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LETTER TO EDITOR

Pathogenic P554S Variant in *TLR3* in a Patient with Severe Influenza Pneumonia

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To the Editor,

Several inborn errors of innate immunity (IEI) in which the activation, amplification, or response to type I interferon (IFN) are perturbed, have been described to underlie life-threatening viral infections. An example is herpes simplex virus (HSV) encephalitis (HSE) caused by impaired toll-like receptor (TLR) 3 pathway responses (due to loss of function mutations in either *TLR3*, *IRF3*, *TBK1*, *TRIF*, *TRAF3*, or *UNC93B1*) [1]. TLR3 deficiency was also described in a patient suffering from recurrent HSV-triggered erythema multiforme and in patients suffering from severe influenza pneumonia [2, 3]. Four additional monogenetic causes of severe influenza pneumonitis have been described: autosomal recessive (AR) complete IRF7 and IRF9 deficiency, and autosomal dominant (AD) IRF3 and GATA2 deficiency, the latter resulting in a broader infectious susceptibility [4]. TLR3 acts as a dsRNA sensor, and TLR3 deficiency leads to impaired type I and type III IFN production in response to the Influenza virus [3]. IRF3 and IRF7 deficiencies impair the amplification of type I and III IFN responses, especially the early production of type I IFN. IRF9 deficiency, on the other hand, results in impaired interferon-stimulated gene factor 3 (ISGF3) formation, which is central to the activation of both type I and type III IFN response pathways. Therefore, increased risk for influenza pneumonia is present in several defects which cause an impaired type I and III IFN signaling. We here describe a child presenting with interstitial pneumonia and respiratory insufficiency following influenza A virus infection, whom we investigated for an underlying IEI.

The patient was born from non-consanguineous parents of Belgian descent. Her past medical history was significant for a short hospitalization at age 1 year for lobar pneumonia, recurrent upper airways infections, dust mite allergy, and congenital hip dysplasia. Her growth and development were normal and she received the childhood vaccines, including live attenuated measles-mumps-rubella, without adverse effects. At age 5 years, she was admitted with fever in the last 4 days, tachypnea, and diffuse crackles and rhonchi on physical examination. A nasal swab PCR-based test was positive for influenza A and culture grew *Haemophilus influenzae*. Chest X-ray was initially normal but evolved into retrocardiac infiltrates and bilateral blunting of the costophrenic angles 8 days after the onset of fever (Fig. 1). Her blood tests revealed a normal complete blood count; mildly elevated CRP (22.5 mg/L, normal <5); normal immunoglobulin levels; normal T, B and NK cell counts; and normal T cell proliferation in response to mitogens (supplementary Table S1). She was started on parenteral antibiotics and oral Tamiflu, but she developed progressive dyspnea and hypoxia on day (D) + 5 after admission, requiring admission to the intensive care unit (ICU) for continuous positive airway pressure ventilation (cPAP) and oxygen therapy. She responded well to therapy and was discharged on D + 10 after admission. Upon resolution of the infection, she showed normal T cell proliferation upon stimulation with influenza in vitro (proliferation index 101, normal >5).

Her subsequent follow-up was uneventful until the age of 15 years, at which time she developed a papulovesicular rash involving the right ear and peri-auricular region. A bacterial swab was negative and HSV-1 infection was clinically diagnosed by her general practitioner. However, HSV

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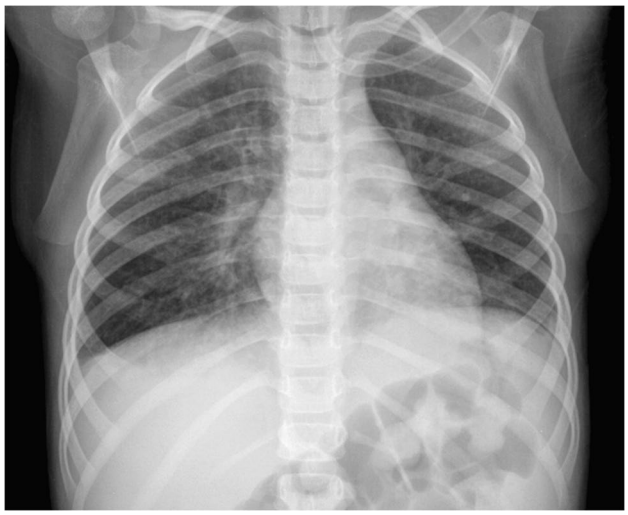

Fig. 1 Chest x-ray of the patient on day 5 from admission to hospital with influenza A pneumonia, showing retro-cardiac infiltrates, diffuse reinforcement of the bronchial tree shadows, and bilateral blunting of the costophrenic angles

serology remains negative. Since she had suffered from VZV as a child, shingles is a more likely cause of the rash. On referral at age 16 years, her blood workup showed low IgM and elevated naïve T cells (85%, reference 35–70). Whole-exome sequencing showed that the patient is heterozygous for a known loss of function variant in *TLR3* (c.1660C > T, P554S) (supplementary Fig. S1; variants of unknown significance are presented in Supplementary Table S2). The variant is also present in heterozygous form in the father, who reportedly never suffered from HSV infections (supplementary Fig. S2).

In the present patient with severe flu, we demonstrated a known pathogenic variant in *TLR3*, P554S, with a dominant negative effect on TLR3 function (1). P554S has a mean allele frequency (MAF) of 0.07% in the European population, where it is only present in heterozygous form, and was previously demonstrated in several patients with HSE [1]. Additionally, the same variant was identified in an adult with coxsackievirus myocarditis. Recently, Lim et al. described three unrelated children with influenza-related acute respiratory distress syndrome carrying the heterozygous loss of function *TLR3* mutations P554S (dominant negative effect, two patients) and P680L (haploinsufficiency, one patient) [3]. The authors show that although TLR3 responses are redundant in leukocytes, patient's fibroblasts and iPSC-derived pulmonary epithelial cells are more susceptible to influenza A infection in vitro, an effect that is rescued by pre-treatment with IFN-α2b or IFN-λ1 [3]. This is in line with the recent description of the role of TLR3 as a rheostat in controlling constitutive IFN-β production in dermal fibroblasts and cortical neurons [5]. Thus, TLR3 plays a non-redundant role in restricting viral replication in early phases of the infection by controlling early IFN-β production, at least in these cells. Interestingly, heterozygous loss of function variants in *TLR3* and other genetic defects in the type I IFN production, response, and amplification pathways have been identified in previously healthy patients with severe COVID-19 pneumonia. These patients and the patients reported by Lim et al. did not suffer from HSE or other severe viral infections, despite positive HSV serology in at least one patient, nor did our patient and her father, who is asymptomatic. Genetic susceptibility to HSE and influenza pneumonia is thus allelic at the *TLR3* locus, with incomplete penetrance for both phenotypes.

In conclusion, we here report the fourth patient with severe influenza pneumonia and a pathogenic variant in *TLR3*. This stresses again the need to investigate children presenting with life-threatening infections for inborn errors of immunity.

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Author Contribution G.B. and I.M. were the principal investigators and drafted the manuscript; A.C. and L.M. performed diagnostic tests; L.D., G.B., and I.M. were involved in the clinical care. All authors revised and corrected the manuscript.

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Data Availability Not applicable.

Code Availability Not applicable.

Declarations

Ethics Approval This study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments and was approved by the Ethics Committee for Research of Leuven University Hospitals (project number S58466).

Consent to Participate The subject has given consent to participate.

Consent for Publication The subject has given consent for findings based on her samples and history to be published.

Conflict of Interest IM holds the CSL Behring Chair in Primary Immunodeficiency in Children, paid to the institution.

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