

A de novo TLR7 gain-of-function mutation causing severe monogenic lupus in an infant

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To the Editor: Childhood-onset systemic lupus erythematosus (cSLE) presents with a more severe phenotype and worse outcomes than adult-onset SLE (1). Genetic factors are understood to have a significant role in cSLE and can occur secondary to a single-gene defect, termed monogenic lupus (2). Recently, germline gain-of-function mutations of TLR7 were shown to cause cSLE, highlighting the role of TLR7 in driving autoimmunity (3). Here, we demonstrate a previously unreported de novo gain-of-function TLR7 variant in a 2-year-old girl with severe SLE and outline a successful approach to disease management. A 13-month-old female infant presented with anti-NMDA receptor encephalitis. Within 10 months, she developed a large pericardial effusion and profound hemolytic anemia and was diagnosed with cSLE. Shortly afterward, she developed inflammatory vasculitis with sudden-onset status epilepticus. Each presentation was life-threatening and required substantial immunosuppression (details in Supplemental Figure 1 and Supplemental Table 1; supplemental material available online with this article; <https://doi.org/10.1172/JCI179193DS1>). Despite clinical improvement, both her interferon-stimulated gene (ISG) signature and neutrophil transcriptional signature remained pathologically elevated (Figure 1, A and B and Supplemental Methods). The very early onset of recurrent, life-threatening immune dysregulation raised suspicion for an inborn error of immunity (IEI), but whole-exome sequencing (Figure 1C) was negative for known pathogenic variants. However, comparison of proband and parental sequences revealed a previously unreported de novo heterozygous missense [...]

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A 13-month-old female infant presented with anti-NMDA receptor encephalitis. Within 10 months, she developed a large pericardial effusion and profound hemolytic anemia and was diagnosed with cSLE. Shortly afterward, she developed inflammatory vasculitis with sudden-onset status epilepticus. Each presentation was life-threatening and required substantial immunosuppression (details in Supplemental Figure 1 and Supplemental Table 1; supplemental material available online with this article; <https://doi.org/10.1172/JCI179193DS1>). Despite clinical improvement, both her interferon-stimulated gene (ISG) signature and neutrophil transcriptional signature remained pathologically elevated (Figure 1, A and B and Supplemental Methods).

The very early onset of recurrent, life-threatening immune dysregulation raised suspicion for an inborn error of immunity (IEI), but whole-exome sequencing (Figure 1C) was negative for known pathogenic variants. However, comparison of proband and parental sequences revealed a previously unreported de novo heterozygous missense mutation in *TLR7*, (c.800C>T, p.P267L), confirmed by Sanger sequencing (Figure 1D). The substitution of this highly conserved proline residue in the leucine-rich repeat (LLR9) ectodomain, lying only 3 residues from the recently described *TLR7*^{Y264H} gain-of-function variant (3), was predicted deleterious.

The encoded product, TLR7, is part of a canonical defense system, linking innate and adaptive immunity. TLR7 predominantly recognizes viral single-stranded RNA in the endosomes of hematopoietic cells. Its signaling leads to activation of interferon regulatory factors to induce interferon synthesis and of NF-κB and MAPK pathways to activate the transcription of proinflammatory cytokines. While loss-of-function *TLR7* variants predispose to severe viral infection (4), *TLR7* gain of function was recently identified in several young children with SLE (3), and polymorphisms affecting *TLR7* expression are also recognized to influence adult-onset SLE risk (5).

We hypothesized that *TLR7* p.P267L causes cSLE through a gain-of-function mechanism. Accordingly, stimulation of patient cells by *TLR7/8* ligand CL097 in vitro led to elevated transcriptional activation of proinflammatory cytokines, TNF-α, IL-1b, and IL-6 (Figure 1E and Supplemental Methods), while CD62L shedding was unaffected (Supplemental Figure 2 and Supplemental Methods). Standard PBMC immunophenotyping was normal, other than reduced B cell numbers reflecting prior B cell-depleting therapy (Supplemental Figure 3 and Supplemental Methods). However, *TLR7* protein

expression was increased, especially in B cells, monocytes, and dendritic cells (Figure 1, F and G, and Supplemental Methods).

To further evaluate the variant's effect on protein function, we first performed transient transfection of WT or mutant *TLR7* into HEK293T cells and documented equivalent protein expression (Figure 1H). Utilizing a cotransfected NF-κB reporter system, *TLR7*^{P267L} generated significantly higher dose-dependent NF-κB signaling than WT *TLR7* in response to two different *TLR7*-specific agonists (Figure 1I and Supplemental Methods). Taken together, these results confirm our hypothesis of gain of function for the *TLR7*^{P267L} variant and imply that increased protein abundance in patient leukocytes (perhaps itself interferon driven) may amplify the effect of higher levels of signaling activity.

Following diagnosis of IEI, JAK inhibitor therapy was introduced to the patient to act as a bridge to a curative procedure in the form of a conditioned maternal TCRαβ/CD19-depleted haploidentical hematopoietic stem cell transplant, which was well tolerated. Nine months after transplant, SARS-CoV-2 infection triggered an autoimmune hemolytic anemia, requiring transfusion and immunomodulation. Over the following 8 months the patient remained positive for red cell antibodies by direct antiglobulin testing on sirolimus and physiological dose corticosteroid, with evidence of smoldering hemolysis and mildly raised ISG signature (final time point in Figure 1, A and B) in the context of 90% donor chimerism. Nonetheless she has remained systemically well and made excellent progress in terms of immune reconstitution, growth, and neurodevelopment (details in Supplemental Figure 1).

Despite intense research, understanding of the etiology of SLE remains incomplete (6). The recognition of rare IEI in patients with cSLE may provide valuable insights into disease mechanism and suggest targets for precision medicine. Our present findings reinforce the importance of innate immunity in SLE pathogenesis while highlighting the curative potential of hematopoietic stem cell transplant in *TLR7* gain of function.

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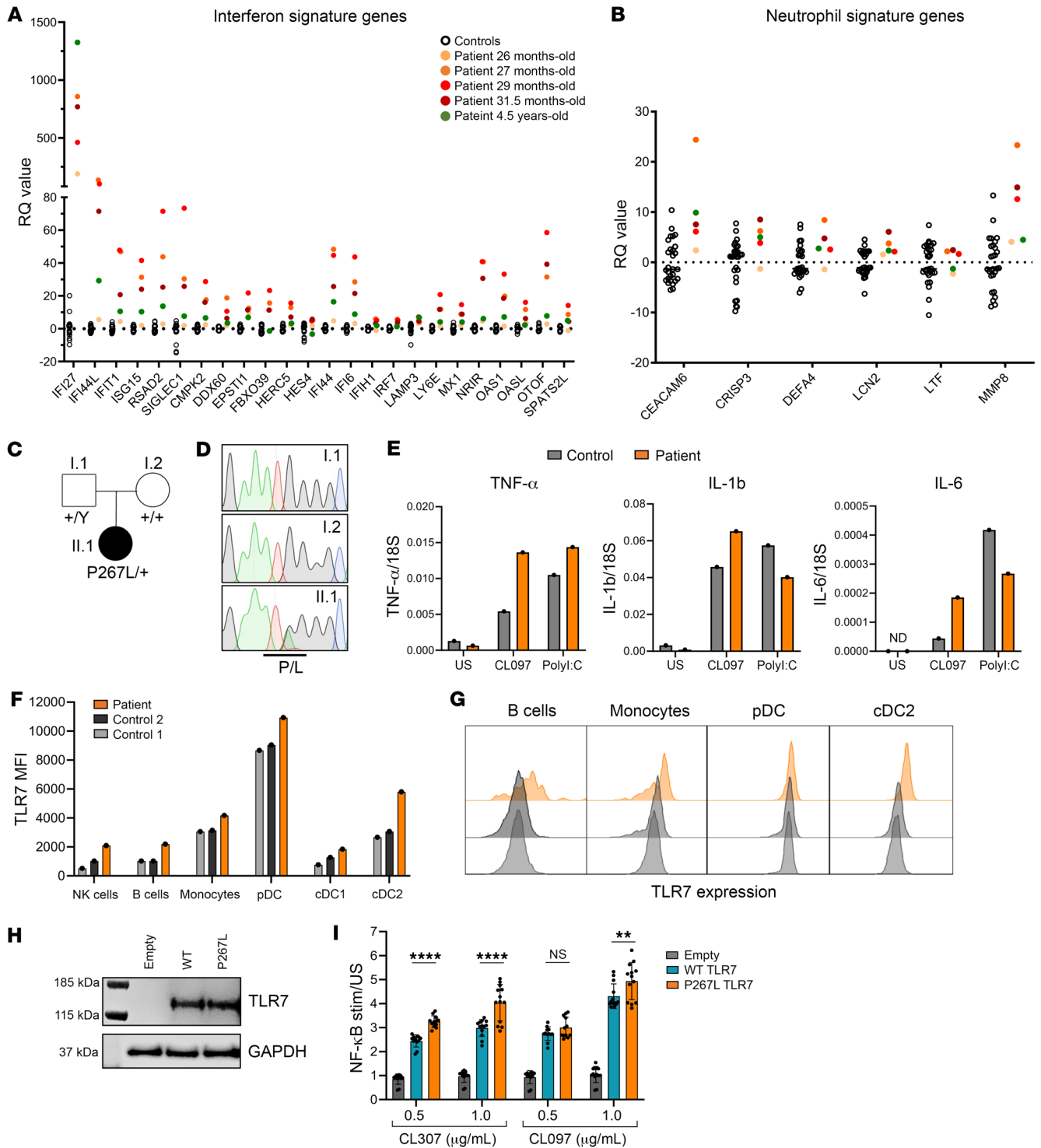


Figure 1. TLR7 gain-of-function mutation in a young child with severe SLE. (A) Persistently pathologically elevated interferon-stimulated gene and (B) neutrophil signature gene transcripts in patient at indicated time points. The y axis shows transcript abundance in arbitrary units (relative quantification [RQ] values) for indicated genes. (C) Pedigree. (D) Capillary sequencing of PCR amplicons. P, proline substituted by L, leucine. (E) Increased transcription of proinflammatory cytokines TNF- α , IL-1b, and IL-6 in patient PBMCs stimulated with TLR7/8 ligand CL097 or polyI:C. US, unstimulated. (F) Increased TLR7 protein expression in patient PBMC subsets quantitated by (G) flow cytometry. Representative of 2 independent experiments. (H) TLR7 protein expression detected by immunoblotting of transfected HEK293T cells 48 hours after transfection. (I) NF- κ B activity by dual luciferase assay after TLR7 plasmid transfection into HEK293T cells and treatment with indicated TLR7 ligands. Luminescence signal normalized to unstimulated cells from 4 independent experiments. Two-way ANOVA, ** $P < 0.01$, **** $P < 0.0001$. Data represent mean \pm SD.

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