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CORRESPONDENCE

Concomitant Ultrarare Mutations in TLR3 and CTPS2 in a Patient with Severe and Recurrent Respiratory Infections in Early Life

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To the Editor,

Here we report the case of an Arab boy with parents of fourth-degree consanguinity (Fig. 1a), with a history of multiple hospitalizations in early life due to recurrent viral lower respiratory tract infections. The boy is the only affected family member. Whole genome sequencing of the boy and family members revealed a biallelic, hypomorphic missense mutation in the TLR3 gene (c.2228G > A, p.Gly743Asp) in the patient (Figure S1), which is ultrarare and predicted to be pathogenic (Table S1). The allele has previously been reported in a single case with herpes simplex encephalitis due to a carrier of two cis variants in TLR3 gene [1]. We also found one of the older, unaffected female siblings to carry the same biallelic TLR3 missense mutation, suggesting incomplete penetrance. Both parents and two other female siblings are heterozygous carriers (Fig. 1a). In addition, we found an ultrarare hemizygous variant in CTPS2 (c.1585T > G, p.Phe529Val) in the patient (Figure S1), which is private to the family. While this CTPS2 variant is predicted to be probably pathogenic (Table S1) and further in silico analysis suggests that the variant may cause local destabilization of the protein structure (Figure S2), comparative immunoblot analyses of activated T cells from the patient, relatives and unrelated healthy donors shows that the CTPS2 protein expression is not altered in the patient (Figure S3). Additional genotyping also including one extended family member revealed that the father, a maternal uncle and two female siblings are hemizygous and homozygous for the wild-type CTPS2 allele, respectively, while the mother and one female sibling are heterozygous carriers of the CTPS2 missense variant (Fig. 1a). Unfortunately, we were unable to genotype additional extended family members to identify another male family member potentially carrying the CTPS2 missense allele. Thus, the significance of this latter variant in CTPS2 remains uncertain. Both variants in TLR3 and CTPS2 were confirmed by Sanger sequencing (Fig. 1b and 1c).

The affected child was born after an uncomplicated pregnancy but required postnatal Neonatal intensive Care Unit (NICU) admission for one week and resuscitation post-birth with 6 h nasal cannula after being diagnosed with transient tachypnea of the newborn. At 7 months of age, the patient suffered from an episode of severe, acute human respiratory syncytial virus (hRSV) bronchiolitis and required emergency room (ER) admission; however, normal microlaryngoscopy and no evidence of an anatomical abnormal airway was detected. At the age of 8 months, he required Pediatric intensive Care Unit (PICU) admission and mechanical ventilation for three days for Human bocavirus (HBoV) bronchiolitis. Further, at one year of age, he was again admitted to intensive care, this time for Human rhinovirus (HRV) bronchiolitis. At the age of 15 months, the patient was presented to the ER with adverse reactions (i.e., severe skin rash with fever and shortness of breath) after receiving Diphteria-Tetaus_Petrussis (DTP), Haemophilus influenzae type b (Hib), Hepatitis B (HepB) and pneumococcal

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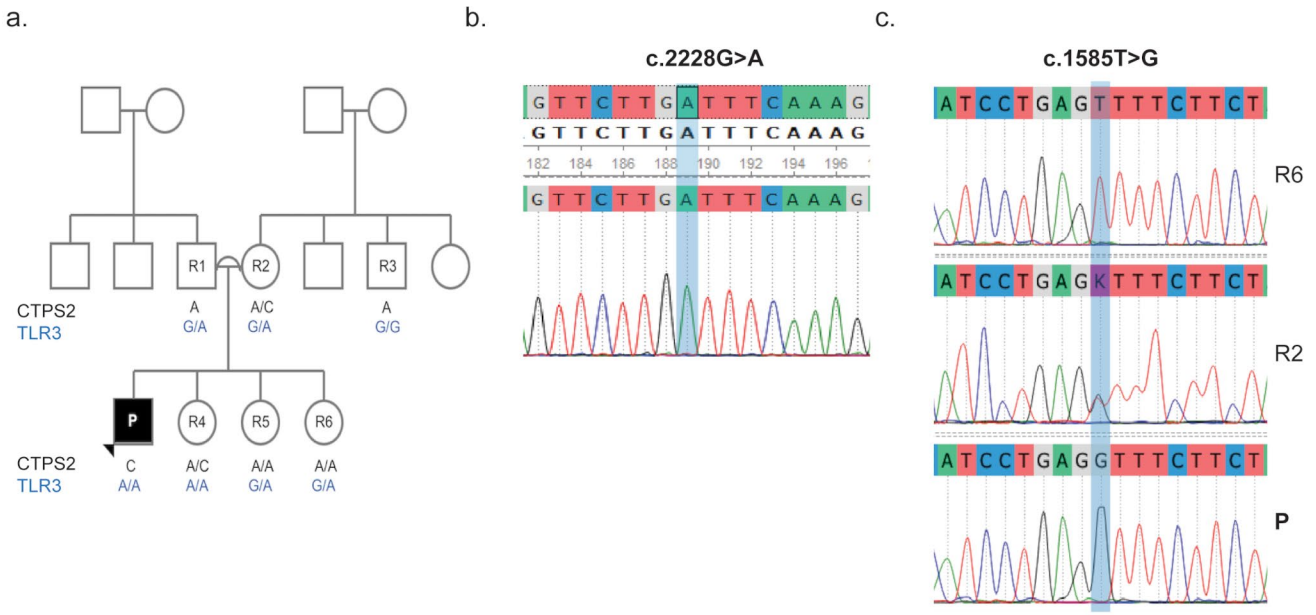

Fig. 1 Identification of a homozygous *TLR3 and hemizygous CTPS2* gene mutation in a patient from consanguineous parents. Parents are first cousins. (**a**) Pedigree and segregation of the *TLR3 and CTPS2* gene mutation. The patient is homozygous for the TLR3 mutation and hemizygous for CTPS2 mutation. (**b**) Sanger sequence analysis of the *TLR3* gene showing the homozygous (c.2228 G > A) mutation; Electropherogram of partial forward sequences of *TLR3* corresponding to the mutation in the patient, The alignment was done on Unipro UGENE 48.0. The reference vs. altered nucleotide position is indicated by a blue box. (**c**) Sanger sequence analysis of the *CTPS2* gene showing the hemizygous (c.1585 T > G) mutation; Electropherograms of partial sequences of *CTPS2* corresponding to the mutation in a relative (R6) $CTPS2^{wt/wt}$ (top), patient (bottom), and a $CTPS2^{wt/mut}$ relative, R2 (middle). The reference vs. altered nucleotide position is indicated by a blue box

13-valent routine vaccination; he was discharged home on the same day as his condition had improved. No additional severe infections or adverse reactions were documented until the last follow up, which was at 4 years of age.

Clinical immunological testing was performed at one and four years of age and revealed mostly intact cellular (Complete Blood Count (CBC) and lymphocyte subsets) and humoral (total IgG, IgA, IgM, IgG subsets and antimicrobial IgG levels) immune function, with slightly increased CD3 + and CD4 + T cell fractions and slightly decreased NK cell subsets at 4 years of age, while total IgE levels were elevated at both time points tested (Table S4). Clinical serological and PCR testing on a sample taken at four years of age showed that the patient was seropositive for Herpes simplex virus (HSV-1 and HSV-2) and VZV IgG, while he was found PCR-negative for Varicella-zoster virus (VZV) and Epstein-Barr virus (EBV). To further elucidate the patient's infection history in early life, we performed large-scale antibody profiling using phage immunoprecipitation-sequencing on a plasma sample obtained from the patient at one year of age. Based on the results from this screen, the patient appeared seropositive for antibodies specific to a variety of common viruses and bacteria, including HRSV, HRV-A, Human adenovirus C (HAdV-C), HSV-1, as well as opportunistic bacteria, such as *S. pneumoniae* and *S. aureus* (Figure S4).

A possible underlying genetic cause for the patient's infection history remains inconclusive. Autosomal dominant TLR3 deficiency is a well-established risk factor in humans predisposing to isolated herpes simplex encephalitis (HSE), severe influenza A virus (IAV) infection manifesting as acute respiratory distress syndrome (IAV-ARDS), multiple recurrences of Herpes zoster ophthalmicus, and critical COVID-19 pneumonia, albeit with incomplete penetrance (Table S2) [2, 3]. Apart from the carrier of two cis variants in HSE patient mentioned above, AR TLR3 deficiency has only been reported in two other HSE patients who were homozygous for a hypomorphic missense variant, namely Arg867Gln and p.Leu297Val [1] (Table S2). Nonetheless, a pathogenic role of the biallelic missense variant found in our patient, although plausible, cannot be ascertained in the absence of other case reports of unrelated patients with a similar genotype. We are also unable to completely rule out a pathogenic role of the hemizygous missense variant in *CTPS2*. The human genome encodes two CTPS proteins, CTPS1 and CTPS2, which share 74% amino acid identity. Thus far, no patients with germline loss-of-function mutations in *CTPS2* have been reported, although there are eight other predicted loss-of-function

variants reported in gnomAD, four of which were found in hemizygote males (Table S3). This suggests a higher degree of immunological redundancy for this gene in comparison to *TLR3* or *CTPS1*. CTPS1 deficient patients exhibit impaired T lymphocyte proliferation and suffer from severe bacterial and viral infections, most notably life-threatening herpes virus infections with VZV and EBV [4, 5], while the patient described here seemed immunocompetent to VZV infection. Loss of CTPS2 function and an either benign or pathogenic role of the variant reported here remains to be established experimentally.

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Author Contributions N.M conceived the study and supervised the project. S.B analyzed the WGS data. S.B and A.G wrote the main manuscript text. S.B prepared Fig. 1 and S1, Tables S1, S2 - S3. T.K prepared Figures S2 - S3. A.G prepared Table S4. N.M reviewed and edited the manuscript, supplemental material and figures. M.A contributed to the clinical data. R.M performed the CTPS1 and CTPS2 immunoblotting. All authors reviewed the manuscript.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Ethics Approval The study protocol was approved by the institutional review board (IRB) of Sidra Medicine (protocol no. 1500746). The procedures used in this study adhere to the principles of the Declaration of Helsinki.

Consent to Participate Written informed consent and parental permission were obtained (and child consent where applicable) from all participants included in the study in accordance with local regulations governing human subject research.

Consent to Publish All the authors have seen and approved the manuscript, which has not been accepted or published elsewhere.
The authors affirm that human research participants provided informed consent regarding publishing their data.

Competing Interests The authors declare no competing interests.

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