and 2 were full sisters with a history of recurrent infections, including: tuberculous lymphadenitis, granulomas, and pneumonias. They had a similarly affected brother. Both patients had an absent rhodamine based respiratory burst, confirming the diagnosis of chronic granulomatous disease (CGD). The parents are distant relatives. Genetic testing was performed at a CLIA-certified commercial laboratory for *NCF2*, *CYBA*, and *NCF1* and was negative. Of note, the commercial *NCF1* screen only examined mutations in exon 2, which harbors the 2GT deletion that causes most reported cases of *NCF1*-related CGD.<sup>4</sup> WGS revealed a homozygous 579G>A substitution causing a premature stop codon (Trp193X) in *NCF1* that had previously been reported as causal for CGD.<sup>5</sup>

Patient 3 was a boy who developed *Pneumocystis jiroveci* pneumonia during the first year of life. There was no family history of PID. Immune evaluation demonstrated absent serum IgG and IgA. He had normal numbers of B, T and natural killer (NK) cells by flow cytometry and had normal T cell proliferative responses to mitogens and antigens. However, the patient lacked any detectable expression of CD40 ligand (CD154) on T cells after stimulation with ionomycin and phorbol myristate acetate, consistent with a diagnosis of X-linked hyper IgM syndrome (HIGM1). CD40 ligand gene (*CD40LG*) sequencing was performed at a CLIA-certified laboratory and no mutations were found, somewhat conflicting with the CD40L expression results. WGS revealed a novel G>T nucleotide substitution in the *CD40LG* on the X-chromosome resulting in a premature stop codon at Glu-230 (Glu230X). Nonsense mutations in neighboring codons (G227X and Q232X) have been previously reported as causes of HIGM1.<sup>6,7</sup> Based on the mutation's absence in control subjects and in Exome Variant Server (EVS, NHLBI GO Exome Sequencing Project (ESP), Seattle, WA [URL: <http://evs.gs.washington.edu/EVS/>]), its predicted functional consequence, and the described disease causing mutations in neighboring codons, this mutation was judged the likely cause of this patient's immune defect.

Patient 4 was a girl who presented in infancy with a history of failure to thrive (FTT) and recurrent infections. Flow cytometry showed that she had no T or B cells, but had an elevated percentage of NK cells. T cell proliferation studies showed no responses to mitogens. A diagnosis of SCID was made. *RAG1* and *RAG2* genes were sequenced and no mutations were identified. WGS revealed a homozygous 82kb deletion spanning exons 1–4 in *DCLRE1C* (see Figure E1A in the Online Repository).

Patient 5 was a girl who presented in infancy with FTT and recurrent infections. Immune studies showed absent T cells and B cells and no T cell proliferative responses to mitogens. All of her lymphocytes were NK cells. These findings led to a diagnosis of SCID. Gene sequencing was performed at a CLIA-certified commercial laboratory for *RAG1*, *RAG2* and *DCLRE1C*, which are known to be associated with NK phenotype SCID<sup>8,9</sup> and the results were negative. WGS revealed a novel heterozygous single nucleotide variant (SNV) observed at an essential splice site in *DCLRE1C*. This splice site mutation (c.362+2T>A) is absent in controls and in EVS, and is predicted to destroy the canonical splice donor site in

intron 5. A splice site variant at the neighboring nucleotide position (c.362+1G>T) is associated with SCID.<sup>9</sup> Further investigation revealed an 82kb deletion involving exons 1–4 in *DCLRE1C* (see Figure E1B in the Online Repository). The combined presence of this partial deletion and the c.362+2T>A (IVS5+2T>A) splice site mutation is the likely cause of SCID in this patient.

Patient 6 was a girl whose brother had combined immunodeficiency with a predominance of NK cells. She was investigated immunologically at an early age and had normal T lymphocyte proliferative responses to mitogens, antigens and allogeneic cells. She did well clinically until around 7 years of age, when she became profoundly lymphopenic and developed very low T-cell function. WGS showed a homozygous essential splice site mutation (c.109+1 G>T) (IVS1+1 G>T) in *DCLRE1C* that was absent in controls. This SNV is extremely rare (MAF=0.00023 in EVS). This splice site mutation is predicted to destroy the canonical splice donor site in intron 1. However, given the rarity of this variant and the lack of consanguinity in the family, homozygosity was considered unlikely. Deletion screening showed that the patient also had the same 82kb hemizygous deletion involving exons 1–4 of *DCLRE1C* observed in patient 5 (see Figure E1C in the Online Repository), which had resulted in miscalling of the SNV as homozygous.

The difference in disease severity between patients 5 and 6 is interesting, as both patients are compound heterozygotes for similar deletion but have different essential splice site mutations. Patient 5 had no T cell function, while patient 6 had normal T-cell function until around 7 years of age. To explain the difference in phenotypic severity, we constructed minigenes for each of the two newly identified *DCLRE1C* variants (IVS1+1 G>T and IVS5+2 T>A) (see Figure E2 in online repository). A decrease in the WT *DCLRE1C* isoform was observed in the IVS1+1 G>T variant construct (Figure 1A). Follow-up quantitative RT-PCR of the wild type and mutant minigene transcript revealed that the variant produced only 16% of the wild type transcript (Figure 1B). The IVS5+2 T>A variant construct resulted in a novel isoform in which exon 5 was skipped (Figure 1C and 1D). Both novel isoforms are predicted to result in abnormal gene and/or protein expression and/or function. We examined the mRNA and protein expression of the full-length WT-*DCLRE1C* cDNA and the IVS5+2 T>A isoform, in which exon 5 is skipped, using FLAG-tagged constructs (Figure 2A). While both isoforms showed similar expression at the mRNA level, only the wild type isoform showed a detectable level of protein (Figure 2B and 2C).

Hence, the IVS1+1 G>T variant found in patient 6 produced some of the canonical wild type isoform, suggesting that this mutation decreases the splicing efficiency. The IVS5+2 T>A variant from patient 5 did not produce any wild type isoform, but did produce another isoform. This result is consistent with a model in which patient 6 produced enough wild type Artemis protein to delay the onset of the patient's combined immunodeficiency, resulting in a less severe phenotype.

The use of NGS is accepted for investigating undiagnosed genetic conditions. Here, we show the value of WGS in PID patients without identified causal mutations.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

We would like to acknowledge the following individuals for the contributions of control samples: Dr. William B. Gallentine, Dr. Erin L. Heinzen, Dr. Aatif M. Husain, Ms. Kristen N Linney, Dr. Rodney A. Radtke, Dr. Saurabh R. Sinha, and Ms. Nicole M. Walley; Dr. Joseph McEvoy, Dr. Anna Need, Mr. Jordan Silver, Ms. Marlyne Silver; Dr. Elizabeth Cirulli; Dr. Julie Hoover-Fong, Dr. Nara L. Sobreira and Dr. David Valle; Dr. Robert Brown; Dr. Ruth Ottman; Dr. Deborah Levy; K. Welsh-Bomer, C. Hulette, J. Burke; Mr. David H. Murdock and the MURDOCK community registry and biorepository; Dr. Vandana Shashi and Ms. Kelly Schoch; Dr. Demetre Daskalakis.

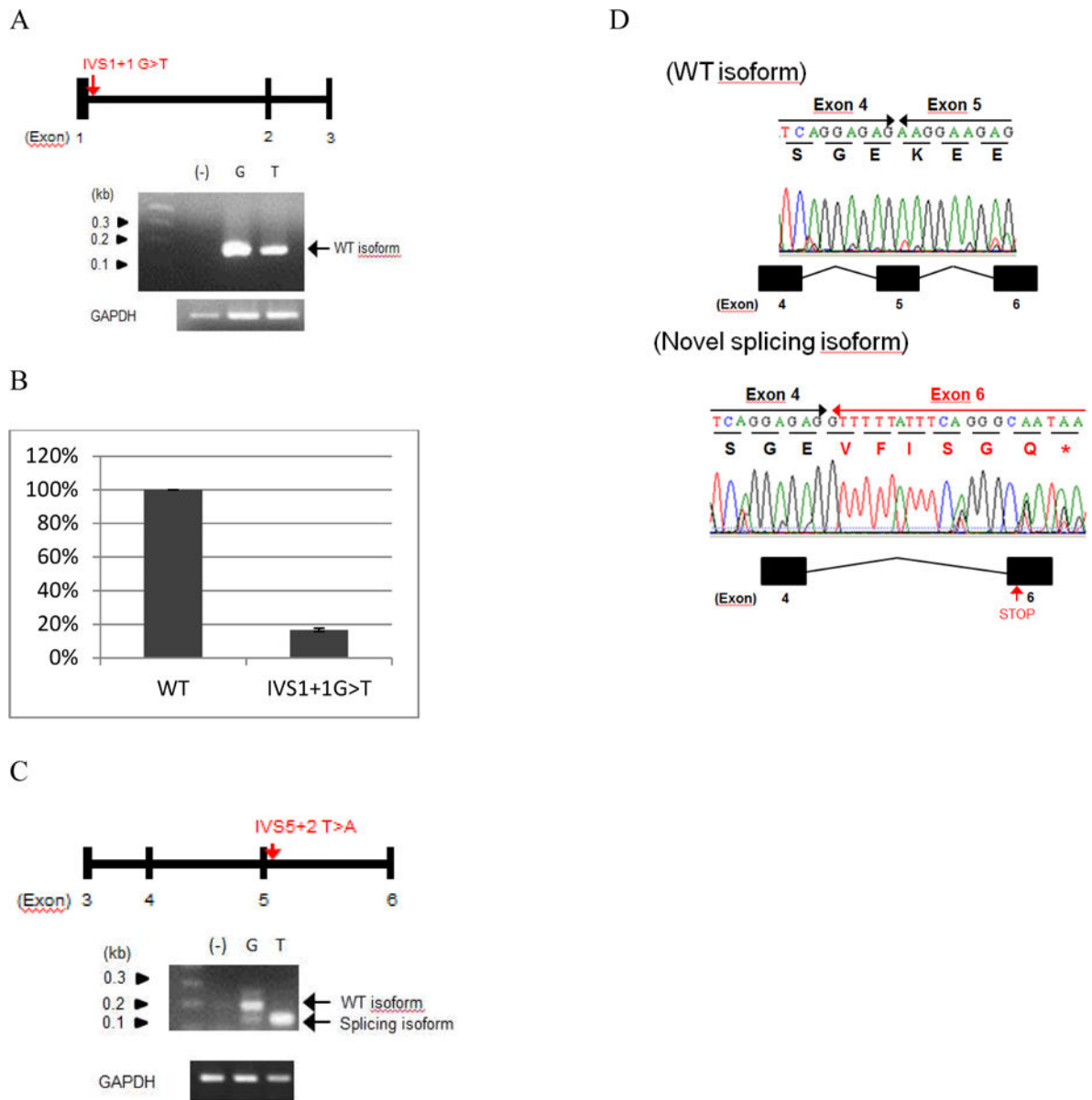
The collection of control samples and the production of sequence data were funded in part by NIAID Grant UO1AIO67854 (Center for HIV/AIDS Vaccine Immunology (“CHAVI”).

The authors would like to thank the NHLBI GO Exome Sequencing Project and its ongoing studies which produced and provided exome variant calls for comparison: the Lung GO Sequencing Project (HL-102923), the WHI Sequencing Project (HL-102924), the Broad GO Sequencing Project (HL-102925), the Seattle GO Sequencing Project (HL-102926) and the Heart GO Sequencing Project (HL-103010).

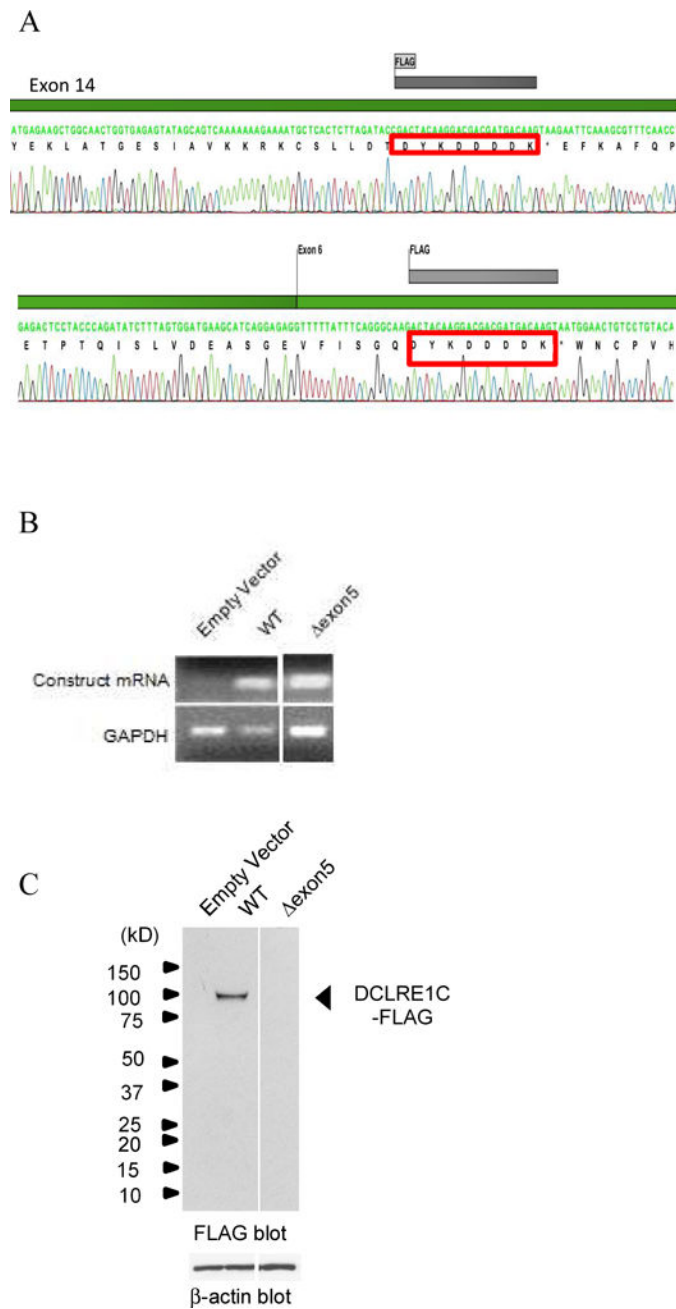
This work was supported by NIH grants (including T32 training grants) and a Senior Fellowship Award from the Clinical Immunology Society/Grifols to TM.

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**Figure 1.** Semi-quantitative PCR and quantitative real-time PCR results of Minigene expression. A) Minigene 1 (IVS1+1G>T) mRNA expression, B) quantitative Taqman results showing only 16% expression of the wild type mRNA in Minigene-1, C) Minigene-5 (IVS5+2T>A) mRNA expression, and D) Sanger sequencing confirmation of IVS5+2T>A resulting in exon 5 skipping.



**Figure 2.** DCLRE1C protein assays. A) FLAG-tag insertion in exon 14 of the wild-type DCLRE1C construct and exon 6 of the  $\Delta$ exon5 construct, B) semi-quantitative PCR showing presence of  $\Delta$ exon5 mRNA expression, C) immunoblotting showing absence of DCLRE1C protein expression in mutant  $\Delta$ exon5 isoform.