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Severe autoimmune lymphoproliferative syndrome phenotype in a pediatric patient with a germline FAS gene variant

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Abbreviation	Full term
ALPS	Autoimmune lymphoproliferative syndrome
ANC	Absolute neutrophil count
DNT	Double-negative T-cell
G-CSF	G-Colony stimulating factor
MMF	Mycophenolate mofetil

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Introduction:

Autoimmune lymphoproliferative syndrome (ALPS) is caused by a disruption of the apoptotic pathways that down-regulate lymphocyte activity.[1-2] Patients present with lymphadenopathy, splenomegaly, autoimmunity, and characteristic laboratory markers including CD3 positive, but CD4 and CD8 negative T-cells, known as double-negative T-cells (DNTs).[3] Additional biomarkers include elevated levels of B12, IgG, IL-10, IL-18, and soluble FAS ligand.[4] The severity of disease is widely variable, with symptoms often presenting in childhood and following a relapsing/remitting pattern.[5] While the lymphocyte proliferation is typically polyclonal and benign, patients with ALPS are also at increased risk of developing lymphoma, with incidence of Hodgkin lymphoma over 100 times greater than in the general population.[6]

Many genetic mutations have been identified in patients with ALPS, most commonly affecting the FAS receptor, its ligand, or caspases 8 and 10, which are downstream in the apoptosis pathway.[7-8] Of note, many healthy family members of patients with ALPS have been found to have similar mutations, suggesting variable penetrance or the need for a "two-hit" process to induce clinical symptoms.[6] We present a patient with clinical features of ALPS and evidence of FAS dysfunction who was found to have a genetic variant of unknown significance in the FAS gene. Given our patient's phenotype and laboratory findings, we posit that this mutation is likely pathogenic and causative of ALPS.

Case Presentation:

The patient presented at age 2 with left inguinal lymphadenopathy. The lymph node biopsy showed pericortical expansion with increase in S-100+ histiocytes, although classical emperipolesis was not identified in either hematoxylin and eosin (H and E) or immunostained sections (Figure 1).

He then developed pancytopenia, with hemoglobin 7.9 g/dl, platelet count 2 k/mcl, and absolute neutrophil count (ANC) 80 k/mcl. He required intravenous immune globulins as well as steroids for persistent thrombocytopenia. At age 4, he developed severe hemolytic anemia with hemoglobin of 2.1 g/dl. He had altered mental status, encephalopathy with electroencephalogram changes, and white matter ischemia on MRI, attributed to hypoxia secondary to severe anemia. He was found to have elevated IL-18 and DNTs, leading to a diagnosis of probable ALPS. After a prolonged course of steroids, he started mycophenolate mofetil (MMF) and his blood counts remained stable for several months.

The patient was lost to follow-up for about 6 months and discontinued MMF during this time. When he returned to care, he was found to have hepatosplenomegaly, increased DNTs (7%), and elevated soluble FAS ligand. He subsequently developed thrombocytopenia with mucosal bleeding, requiring intravenous methylprednisolone. After restarting MMF, he developed neutropenic gingivitis, requiring granulocyte-colony stimulating factor therapy. He was then switched from MMF to sirolimus. Blood counts normalized and laboratory testing after 6 months showed normalization of DNTs, B12, and IL-18 levels.

Genetic testing in unsorted blood cells was performed, and he was found to carry a heterozygous variant in FAS, Exon 9, c.857G>A (p.Gly286Glu). This sequence change replaces glycine, which is neutral and nonpolar, with glutamic acid, which is acidic and polar, at codon 286 of the FAS protein (p.Gly286Glu).This variant is not present in population databases. Algorithms developed to predict the effect of this missense

change on protein structure and function were inconclusive (SIFT: "Not Available"; PolyPhen-2: "Possibly Damaging"; Align-GVGD: "Not Available"). A FAS-mediated apoptosis assay revealed significantly reduced apoptosis cell loss at 8% (normal:57-100%).

Discussion:

We present a patient who meets clinical diagnostic criteria for ALPS[9] and has a heterozygous germline FAS variant of previously unknown significance. His clinical phenotype was severe and included lymphadenopathy, recurrent autoimmune cytopenias, elevated levels of DNTs, and defective lymphocyte apoptosis. He also met several secondary accessory criteria, including elevation of soluble FAS ligand, IL-18, and B12 levels, and typical immunohistological findings. While he had severe autoimmune cytopenias, he has never had elevation of IgG. Lymph node biopsy showed S100+ histiocytosis, characteristic of ALPS.[10]

The FAS variant found in our patient is not known to be pathogenic. The variant is on Exon 9 of the FAS receptor gene, which generally encodes the death domain of the receptor.[6,11] This is consistent with the finding of impaired FAS-mediated apoptosis. There has been one prior report of a similar phenotype with this genetic variant,[12] which combined with our patient's presentation, provides evidence that this variant is pathogenic and is associated with severe ALPS-FAS phenotype.

The variation in ALPS phenotypes likely relates to the range of genetic variants associated with the disease. Our patient and the previously reported patient with the same mutation share many features: both presented in toddlerhood with lymphadenopathy followed by recurrent cytopenias.[12] The severity of our patient's cytopenias, with acute, life-threatening drops in hemoglobin and platelets, is remarkable and is consistent with prior work identifying severity and frequency of cytopenias as predictors of defects in FAS-mediated apoptosis. [13] Unlike the case reported by Gu et al., our patient did have short-term response to steroids. Both had good response to sirolimus, with stabilization of blood counts and normalization of DNTs. It is not clear whether severity of cytopenias is predictive of lymphoma risk or whether disease control with sirolimus mitigates this risk. [14] Continued identification of genetic variants and associated disease course is needed to help with future risk stratification.

TABLE 1 ALPS diagnostic findings identified in our patient

Diagnostic Criterion	Finding
Required criteria	
Evidence of Lymphoproliferation	Inguinal lymphadenopathy without infectious cause requiring lymph node resection
Double negative T cells	CD3+ CD4-CD8- T cells elevated to 7%
Primary accessory criteria	
Defective lymphocyte apoptosis	FAS-mediated apoptosis decreased at 8% (normal range 57-100%)
Pathogenic FAS	*FAS mutation identified, although not known to be pathogenic
Secondary accessory criteria	
Elevated biomarkers	Elevated B12, soluble FAS ligand, IL-2, IL-10, and IL-18
Autoimmune cytopenias	Severe anemia (Hemoglobin low of 2.1 g/dL), neutropenia (Absolute neutrophil count lo
Immunohistologic findings	Lymph node biopsy showed histiocytosis, positive for CD68 and S100

Figure Captions:

Figure 1. Lymph node pathology and flow cytometry results

The excision biopsy of inguinal lymph node shows a paracortical expansion with a mixture of small mature lymphocytes and many histiocytes (Panel A shows 20x and B shows 200x; H&E stain), which are highlighted by S100 immunostaining (Panel C, 200x). Panel D displays flow cytometry data showing increased (˜16%) double negative T-cells (CD4-, CD8-).

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