Novel NLRC4 Mutation Causes a Syndrome of Perinatal Autoinflammation With Hemophagocytic Lymphohistiocytosis, Hepatosplenomegaly, Fetal Thrombotic Vasculopathy, and Congenital Anemia and Ascites

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Abstract
Autoinflammatory diseases are caused by pathologic activation of the innate immune system. Primary hemophagocytic lymphohistiocytosis (HLH) is an aggressive syndrome of excessive immune activation caused by monogenic mutations resulting in cytotoxic cell defects and subsequent failure to eliminate activated macrophages. Secondary HLH is often diagnosed in cases without a known Mendelian inheritance. However, some cases of “secondary” HLH have been shown to harbor mutations with partial dysfunction of the cytotoxic system. Recently, macrophage intrinsic abnormalities caused by NLRC4 inflammasome mutations have been linked to autoinflammation and recurrent macrophage activation syndromes resembling a primary HLH. We report a case of a former 28-week preterm infant with congenital anemia, ascites, and a heavy edematous placenta with fetal thrombotic vasculopathy, who developed hepatosplenomegaly and unexplained systemic inflammation with laboratory features of HLH in the early postnatal course and died at 2 months of age. Postmortem examination confirmed the hepatosplenomegaly with marked sinusoidal hemophagocytosis, along with striking hemophagocytosis in the bone marrow and lymph nodes. There was extensive acute and chronic ischemic bowel disease with matted bowel loops, fibrous adhesions, and patchy necrotizing enterocolitis features. Whole exome sequencing analysis demonstrated a novel mosaic heterozygous NLRC4 512 C>T (p.Ser171Phe) de novo mutation predicted to cause a dominant, gain-of-function mutation resulting in a constitutively active protein. The assembly of NLRC4-containing inflammasomes via an induced self-propagation mechanism likely enables a perpetuating process of systemic macrophage activation, presumed to be initiated in utero in this patient.

Keywords
autoinflammation, hemophagocytic lymphohistiocytosis (HLH), fetal thrombotic vasculopathy, NLRC4, inflammasome, congenital anemia, congenital ascites, prematurity, hepatosplenomegaly, macrophage activation
Introduction

A subset of autoinflammatory diseases, including hemophagocytic lymphohistiocytosis (HLH), are characterized by hyperactivation of the innate immune system without apparent provocation, lacking involvement of either autoantibodies or autoreactive T cells. Our understanding of pathogenesis in autoinflammatory diseases has been expanded by the discoveries of causative mutations underlying several monogenic autoinflammatory diseases, including those associated with the inflammasome. Among them, dominant mutations in the NOD-like receptor (NLR) genes, NLRP3 and NLRP12, have been linked to cryopyrin-associated periodic syndromes including familial cold autoinflammatory syndrome (FCAS) and Muckle–Wells syndrome.4,5 Dominant missense mutations of a third NLR gene, NLRC4 (also known as IPAF or CARD12), have recently been found in patients with FCAS (p.His443Pro),6 autoinflammation with recurrent macrophage activation syndrome (MAS) (p.Trim37Ser),7 and a syndrome of autoinflammation with enterocolitis (p.Val341Ala).8 In normal individuals, microbial ligands activate NLR proteins including NLRP3 and NLRC4 in macrophages, resulting in the assembly of inflammasomes which are large protein complexes capable of converting precursor IL-1β and IL-18 to their active forms. The reported activating NLRC4 mutations are believed to cause ligand-independent, spontaneous inflammasome assembly and subsequent excessive production of pro-inflammatory cytokines IL-1β and IL-18, leading to fever, inflammatory cell death, and tissue damage, with variable clinical presentations in different patients.6–8

HLH refers to a life-threatening syndrome in which an intense, uncontrolled immune response, triggered in most cases by infectious agents, leads to severe hyperinflammation.9 HLH is divided into primary (or familiar) and secondary (or reactive) HLH, both of which are characterized by fever, hepatosplenomegaly, cytopenias, hypertriglyceridemia, hypofibrinogenemia, hemophagocytosis in reticuloendothelial organs, and low natural killer-cell activity.10 Primary HLH exhibits Mendelian inheritance and is associated with mutations resulting in lymphocyte cytotoxic defects.9–11 Recently, an expansion of this category has been proposed to include Mendelian inherited conditions related to abnormal activation of inflammasome.12 These include the autoinflammatory diseases with NLRC4 mutations which have been described as syndromes related to macrophage activation without cytotoxic defects or other immunodeficiencies with HLH-like features.7,8 On the other hand, secondary or reactive HLH is diagnosed when a similar set of clinicopathologic criteria are met in a patient without a known Mendelian genetic defect. However, the category of “secondary” HLH is gradually shrinking as more genetic alterations are found in this group.13 In both primary and secondary HLH, failure to remove activated macrophages leads to cytokine storm with extremely high levels of interferon γ, tumor necrosis factor α, IL-6, IL-8, IL-12, and soluble IL-2 receptor (CD25).

Prenatal/antenatal hyperinflammatory presentations are rare and thus few studies have shown correlation with placental findings. Our case showed evidence of placental fetal thrombotic vasculopathy (FTV), which is a chronic vaso-occlusive disorder characterized by thromboses (occlusive and/or non-occlusive) of the chorionic plate or stem villous vessels and death of the downstream villous parenchyma.14,15 Fetal FTV is associated with high rates of obstetric and perinatal complications such as fetal growth restriction, infarctions in fetal organs, and fetal neurologic complications.16 FTV can develop in the context of vascular endothelial damage (eg, as seen with the fetal inflammatory response to infected amniotic fluid) or vascular stasis (eg, as seen with chronic or intermittent umbilical cord obstruction). These predisposing factors are absent from many if not most placentas, in which case, the etiology is rarely determined. A relation to maternal thrombophilias has been postulated, but this hypothesis remains controversial.

There is well-documented evidence linking inflammation and thrombosis.17,18 These data support the hypothesis that a hyper-inflammatory state can incite or drive local tissue thrombosis, with the thrombosis then amplifying inflammation through major cell types including macrophages, platelets, vascular endothelium, and smooth muscle.18 Interestingly, the father of a described index case8 with a NLRC4 activating mutation had a reported systemic inflammatory episode with pancytopenia and disseminated intravascular coagulation. To our knowledge, placental FTV has not previously been reported in NLRC4 related cases.

Here, we describe a former 28-week preterm infant with autoinflammation and features of HLH, first met on day of life 11 (Table 1) associated with a novel NLRC4 gain of function mutation (Table 2). The clinical presentation is significant for congenital anemia and ascites status post subsequent intrauterine transfusions and paracentesis, FTV of the heavy, edematous placenta, subsequent acute and chronic enteropathy and enterocolitis postnatally, and an atypical clinical course with diagnostic laboratory features of HLH (Table 1).

Case Report

A 30-year-old G2P1 woman was found by prenatal ultrasound to have a singleton fetus with echogenic bowel at 16–18 weeks. On follow-up ultrasound, the fetus was noted to have severe fetal anemia by middle cerebral artery Doppler assessment, severe ascites, and placentomegaly...
at 26–28 weeks. Amniocentesis showed normal chromosome analysis. At 27 weeks, cordocentesis was performed with transfusion of 70 mL of red blood cells and 10 mL of platelets, and paracentesis removed 320 mL of ascites. Of note, the mother was found to have enlarged bilateral ovaries and elevated beta hCG levels. Maternal serologies were negative for infection. The mother was parvovirus IgG positive and IgM negative, indicating prior exposure but no active infection. A cystic fibrosis screen was negative. At 28 weeks’ gestation, the fetus was delivered via emergent cesarean section for fetal distress and rapid reaccumulation of ascites. The mother’s enlarged bilateral ovaries were consistent with theca-lutein cysts (up to 18 cm in diameter) and her beta hCG levels normalized appropriately after delivery.

The neonate was appropriately grown at 1426 g. The placental examination was significant for a heavy edematous placenta which weighed 476 g, above the 90th percentile for gestational age. Histologic examination showed thromboses, both non-occlusive and occlusive, involving multiple fetal chorionic plate and stem villous vessels (Figure 1(c)). Multiple foci of avascular villi and villous stromal vascular karyorrhexis were noted (Figure 1(d)). These findings were diagnostic of FTV. At birth, the neonate had no respiratory effort, requiring immediate intubation and 1 dose of surfactant. Abdominal distention and ascites were noted. An abdominal x-ray showed no definite free air or abnormal calcifications. Paracentesis removed 200 ml of serosanguineous ascites. The neonate received multiple transfusions

### Table 1. Relevant Clinical, Laboratory, and Histopathological Findings in the Current Case Regarding the HLH-2004 Diagnostic Criteria.

<table>
<thead>
<tr>
<th>Diagnostic Criteria for HLH (5 out of 8 criteria needed)</th>
<th>Relevant Findings in the Index Case</th>
<th>When Identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>Yes</td>
<td>DOL 10</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>Yes</td>
<td>Birth</td>
</tr>
<tr>
<td>Cytopenias (affecting ≥ 2 of 3 lineages in the peripheral blood):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin &lt; 90 g/L (in infants &lt; 4 weeks: hemoglobin &lt; 100 g/L)</td>
<td>Yes (intrauterine transfusion required)</td>
<td>In Utero</td>
</tr>
<tr>
<td>Platelets &lt; 100 x 10^9/L</td>
<td>Yes (intrauterine transfusion required)</td>
<td>In Utero</td>
</tr>
<tr>
<td>Neutrophils &lt; 1.0 x 10^9/L</td>
<td>Yes</td>
<td>DOL 14</td>
</tr>
<tr>
<td>Hypertriglyceridemia and/or hypofibrinogenemia:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting triglycerides ≥ 265 mg/dL</td>
<td>Yes</td>
<td>DOL 38</td>
</tr>
<tr>
<td>Fibrinogen ≤ 1.5 g/L</td>
<td>No</td>
<td>N/A</td>
</tr>
<tr>
<td>Hemophagocytosis in bone marrow or spleen or lymph nodes</td>
<td>Yes (hemophagocytosis in all 3 organs)</td>
<td>Autopsy</td>
</tr>
<tr>
<td>Low or absent NK-cell activity</td>
<td>Borderline</td>
<td>DOL 11</td>
</tr>
<tr>
<td>Ferritin ≥ 500 µg/L</td>
<td>Yes</td>
<td>DOL 2</td>
</tr>
<tr>
<td>Soluble CD25 ≥ 2400 U/mL</td>
<td>Yes</td>
<td>DOL 11</td>
</tr>
</tbody>
</table>

DOL, day of life; HLH, hemophagocytic lymphohistiocytosis; NA, Clinical information not available; NK, natural killer.

### Table 2. Summary of Reported Cases With NLRC4 Mutations.

<table>
<thead>
<tr>
<th>Nucleotide</th>
<th>Amino Acid</th>
<th>Protein Domain</th>
<th>Zygosity</th>
<th>Predicted Mutant Type</th>
<th>Phenotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.1009A &gt; T</td>
<td>p.Thr337Ser</td>
<td>HD1</td>
<td>Het</td>
<td>Gain-of-function</td>
<td>Autoinflammation with recurrent MAS-HLH in a child of European ancestry</td>
<td>Canna SW et al., 2014</td>
</tr>
<tr>
<td>c.1022T &gt; C</td>
<td>p.Val341Ala</td>
<td>HD1</td>
<td>Het</td>
<td>Gain-of-function</td>
<td>Autoinflammation with enterocolitis in a USA family</td>
<td>Romberg N et al., 2014</td>
</tr>
<tr>
<td>c.512C &gt; T</td>
<td>p.Ser171Phe</td>
<td>NBD</td>
<td>Het</td>
<td>Gain-of-function</td>
<td>Perinatal autoinflammation with MAS-HLH and FTV</td>
<td>Current case</td>
</tr>
</tbody>
</table>

FCAS, familial cold autoinflammatory syndrome; FTV, fetal thrombotic vasculopathy; HD1, helical domain 1; Het, heterozygous; MAS-HLH, macrophage activation syndrome—hemophagocytic lymphohistiocytosis; NBD, nucleotide-binding domain; WHD, winged-helix domain.
for anemia and thrombocytopenia. Total parenteral nutrition was started on day 6 of life. Later, she underwent multiple therapeutic and diagnostic paracenteses which were non-diagnostic with negative cultures.

An evaluation for HLH was pursued, given symptoms of fever, cytopenias, and splenomegaly (Table 1). She was found to have hyperferritinemia (ferritin 1945 ng/ml) on day 2 of life. Soluble interleukin-2 receptor levels were elevated (8525 units/ml, range 334–3026 units/mL) on day 11 of life. However, a bone marrow biopsy with aspirate and CD163 immunostain performed at the time was inconclusive for hemophagocytosis given the limited biopsy specimen. Natural killer cell function was borderline. She also had hepatomegaly with a conjugated hyperbilirubinemia (peak total bilirubin 15 mg/dL, direct-bilirubin 8.3 mg/dL on day 6 of life).

An exhaustive genetic-metabolic workup excluded storage disorders including Niemann-Pick type A, B, and C, Sialidosis, Wolman disease, and amino acid metabolic disorders associated with HLH. Infectious etiologies were excluded including viral, bacterial, and fungal infections. A liver biopsy at 8 weeks showed cholestasis with mild cholangiolar proliferation along with periporal hepatocyte ballooning and focal vacuolization. Electron microscopy showed nonspecific findings of previous hepatic injury.

She remained intubated for respiratory distress syndrome and continued to receive multiple transfusions for her persistent anemia and thrombocytopenia. On day of life 60, she acutely decompensated with severe acidosis, hyperkalemia, and respiratory failure. Resuscitation attempts were unsuccessful and she expired.

Postmortem examination was significant for a protuberant abdomen with diffusely matted bowel loops (Figure 1(a)). Significant mesenteric/serosal fibrous adhesions were present, encasing most of the small bowel and portions of the ovaries and fallopian tubes, with fibrous extension around the gallbladder fossa and extrahepatic biliary tree (Figure 1(b)). Numerous hemosiderin-laden macrophages were seen in the bowel wall and mesentery, indicating blood breakthrough. These findings suggest chronic bowel injury with resultant mesenteric/peritoneal irritation. Acute mucosal injury was also present focally, showing a patchy necrotizing enterocolitis (NEC)-like pattern with acute mucosal necrosis and granulation tissue formation. The latter seemingly narrowed and focally occluded parts of the bowel lumen. Thromboses of the small bowel submucosal vessels along with mixed inflammatory infiltrates, fibrinous necrosis, and fibrosis were noted (Figure 1(c)).
The liver showed hepatomegaly (290 g, age expected 96 g) with a small gallbladder. There was perihilar fibrosis with pigment laden macrophages also present. The liver did not show classic features of a primary HLH involvement, as described by Chen et al., but there were enlarged sinusoidal macrophages with hemophagocytosis and increased portal macrophages. There was striking ductular proliferation with canalicular and hepatocellular cholestasis, portal fibrosis, and early bridging fibrosis. Extramedullary hematopoiesis was also evident.

The pancreas showed striking interlobular fibrosis. The main pancreatic ducts were dilated with inspissated mucin. There were normal numbers of islet cells and no evidence of inspissated mucin in the distal ducts or acini.

The spleen was enlarged (56 g, aged expected 8 g) with sinusoidal hemophagocytosis and red blood cell congestion. Histologic examination also demonstrated erythro and hemophagocytosis involving the bone marrow and lymph nodes.

The heart showed mildly increased right ventricular wall thickness (0.3 cm) and mildly enlarged right ventricular outflow tract. A small non-occlusive right atrial wall thrombus (0.4 × 0.3 cm) with focal myocyte infarction and dystrophic calcifications were present, likely related to previous central line placement.

The lungs showed bilateral necrotizing bronchopneumonia with focal hemorrhagic nodularity. Postmortem lung cultures were positive for Enterobacter cloacae and Staphylococcus aureus, while postmortem aerobic and anaerobic blood cultures were positive for Enterococcus. Developmentally, the lungs showed mild alveolar maturation delay.

The brain (183.5 g) was grossly normal but pontosubicular necrosis without evidence of chronic ischemic injury was noted. There was no evidence of hemophagocytosis.

Whole-exome sequencing was sent on the patient’s blood premortem and identified a mosaic heterozygous mutation in the NLRC4 gene 512C>T, resulting in a p.Ser171Phe substitution in a highly conserved region of the NLRC4 nucleotide-binding domain (NBD) (Figure 2(a)). Both wildtype and variant alleles were confirmed by Sanger sequencing. Parental DNA testing (eg, saliva) was analyzed by Sanger sequencing, and both parents were negative for the identified mutation, indicating our patient had a de novo NLRC4 mutation. Protein conformational analysis based on the published crystal and cryo-electron microscopy structures of the NLRC4 protein indicated that this mutation substitutes a bulky, hydrophobic phenylalanine for a small, hydrophilic serine residue at the Adenosine diphosphate (ADP)-binding
interface within the conserved NBD region (Figure 2(b), 2(c)). The inactive state of NLRC4 depends on an auto-inhibitory mechanism requiring stable interaction between its NBD and the winged-helix domain (WHD) mediated by ADP binding and stabilized by the helical domain 1 (HD1). The steric effects created by this mutation might severely destabilize the NBD–WHD interaction, leading to the adaptation of an active, opened conformation in the absence of ligand binding.

Discussion
Autoinflammatory/hyperinflammatory diseases have gradually segregated from autoimmune diseases and have emerged as a distinct diagnostic group. We are just beginning to understand the underlying molecular and genetic mechanisms. Newly discovered mutations in members of the NLR gene family including NLRP3 mutations in cryopyrin-associated periodic syndromes (OMIM*606416)24 and NLRC4 in autoinflammation (OMIM*606831)25 have expanded the list of genes responsible for autoinflammatory diseases and possibly also expanding the spectrum of HLH. To date, 3 distinct NLRC4 mutations have been reported (Table 2).6–8 Functional data have shown that gain of function mutations lead to elevated levels of IL-18, similar to other pathologic hyper-inflammatory conditions.7

In this case, there was a mosaic heterozygous mutation which indicates the presence of genotypically distinct cell lines in the same individual where a proportion of cells carry a single mutation (heterozygous, in this case with a dominant effect) while other cells do not carry the change in the DNA and are normal; however, both cell lines are derived from a single zygote. In this case, the NLRC4 mosaic ratio was 74 normal references reads and 25 abnormal variant reads. Different mosaic ratio may be found in distinct organs and tissues based on the proportion of cells carrying a mutation. In this case, mutation analysis was performed on the blood of the proband, while saliva only was submitted on the parents. Therefore, it is not clear what the mosaic ratios are in different organs and tissues.

A mosaic mutation could have been missed by standard Sanger sequencing. Next generation sequencing and whole exome sequencing are better tools in these case for detecting somatic mosaicism of variable degrees, even as low as 5% in some cases. Low-grade mosaicism may still be associated with severe disease, as in this case. This information should be provided during genetic counseling. There is evidence that low-grade somatic mosaicism, with as few as 5% of hematopoietic cells carrying the NLRP3 mutation, resulted in a severe neonatal onset multisystem disorder phenotype.26 It is speculated that activated macrophages carrying an NLRP3 mutation could still release inflammasome particles, while retaining extracellular enzymatic activity, such that when phagocytozed by neighboring macrophages, inflammasome activity is transferred to unstimulated/wild-type cells.27,28

Interestingly, all previously reported NLRC4 mutations6–8 are heterozygous, missense mutations and are mapped to the critical amino acid residues located at the ADP-bound NBD-WHD-HD1 interaction interface. Specifically, the p.Thr337Ser mutation in HD1 reported to present as autoinflammation and “MAS-HLH” in 1 patient was predicted to destabilize HD1 interaction with NBD residues 170 and/or 173.7 In comparison, the mutation found in our case, p.Ser171Phe, is mapped to the immediately adjacent NBD residue 171. The p.Val341Ala mutation found in kindred, affecting 3 individuals with a syndrome of enterocolitis and autoinflammation also maps to the HD1 with similar predicted destabilizing effect.8 The p.His443Pro mutation affecting 13 individuals in a Japanese non-consanguineous family with FCAS maps to the WHD, where the His443 residue appears to hydrogen bond with the β-phosphate group of the ADP molecule.6 In vitro study has demonstrated that disruption of the His443-ADP interaction facilitates conformational changes in the WHD and weakens ADP binding, therefore promoting NLRC4 activation.20

Recently solved macromolecular structure of the NLRC4 inflammasome lends further support that a small number of NLRC4 proteins with active conformation rendered by the activating mutations appear to be sufficient to assemble fully functional inflammasomes via a nucleated polymerization process where activated NLRC4 catalyzes the activation of inactive protein.22,23

The clinical presentations vary considerably among the reported patients with NLRC4 mutations, even in those of the same kindred with identical mutations. For example, a 43-year-old father who carried the p.Val341Ala mutation had a more variable course including extended hospitalization for fever and diarrhea during infancy, and his gastrointestinal symptoms resolved by 1 year of age. During adulthood, he had erythematous skin plaques, joint pains with fevers, and one late episode of acute respiratory distress syndrome with subarachnoid hemorrhage, hematochezia, and features of HLH. His newborn son who carried the same NLRC4 mutation, however, presented at 1 week of life with fever, secretory diarrhea, and features of HLH, and died on day 23 from diffuse alveolar hemorrhage.8 Notably, while patients with NLRC4 mutations (including our case) presented with findings consistent with HLH-like features with macrophage activation, the p.His443Pro mutation affecting the His443-ADP interaction appears to consistently manifest as FCAS with infantile onset, self-limiting episodes of fever and rash frequently induced by exposure to cold temperature. Our case presented with evidence of intrauterine disease (anemia, ascites, and placentomegaly at 26–28 gestational weeks with FTV), likely representing an
extreme form within the spectrum of autoinflammation and HLH-like symptoms. It is plausible that hyperinflammation may have initiated or accelerated the development of the placental FTV. In this case, FTV may have resulted in a chronic fetal hypoxic state in utero in which the mesenteric organs may have been preferentially targeted from redistribution of blood flow to the vital organs (eg, heart and brain). Bleeding into the peritoneal cavity and bowel lumen from an in utero vascular/ischemic-type bowel insult may have incited both the anemia and isolated ascites. Interestingly, NLRC4 has been recently identified as driver of sterile inflammatory responses in the brain with NLRC4-dependent mechanisms contributing to ischemic brain injury. While the degree of pontosubicular necrosis seen in this case is not unexpected for an infant in this clinical setting, it is possible that the NLRC4 gain of function mutation may have affected the development or severity of cerebral and possible systemic ischemic injury.

Most of the clinical and pathological findings in our patient can be attributed to the underlying chronic hyperinflammatory state that appeared to have initiated in utero, without apparent maternal or fetal triggers. The finding of severe ascites and anemia at 26–28 weeks and diffusely matted bowel loops at autopsy with patchy areas of NEC-like changes suggested severe, predominantly chronic inflammation of the bowel with ischemic change. Various degrees of inflammation involving the intestines were also a major feature associated with 2 of the 3 reported NLRC4 mutations, with neonatal or infantile onset. Further, genotype-phenotype correlation of the diseases caused by NLRC4 mutations requires accumulation of more studies of NLRC4 patients with detailed clinical and molecular characterization.

Of note, assessment of mucosal inflammation in our patient was hampered by the marked autolysis at autopsy. Translocation of bacteria through the necrotic bowel wall near the end of life likely contributed to the focal areas of active NEC and bacteremia present only in postmortem cultures. The chronic inflammation and fibrotic changes in other organs including the liver and pancreas are likely also related to the underlying chronic inflammatory process. Placentomegaly with FTV has not been previously described in association with fetal/neonatal autoinflammatory disease or HLH to our knowledge. Interestingly, it has been shown that activation of NLRP3 inflammasomes in platelets (which express receptors for IL-1β) and subsequent release IL-1β may play a key role linking coagulation to the inflammatory response. Therefore, the placental FTV and microthrombi in other organs seen in our patient may possibly be related to the increased IL-1β signaling activities and a hyperinflammatory state.

As discussed above, one key pathologic outcome in both NLRC4 and NLRP3 autoinflammatory diseases is the overproduction of IL-1β and/or IL-18 by the inflammasomes in macrophages, driving excessive systemic inflammation. The proven success of IL-1β blockade therapy in NLRP3 diseases has prompted a trial in one of the reported NLRC4 cases, which also appeared to be effective. The other reported NLRC4 cases were not treated with IL-1β targeted drugs either because of a mild, self-limiting course, it was not suggested, or there was lack of patient consent. The diagnosis of NLRC4 autoinflammatory disease in our case was not made ante-mortem. This patient had a highly unusual clinical presentation and there is a paucity of data on the impact of this genetic alteration, with only 3 reports published to date. Retrospectively, measurements of serum cytokine levels including IL-1β and IL-18 would likely be informative and help guide the differential diagnosis and formulation of therapy strategies, although the normal laboratory ranges of IL-1β and IL-18 levels in perinatal period are not yet established. Collectively, there is much to be learned regarding the nature of autoinflammatory diseases such as NLRC4 mutations and potential overlap in clinical features with HLH. The diagnosis of a perinatal case is extremely challenging, requiring a high degree of suspicion when an unexplained hyperinflammatory state is encountered, and requiring the institution of early molecular testing and an interdisciplinary collaborative effort to interpret novel mutations in the context of the clinical presentation.

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