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

Jarmila Stremenova Spegarova¹, Praisoody Sinnappurajar², Dalila Al Julandani², Rokas Navickas¹, Helen Griffin¹, Manisha Ahuja¹, Angela Grainger¹, Katie Livingstone³, Gillian I Rice⁴, Fraser Sutherland³, Corinne Hayes⁵, Simon Parke⁵, Lewis Pang⁶, Marion R Roderick^{7,8}, Mary Slatter⁹, Yanick Crow³, Athimalaipet V Ramanan^{2,8}, Sophie Hambleton^{1,9}

¹Translational and Clinical Research Institute, Medical School, Newcastle University, Newcastle upon Tyne, UK. ²Department of Paediatric Rheumatology, Bristol Royal Hospital for Children, Bristol, UK. ³MRC Human Genetics Unit, Institute of Genetics and Cancer, University of Edinburgh, Edinburgh, UK. ⁴Division of Evolution and Genomic Sciences, School of Biological Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester Academic Health Science Centre, Manchester, UK. ⁵Department of Paediatrics and ⁶Exeter Genomics Laboratory, Royal Devon University Healthcare NHS Foundation Trust, Exeter, UK. ⁷Department of Paediatric Immunology, Bristol Royal Hospital for Children, Bristol, UK. ⁸Translational Health Sciences, University of Bristol, Bristol, UK. ⁹Great North Children's Hospital, Royal Victoria Infirmary, Newcastle upon Tyne, UK.

Correspondence to: Sophie Hambleton, Newcastle University Translational & Clinical Research Institute, Framlington Place, Newcastle upon Tyne, NE2 4HH, UK, sophie.hambleton@newcastle.ac.uk, +44 191 208 7139

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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 private *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 female infant presented aged 13 months with anti-NMDA receptor encephalitis. Within ten months, she acquired a large pericardial effusion and profound haemolytic anaemia and was diagnosed with cSLE. Shortly after, she developed inflammatory vasculitis with sudden onset status epilepticus. Each presentation was life-threatening and required significant immunosuppression (details in Supplementary Figure S1 and Table 1). Despite clinical improvement, both her interferon-stimulated gene (ISG) signature and neutrophil transcriptional signature remained pathologically elevated (Figure 1, A and B).

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 private *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, Toll-like receptor-7 (*TLR7*), is part of a canonical defence system, linking innate and adaptive immunity. *TLR7* predominantly recognises viral single-stranded RNA in the

endosomes of hematopoietic cells. Its signalling leads to activation of interferon regulatory factors to induce interferon synthesis, and of NF- κ B and MAPK pathways to activate the transcription of pro-inflammatory 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 pro-inflammatory cytokines, TNF- α , IL-1 β and IL-6 (Figure 1E), while CD62L shedding was unaffected (Supplementary Figure S2). Standard PBMC immunophenotyping was normal other than reduced B cell numbers, reflecting prior B cell depleting therapy (Supplementary Figure S3). However, TLR7 protein expression was increased, especially in B cells, monocytes and dendritic cells (Figure 1, F and G).

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 co-transfected NF- κ B reporter system, TLR7^{P267L} generated significantly higher dose-dependent NF- κ B signalling than WT in response to two different TLR7-specific agonists (Figure 1I). 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 unit signalling activity.

Following the IEI diagnosis, JAK inhibitor therapy was introduced to bridge the patient to a curative procedure in the form of a conditioned maternal TCR $\alpha\beta$ /CD19-depleted haploidentical hematopoietic stem cell transplant (HSCT), which was well-tolerated. Nine months post-

transplant, SARS-CoV2 infection triggered an autoimmune hemolytic anemia, requiring transfusion and immunomodulation. Over the following 8 months she remained DAT-positive on sirolimus and physiological dose corticosteroid, with evidence of grumbling hemolysis and mildly raised ISG signature (final time point in Figure 1A, 1B) 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 Figure S1).

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 HSCT in TLR7 gain-of-function.

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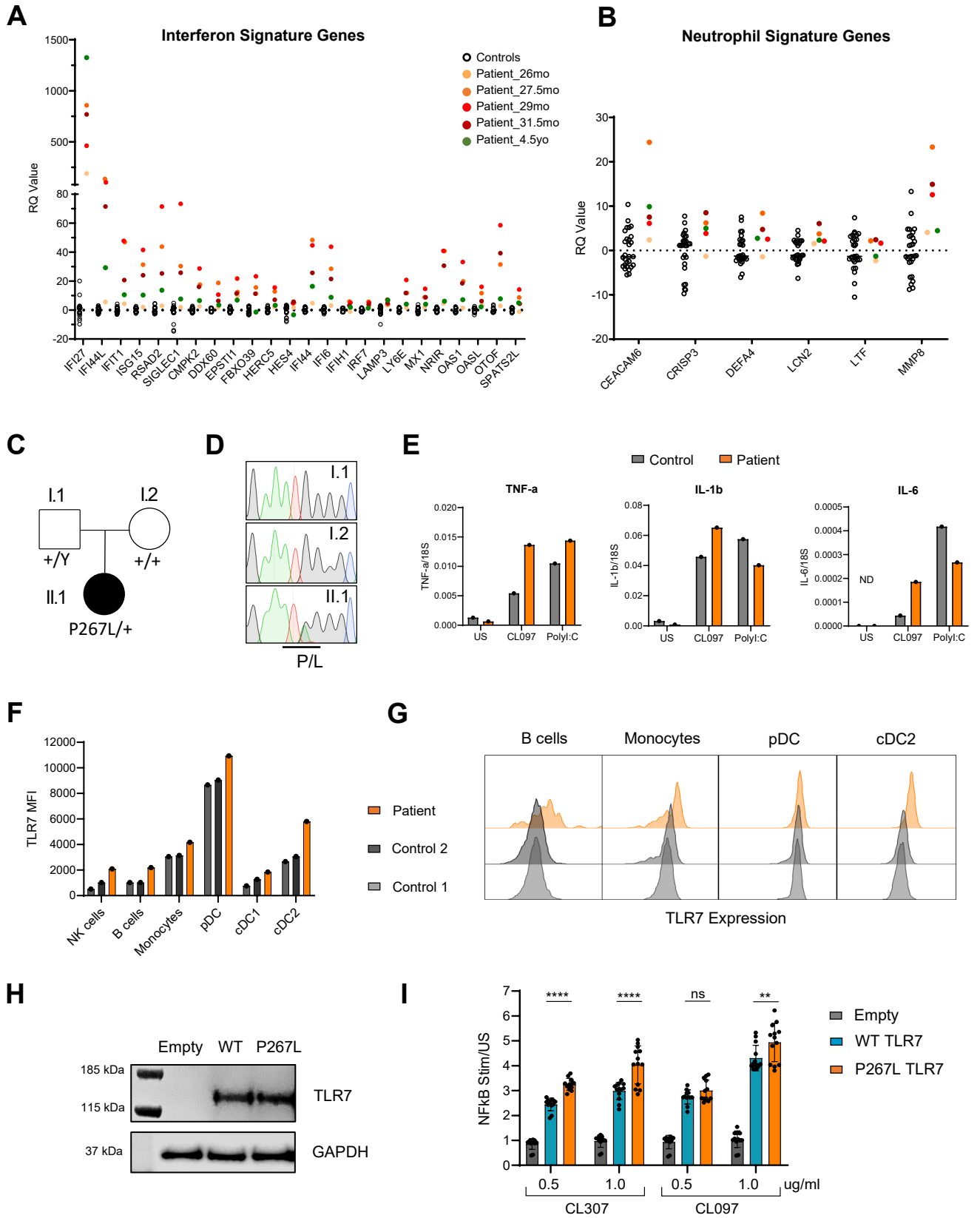


Figure 1. TLR7 gain-of-function mutation in a young child with severe SLE. (A) Persistently pathologically elevated interferon stimulated gene and (B) Neutrophil signature gene transcripts in patient at indicated timepoints. y-axis: transcript abundance in arbitrary units (RQ values) for indicated genes, mo: months-old, yo: years-old. (C) Pedigree. (D) Capillary sequencing of PCR amplicons. (E) Increased transcription of pro-inflammatory cytokines TNF- α , IL-1 β and IL-6 in patient's PBMC stimulated with TLR7/8 ligand CL097 or PolyI:C. US: Unstimulated. (F) Increased TLR7 protein expression in patient's PBMC subsets quantitated by: (G) flow cytometry. Representative of two independent experiments. (H) TLR7 protein expression detected by immunoblotting of transfected HEK293T cells 48h post-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 normalised to unstimulated cells (US) from four independent experiments. 2way ANOVA, ****: $p < 0.0001$, **: $p < 0.01$, ns: non-significant. Data represent mean \pm SD.