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Interstitial lung disease caused by STING-associated vasculopathy with onset in infancy (SAVI)

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A full term, male patient was born in the UK to parents of Hazars ethnic background, weighing 3.5kg (50th percentile) and regaining his birth weight by day 12. There was no significant family history and he had two healthy siblings. He presented to hospital at five weeks of age with fever, respiratory distress, tachypnea, and CRP 59 (reference range, <5mg/L). Over the next six months he had a persistent cough and three further admissions. Chest radiographs showed persistent perihilar changes; he received multiple courses of antibiotics although tests for bacterial and viral infections were consistently negative.

He was referred for specialist assessment at seven months of age with ongoing respiratory symptoms, intermittent fever and failure to thrive (weight 5.59kg, 0.4th percentile). He was tachypneic with moderate respiratory distress, but normal oxygen saturations in room air. He had clusters of raised maculopapular, erythematous lesions on his neck, back, arm and legs first noted at five weeks of age (*Figure 1A*). Some of the features of this case have been previously reported in the form of an abstract (Pellowe 2015).

Initial investigations found CRP 10mg/L, microcytic anemia with mean cell volume 66.5fL (reference range, 68-84fL) and hemoglobin 100g/L (reference range, 111-141g/L). White blood cell count, renal function, liver function, thyroid function and sweat chloride were normal. Chest radiograph showed increased interstitial and peri-bronchial markings throughout both lungs. Chest computed tomography (CT) showed features of interstitial lung disease (ILD, *Figure 1B*).

Immunological testing showed raised IgG (13.8g/L, reference range, 3.0-9.0g/L) and IgA levels (1.2g/L, reference range, 0.2-0.7g/L), abnormal lymphocyte proliferation, highly positive anti-nuclear antibody (ANA) screen with positive SS-A(Ro) and raised plasma

viscosity (1.77 mPa.s, reference range, 1.50-1.72 mPa.s). Extensive investigations for bacteria, viruses and fungi were performed on peripheral blood, skin biopsy and bronchoalveolar lavage samples; all were negative.

A lung biopsy showed active inflammation, with type II pneumocyte hyperplasia, epithelial hyperplasia of the bronchioles and surrounding lymphocytic infiltrate (*Figure 1C*). Ultrastructure examination with electron microscopy showed endothelial tuboreticular inclusions which are suggestive of excess type I interferon, either due due to exogenous treatment or endogenous overproduction (*Figure 1D*).

Given the constellation of ILD, systemic inflammation and persistent rash since five weeks of age, a diagnosis of STING-associated vasculopathy with onset in infancy (SAVI) was considered. Genetic analysis by Sanger sequencing confirmed a heterozygous somatic mutation (c.463G>A, p.V155M) in exon 5 of the *TMEM173* (NM_198282.3), the gene encoding STING. Genetic testing of the parents showed that the mutation had occurred *de novo*. Whole blood gene expression studies demonstrated a strong interferon signature, with interferon scores similar to other SAVI patients (*Figure 2*).

Treatment with three days of intravenous methylprednisolone (10mg/kg) yielded no clinical improvement. He gained weight with supplemental nasogastric feeding, but had persistent tachynpea, and subsequently became hypoxemic requiring low flow oxygen therapy. He then commenced monthly intravenous immunoglobulin (IVIg) at 2gms/kg; after four months of treatment there has been some clinical improvement, with reduced tachypnea and a stable oxygen requirement.

The clinical syndrome of SAVI is characterized by early onset (<8 weeks of age) cutaneous vasculitis, fevers, ILD and systemic inflammation. On laboratory testing, common features include positive autoantibodies (particularly a persistently raised ANA), and raised IgG and IgA (Liu 2014); all features observed in our patient. Our patient developed a more central rather than peripheral (ears, nose, digits) rash exacerbated by cold exposure more commonly seen in SAVI patients (Munoz 2015).

Of the previously described cases, 11 out of 12 patients had clinically apparent lung disease (Liu 2014, Jeremiah 2015, Munoz 2015, Omoyinmi 2015). Importantly, three of these patients have died in adolescence from pulmonary complications (Liu 2014, Omoyinmi 2015). Lung toxicity from exogenous type I interferon treatment has been reported in patients with multiple sclerosis (Petousi 2012, Chakravarty 2012) and chronic hepatitis C virus infection (Slavenburg 2010). Despite the documented link between chronic type I interferon exposure and lung pathology, it is perplexing that SAVI is currently the only known type I interferonopathy where lung involvement is a common and major clinical feature.

Autosomal dominant, gain-of-function mutations in the *TMEM173* gene underlie the pathogenesis of SAVI. *TMEM173* encodes STING protein, an adaptor molecule linking sensing of foreign (viral and bacterial) DNA to the production of type I interferons as part of the innate immune response. These gain-of-function mutations lead to constitutive activation of STING and upregulated type I interferon production. STING is expressed in alveolar macrophages, bronchial epithelium and alveolar type II pneumocytes (Liu 2014) which presumably explains the specific lung pathology seen in SAVI.

In addition, *in vitro* studies show STING also acts directly on endothelial cells, causing inflammation, and initiating the coagulation cascade. Thus, these *TMEM173* mutations are postulated to mediate chronic vessel endothelium inflammation leading to the vasculitic rash and vaso-occlusive processes seen in SAVI (Liu 2014).

The predominance of early and significant respiratory symptoms, lack of characteristic peripheral rash in this patient and the variable phenotype described in family members with the same, inherited *TMEM173* mutation (Jeremiah 2014) illustrates the variable phenotype of SAVI patients; further research into the genotype-phenotype correlation and prognosis is warranted.

Treatment options in SAVI remain limited. Liu *et al* reported a lack of response to glucocorticoids, disease-modifying anti-rheumatic drugs and biological therapies. Isolated improvement in ILD has been documented in one patient following pulsed methylprednisolone and mycophenolate mofetil (Munoz 2015). Given the pathogenesis in SAVI, there is potential benefit from Janus kinase (JAK) inhibitors to block type I interferon signaling despite constitutively activated STING. The JAK inhibitor baricitinib has shown benefit in adult patients with a range of inflammatory conditions but there is limited paediatric data. An approved clinical protocol to assess the therapeutic benefit of baricitinib in patients with either SAVI or chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperatures (CANDLE) interferonopathy is ongoing, and has recently published encouraging results in patients with CANDLE (ClinicalTrials.gov number, NCT01724580, Monteleagre 2015).

To conclude, a diagnosis of SAVI has significant implications for patients and families, not only from the high mortality risk, but also the significant morbidity. The respiratory component of the disease may predominate and pulmonary complications have been the cause of death in previous reports. In the context of early age of onset, ILD, failure to thrive, fevers and rash, we urge respiratory pediatricians to consider SAVI as a differential diagnosis and to send testing for *TMEM173* mutations and interferon gene signature to confirm the diagnosis.

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Figure legends

Figure 1: (A) Raised, papular, erythematous rash on the trunk. (B) Chest CT scan showing widespread, slightly nodular interstitial opacification in both lungs. There are a number of small (<1cm) peripheral cysts within the right lower and middle lobes. (C) Light microscopy microphotograph of lung biopsy (low magnification, hematoxylin and eosin stain), showing solid area composed of mixed infiltrate (left arrow) and chronic inflammation (right arrow). (D) Electron microscopy photograph of lung endothelium, showing tuboreticular inclusions (arrow).

Figure 2: Gene expression of selected interferon response genes (IRGs) were determined by Nanostring (NanoString Technologies, Seattle, WA) and an IFN-score was calculated for healthy controls, patients with varying autoinflammatory conditions and our patient (patient O6226). Standardized IFN score (y axis) is the sum of 6 Nanostring counts that were standardized by subtracting the mean of healthy controls and dividing by standard deviation of the healthy controls. IRGs assessed in this study were: *IFI27, IFI44, IFI44L, ISG15, RSAD2 and USP18*. Mean and standard deviation of the IFN score is depicted in parenthesis for each group of individuals. NOMID, neonatal onset multisystem inflammatory disease (mediated by excess IL-1); CANDLE, chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature syndrome (mediated by excess IFN); SAVI, STING-associated vasculopathy with onset in infancy (mediated by excess IFN).