In utero and early postnatal presentation of autoimmune lymphoproliferative syndrome in a family with a novel *FAS* mutation

Autoimmune lymphoproliferative syndrome (ALPS; MIM 601859) is a congenital disease of defective T-cell apoptosis and autoimmunity, most often caused by mutations in the *FAS* gene. The hematologic manifestations of ALPS include chronic lymphadenopathy, splenomegaly, multilineage cytopenias secondary to sequestration and autoimmune destruction, and an increased risk of B-cell lymphoma.[1-3] Onset is typically early in life with a median age of 11.5 months.[4] We report a family with a novel *FAS* mutation, in which the proband presented with onset of ALPS at three weeks of age and her brother *in utero* at 36 weeks gestation. To our knowledge, these are among the earliest documented presentations of ALPS. Based on these cases, we now recommend obstetricians to assess fetal spleen size by third trimester ultrasound in mothers with ALPS or a family history of ALPS, in addition to monitoring both mother and child for autoimmune hemolytic anemia and thrombocytopenia.

The proband, (NIH ALPS Family n. 323.1) was born at 41 weeks gestation. She presented at three weeks of age with pallor, vomiting, lethargy, jaundice, splenomegaly and lymphadenopathy. Prior to diagnosis, she developed worsening lymphadenopathy and massive splenomegaly. Lymph node biopsy showed few germinal centers and expansion of the paracortical zone with mature T cells admixed with plasma cells and immunoblastic cells. Flow cytometry of peripheral blood showed 24% $CD3^+$ T cells were $CD4^-/CD8^-$ (double negative T cells; DNT) with increased alpha/beta population (60%). Fas mediated apoptosis assay showed cell kill of only 1.3% compared to normal control of 71.2%, consistent with ALPS. Given these findings, the clinical presentation and history, a diagnosis of ALPS was made according to the 2010 diagnostic criteria.[5]

Sanger sequencing of the proband's *FAS* gene (GenBank accession number M67454.1) in genomic DNA extracted from peripheral mononuclear blood cells revealed two variants: heterozygous c.761T>G, p.Val254Gly missense mutation and heterozygous c.642C>T single nucleotide polymorphism (SNP, rs2234978, average minor allele frequency T=0.236) (notation based on NM_000043). The c. 642C>T synonymous SNP is considered benign.[6] The missense variant c.761T>G, to our knowledge, has not been reported previously but is located in the FAS death domain at an amino acid position that is biochemically conserved across species. Bioinformatic tools SIFT, SNP3D and PolyPhen predict c.761T>G as deleterious. Family studies revealed that the c.761T>G variant was paternally inherited and the c.642C>T SNP was maternally inherited.

The proband's father was asymptomatic until 31 years of age (about a year after the proband's birth), at which time he developed an unusual maculopapular rash. Skin biopsy showed *pityriasis lichenoides et varioliformis acuta* (PLEVA). The cause of this is unknown but it is considered to be a benign form of a T-cell lymphoproliferative disorder. He had an increased DNT population of 14% but, interestingly, apoptosis testing showed no defect using Annexin V and 7-AAD assays. This is contrary to previous asymptomatic carriers who would normally still show apoptotic evidence of the gene defect.[7-9] The moth-

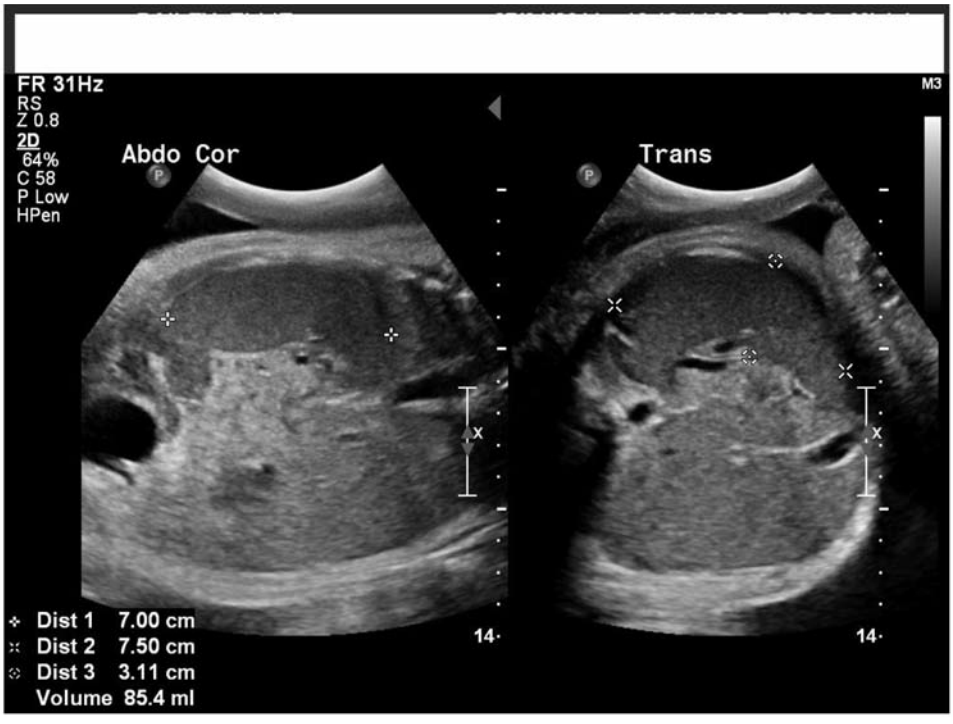

Figure 1. Splenomegaly with span 7 cm extending into the pelvis.

er had normal DNT cell numbers and apoptosis assay.

The proband's brother (NIH Patient Family n. 323.4) was diagnosed *in utero* at 36 weeks gestation when a fetal ultrasound identified hepatosplenomegaly (Figure 1), mild cervical lymphadenopathy, cardiomegaly, polyhydramnios and anemia (based on middle cerebral artery flow). DNT CD3$^+$ population was elevated at 21%. His *FAS* variants were identical to his sibling. Reduced penetrance and variable phenotypic expression are seen in ALPS pedigrees, despite the affected individuals sharing the same *FAS* mutation.[10,11] Recent data suggest that both environmental factors and variants at other loci may be responsible for this.[12] The novel genetic change we describe here lies in the intracellular region of the *FAS* gene, in exon 9, which encodes the death domain of the Fas protein. Mutations affecting the intracellular domain are associated with a higher penetrance than extracellular *FAS* mutations.[8] As the father was not affected until later in life, and the maternally inherited SNP appears to be benign,[6] other factors clearly contributed to the severe phenotype of the proband and her sibling. Conceivably, the affected members of this family could have additional somatic changes to the *FAS* gene, as somatic changes in this gene have been implicated in the progression of ALPS.[9] A cryptic deleterious maternally inherited FAS variant in the promoter or other non-coding region, for example, may be present but not detected by our sequencing. Alternatively, other mutations to proteins in the signaling cascade, such as FAS-ligand or FAS associated signaling proteins procaspase-8/10 could contribute to the family phenotype. These possibilities warrant further genetic analyses, but are beyond the scope of this paper.

In summary, we present a novel death domain mutation in the *FAS* gene associated with *in utero* and early neonatal onset ALPS. It is an important differential to consider in antenatal and neonatal diagnosis of splenomegaly. Significant morbidity and, possibly mortality, may be avoided if ALPS is included in an early differential. Given our experience, third trimester ultrasound is now suggested in mothers with ALPS or a family history of ALPS to assess fetal spleen size. Both mother and child must be monitored for autoimmune hemolytic anemia and thrombocytopenia during pregnancy and the postnatal period.

Jordan R. Hansford,[1] Manika Pal,[1] Nicola Poplawski,[1,2,3] Eric Haan,[2,3] Bernadette Boog,[3,4] Antonio Ferrante,[3,4] Joie Davis,[5] Julie E. Niemela,[6] V. Koneti Rao,[5] and Ram Suppiah[1]

[1]Women's and Children's Hospital, North Adelaide, South Australia, Australia; [2]SA Clinical Genetics Service, SA Pathology, Adelaide, South Australia, Australia; [3]Discipline of Pediatrics, University of Adelaide, Adelaide, South Australia, Australia; [4]Department of Immunopathology, SA Pathology, Women's and Children's Hospital, North Adelaide, South Australia, Australia; [5]ALPS Unit, LCID/NIAID, National Institutes of Health, Bethesda, USA; and [6]Department of Laboratory Medicine, Warren Grant Magnuson Clinical Center, National Institutes of Health, Bethesda, MD, USA

Correspondence: ram.suppiah@health.sa.gov.au
doi:10.3324/haematol.2012.070524

Acknowledgments

The authors would like to thank Dr. Patrick Quinn for his work in helping to diagnose and manage this family. JH and MP contributed equally to this manuscript.

Trial registration

There was no clinical trial involved in this work. Consent was obtained from the family for genetic studies and publication of results.

Funding

No external funding or financial support was provided to carry out this work.

Authorship and Disclosures

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

References

1. Le Deist F, Emile JF, Rieux-Laucat F, Benkerrou M, Roberts I, Brousse N, et al. Clinical, immunological, and pathological consequences of Fas-deficient conditions. Lancet. 1996;348(9029):719-23.
2. Sneller MC, Straus SE, Jaffe ES, Jaffe JS, Fleisher TA, Stetler-Stevenson M, et al. A novel lymphoproliferative/autoimmune syndrome resembling murine lpr/gld disease. J Clin Invest. 1992;90(2):334-41.
3. Sneller MC, Wang J, Dale JK, Strober W, Middelton LA, Choi Y, et al. Clincal, immunologic, and genetic features of an autoimmune lymphoproliferative syndrome associated with abnormal lymphocyte apoptosis. Blood. 1997;89(4):1341-8.
4. Rao VK, Oliveira JB. How I treat autoimmune lymphoproliferative syndrome. Blood. 2011;118(22):5741-51.
5. Oliveira JB, Bleesing JJ, Dianzani U, Fleisher TA, Jaffe ES, Lenardo MJ, et al. Revised diagnostic criteria and classification for the autoimmune lymphoproliferative syndrome (ALPS): report from the 2009 NIH International Workshop. Blood. 2010;116(14):e35-40.
6. Niemela JE, Hsu AP, Fleisher TA, Puck JM. Single nucleotide polymorphisms in the apoptosis receptor gene TNFRSF6. Mol Cell Probes. 2006;20(1):21-6.
7. Bleesing JJ, Brown MR, Straus SE, Dale JK, Siegel RM, Johnson M, et al. Immunophenotypic profiles in families with autoimmune lymphoproliferative syndrome. Blood. 2001;98(8):2466-73.
8. Jackson CE, Fischer RE, Hsu AP, Anderson SM, Choi Y, Wang J, et al. Autoimmune lymphoproliferative syndrome with defective Fas: genotype influences penetrance. Am J Hum Genet. 1999;64(4):1002-14.
9. Magerus-Chatinet A, Neven B, Stolzenberg MC, Daussy C, Arkwright PD, Lanzarotti N, et al. Onset of autoimmune lymphoproliferative syndrome (ALPS) in humans as a consequence of genetic defect accumulation. J Clin Invest. 2011;121(1):106-12.
10. Straus SE, Jaffe ES, Puck JM, Dale JK, Elkon KB, Rosen-Wolff A, et al. The development of lymphomas in families with autoimmune lymphoproliferative syndrome with germline Fas mutations and defective lymphocyte apoptosis. Blood. 2001;98(1):194-200.
11. Straus SE, Sneller M, Lenardo MJ, Puck JM, Strober W. An inherited disorder of lymphocyte apoptosis: the autoimmune lymphoproliferative syndrome. Ann Intern Med. 1999;130(7):591-601.
12. Fuchs H, Posovszky C, Lahr G, van der Werff ten Bosch J, Boehler T, Debatin KM. Residual CD95-pathway function in children with autoimmune lymphoproliferative syndrome is independent from clinical state and genotype of CD95 mutation. Pediatr Res. 2009;65(2):163-8.