

Letter to the Editor

A new case of Fas-associated death domain protein deficiency and update on treatment outcomes

To the Editor:

We present a new case of Fas-associated death domain protein (FADD) deficiency¹ (MIM 613759) in a 3-year-old girl of Pakistani descent. She was well until age 6 months, when she developed pneumococcal meningitis from which she recovered fully with antibiotic therapy. The family history was notable for the death of 2 male siblings from pneumococcal meningitis in infancy, while another female sibling had died at age 4 months from congenital cardiac abnormalities (Fig 1). Over the following 12 months, she had 2 further hospitalizations with fever, irritability, and drowsiness, on one occasion requiring intensive care owing to worsening encephalopathy and seizures. Extensive investigations showed no infective cause for her deterioration, but cranial magnetic resonance imaging (MRI) of the head demonstrated nonspecific white matter changes (Fig 2, G) and electroencephalography showed an encephalopathic picture with diffuse slow waves. She was also found to have mild liver dysfunction (alanine transaminases, 76 IU/L; reference, <40). With supportive care, she recovered fully from these episodes, although with a degree of global developmental delay and an abnormal cranial MRI.

Her immunological workup showed a normal immunoglobulin profile and complement studies, but suboptimal anti-pneumococcal antibody titers despite repeated Prevenar-13 vaccination (see Fig E1 in this article's Online Repository at www.jacionline.org). This was determined by measuring the total anti-pneumococcal antibody levels, rather than serotype-specific responses. Lymphocyte numbers were grossly normal except for marginal elevation of CD4⁺CD8⁻ (double-negative) T cells (DNTs), which measured 3% on 1 occasion (see Table E1 in this article's Online Repository at www.jacionline.org). T-cell proliferative responses to phytohemagglutinin and anti-CD3 were within normal limits and lipopolysaccharide-induced CD62L shedding was normal; an autoimmune screen was negative. Because the diagnosis at this point was not obvious, and in view of the compelling family history, consent was sought and given for whole exome sequencing. Targeted capture using the Agilent SureSelect All Exon v4 exome enrichment kit was performed using peripheral blood DNA from the proband and her parents before sequencing on an Illumina HiSeq. Reads were aligned to the human GRCh37 assembly using novoalign (Novocraft Technologies, Selangor, Malaysia) followed by processing and variant calling using Picard² and GATK.³ Variant consequences were annotated using the Ensembl Variant Effect Predictor⁴ and bespoke scripts used to identify rare and potentially damaging variants. We identified a list of 12 candidate homozygous variants (see Table E2 in this article's Online Repository at www.jacionline.org) and noted among them the previously described c.315T>G;p.C105W variant in *FADD*.¹ This missense mutation was shown to impair FADD's interaction with FAS (CD95), leading to defective FAS-mediated apoptosis *in vitro* and a distinctive clinical syndrome. Homozygosity for this variant in the patient, and heterozygosity in both parents, was confirmed by Sanger sequencing (Fig 1).

On reflection, the new case showed an almost identical pattern of clinical and biological features to that previously

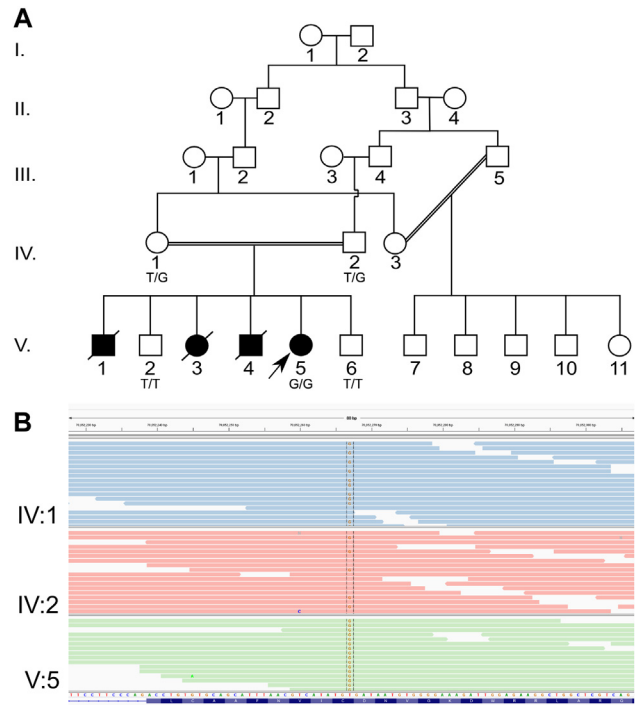


FIG 1. Identification of *FADD* mutations in the new case of *FADD* deficiency. **A**, Pedigree of the family with affected individuals shaded black. The genotypes for the *FADD* mutation identified (NM_003824.3:c.315T>G; NP_003815.1:p.Cys105Trp) are given for individuals for whom samples were available for Sanger sequencing. **B**, *FADD* mutation identified through whole exome sequencing of the parent-child trio (IV:1, IV:2, and V:5). A snapshot from Integrative Genomics Viewer⁶ is shown for the mutation *FADD* mutation.

described in *FADD* deficiency.¹ Although affected children with *FADD* deficiency have several biological features in common with autoimmune lymphoproliferative syndrome (impaired Fas-dependent apoptosis, increased DNTs, elevated serum levels of IL-10 and FasL), their clinical phenotype differs. They display functional hyposplenism and consequent susceptibility to invasive pneumococcal disease, together with recurrent episodes of fever, encephalopathy, and mild liver impairment. These episodes can be triggered by viral infection (eg, varicella zoster virus, EBV, measles-mumps-rubella vaccine, and parainfluenza infection); however, this has not been confirmed in each instance. The striking similarity between the present and previous cases, in apparently unrelated families from the same community, implies that *FADD* deficiency causes all aspects of this particular clinical phenotype. Fatal early onset invasive pneumococcal disease is a particular risk. Therefore, *FADD* deficiency should be suspected in any child with early pneumococcal disease and/or episodes of encephalopathy with hepatitis, especially if associated with elevated DNTs and relative lymphocytosis.

The pedigrees of both affected families are remarkable for the death of multiple affected or putatively affected individuals in early childhood. In view of the seemingly dismal prognosis and the likelihood of an immunologic component to the condition, hematopoietic stem cell transplantation (HSCT) was offered as a

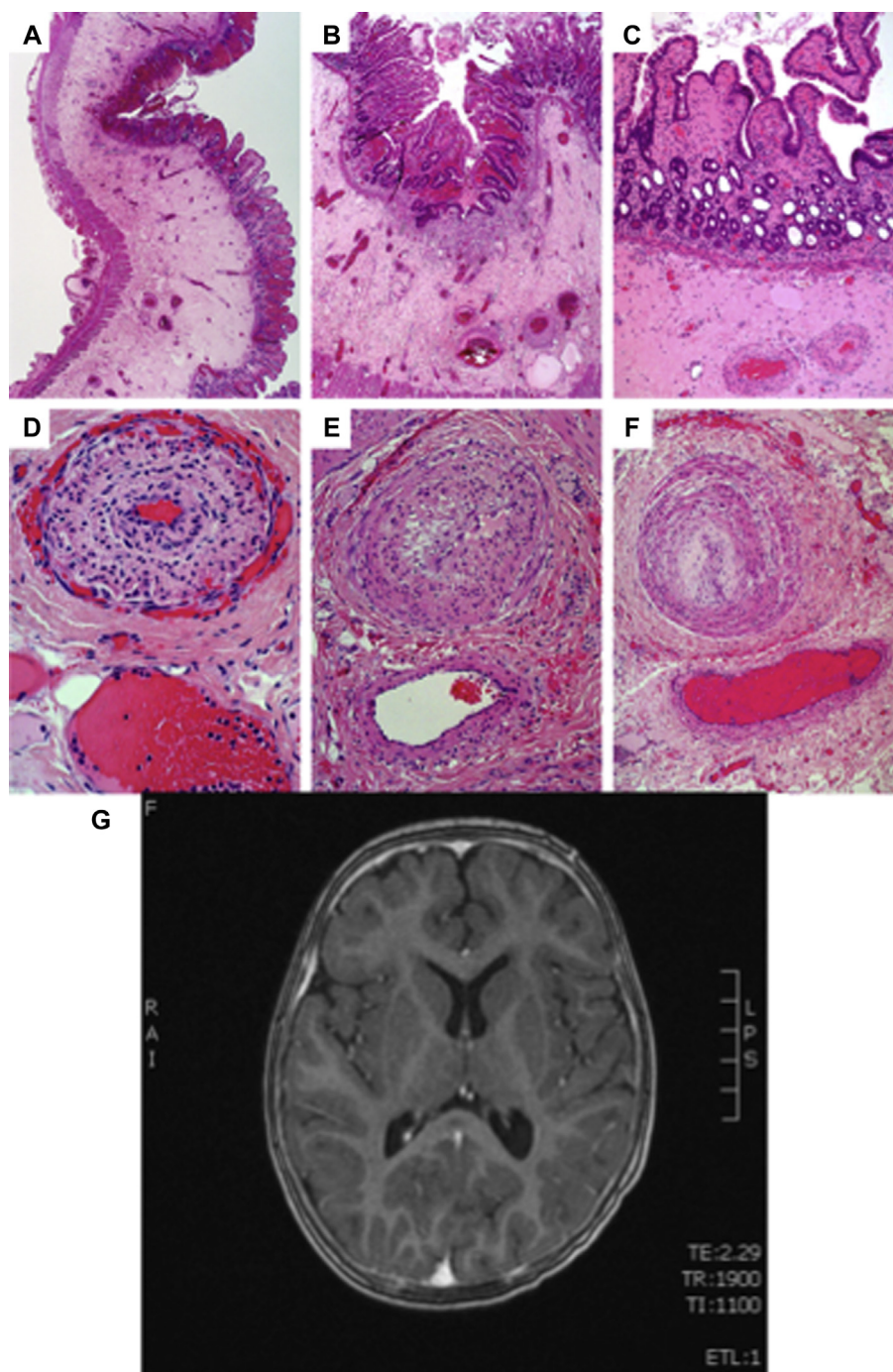


FIG 2. Gastrointestinal pathology post-HSCT in a previous case of FADD deficiency. The small intestine showed marked mucosal and submucosal hemorrhage and edema (**A**) with multiple ischemic lesions. Acute lesions (Fig 2, **A**) showed mucosal necrosis with ischemic damage to the muscularis propria, leading to multiple perforations. Some ischemic lesions healed with granulation tissue (**B**), and some more chronic/mild lesions characterized by mucin depletion and crypt withering were present (**C**). Within the submucosa, subserosa, and mesentery, there were many small or medium-sized arteries showing moderate to almost complete luminal narrowing as a result of an obliterative arteriopathy characterized by variable medial and intimal hyperplasia and fibrosis, with focal myxoid change and erythrocyte extravasation (**D-F**). **G**, MRI brain: Encephalitis protocol. There are multiple punctate high T2 signal lesions within the corona radiata bilaterally that demonstrate restricted diffusion. Further, more subtle lesions are demonstrated within the cerebellar white matter. Restricted diffusion is also demonstrated within the splenium and the rostrum of the corpus callosum. No evidence of microhemorrhage on the gradient echo sequence.

potentially life-saving procedure in both cases and we now report initial outcome.

The previously described child with FADD deficiency¹ experienced progressively more severe episodes of encephalopathy in early childhood, culminating in a prolonged period of coma from which she partially recovered despite a markedly abnormal MRI. She underwent fully conditioned matched related donor HSCT at age 2 years, which was well tolerated. Gradual decline in donor chimerism prompted a top up of stem cells 10 months later, resulting in stable mixed chimerism (29% T, 5% B, 12% myeloid). She had 1 brief episode of unresponsiveness but no recurrence of encephalopathy and her neurodevelopment is slowly progressing. She also has remained free of major septic episodes such as pneumococcal infection. However, she has significant ongoing gut dysfunction marked by intermittent gastrointestinal bleeding and abdominal pain from 4 months after transplant, including 2 life-threatening episodes. This began with severe abdominal pain and diarrhea that evolved into perirectal bleeding and eventually shock with small-bowel perforation requiring bowel resection. Histopathologic examination showed a striking vasculopathy as described previously in very few post-HSCT patients⁵ (Fig 2). Intercurrently, she had an isolated episode of autoimmune hemolytic anemia and thrombocytopenia that was treated with immunomodulation including high-dose intravenous immunoglobulin and rituximab. Following a second episode of gastrointestinal bleeding, repeat biopsies confirmed an obliterative arteriopathy and the patient was commenced on sirolimus and amlodipine, gut rest with parenteral nutrition, and gut decontamination. There has been a general improvement in terms of bleeding frequency and abdominal pain, but this patient remains on partial parenteral nutrition some 4 years posttransplant and recently developed a vasculopathic ulcer of the abdominal wall. It is unclear whether the obliterative arteriopathy is a complication of HSCT, a bone marrow-independent manifestation of FADD deficiency, or perhaps an immunologically driven process in the context of incomplete donor chimerism.

The later patient received a fully matched sibling donor HSCT, following which she has made a steady and largely uneventful recovery to date: 6 months posttransplant she is fully engrafted with evidence of ongoing immune reconstitution and no graft versus host disease. She has persistent leukodystrophy on MRI and mild developmental delay mainly affecting her speech. No gastrointestinal problems similar to the first case have been encountered.¹

Beyond pneumococcal prophylaxis, the further management of FADD deficiency remains challenging because of its extreme rarity together with our incomplete understanding of pathogenesis. Following HSCT, neither patient has experienced further episodes

of parainfectious encephalopathy or pneumococcal disease so far. However, it is not clear what other clinical manifestations may result from FADD deficiency, nor whether HSCT will prove effective in preventing such complications.

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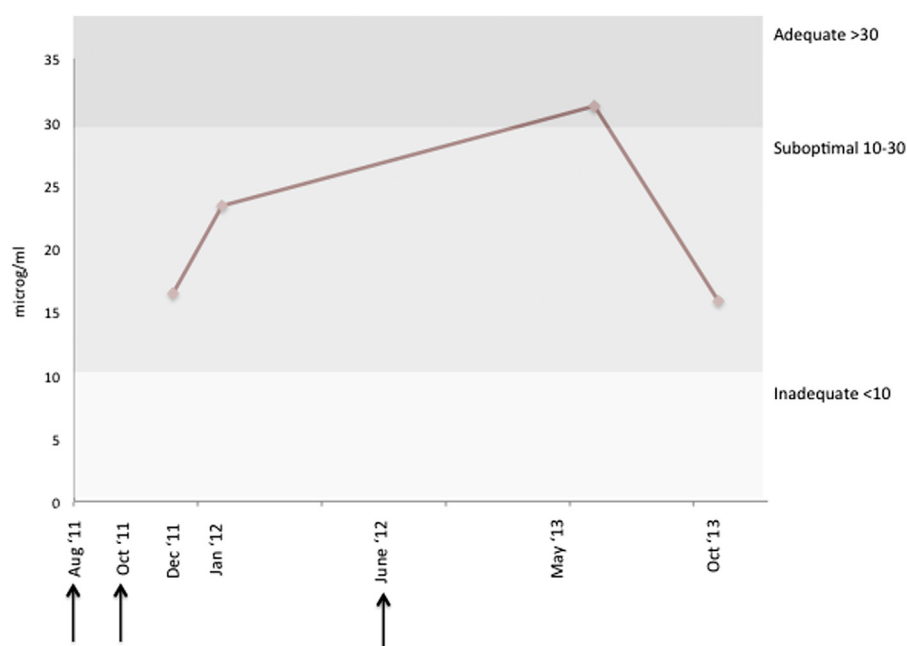


FIG E1. Anti-pneumococcal antibody levels before and after vaccination with Pevnar 13. Arrow indicates the time of vaccination. The response to vaccination was determined using the Binding Site pneumococcal capsular assay, which measures total anti-pneumococcal antibody response against the following serotypes: 1-5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F, and 33F (Danish nomenclature).

TABLE E1. Results of immunological investigations

Lymphocyte subsets	December 21, 2011	December 10, 2012	January 30, 2013	Ref. range (10 × 9/L)
T cells	4.675	6.381	6.886	1.600-6.700
CD4	3.175	4.212	4.995	1.000-4.600
CD8	1.297	1.843	1.548	0.400-2.100
CD19	0.996	4.578	2.761	0.600-2.700
Natural killer	0.234	0.403	0.185	0.200-1.200
DNTs	<2%	3%	<2%	<2%
CD4/CD8	2.45	2.29	3.23	1.07-1.87

Immunoglobulins	December 7, 2012	October 8, 2013	Reference ranges
IgG	4.2	6.6	2.4-8.8
IgA	0.29	0.52	0.10-0.5
IgM	0.47	0.56	0.2-1.0
IgG ₁	3.49	5.39	1.51-7.92
IgG ₂	0.53	0.8	0.26-1.36
IgG ₃	0.172	0.339	0.093-0.920
IgG ₄	0.116	0.155	0.004-0.464

Other investigations	
PHA and anti-CD3	Normal
TLR2 and TLR4 expression	Normal
CD 62L shedding	Normal
Complement studies	Normal
Autoimmune screen	Negative

TABLE E2. Homozygous variants identified by exome sequencing in Family x, individual V:5

Symbol	cDNA	Protein
LCE2A	NM_178428.3:c.96C>A	NP_848515.1:p.Cys32Ter
EFNA4	NM_182689.1:c.250G>A	NP_872631.1:p.Glu84Lys
CD5L	XM_005245602.1:c.593G>A	XP_005245659.1:p.Arg198Gln
OR6K6	NM_001005184.1:c.206G>A	NP_001005184.1:p.Gly69Glu
SENP5	NM_152699.4:c.791A>G	NP_689912.2:p.Gln264Arg
FRAS1	NM_025074.6:c.8440G>A	NP_079350.5:p.Ala2814Thr
FRAS1	NM_025074.6:c.11815G>A	NP_079350.5:p.Ala3939Thr
LIN54	XM_005262749.1:c.754C>G	XP_005262806.1:p.Arg252Gly
FLNC	NM_001458.4:c.1577G>A	NP_001449.3:p.Arg526Gln
FADD*	NM_003824.3:c.315T>G	NP_003815.1:p.Cys105Trp
RELT	NM_152222.1:c.995C>T	NP_689408.1:p.Ala332Val

*Previously described variant in FADD.

TABLE E3. Clinical information related to HSCT outcomes

	Patient 1	Patient 2
Type of HSCT	Related (sibling) bone marrow Fully matched CD34 ⁺ 4.8 × 10 ⁶ /kg	Related (grandfather) PBSC Fully matched CD34 ⁺ 10.6 × 10 ⁶ /kg
Conditioning	Treosulphan 42 g/m ² Fludarabine 150 mg/m ² Thiotepa 5 mg/kg	Treosulphan 42 g/m ² Fludarabine 150 mg/m ² Alemtuzumab day −8 → day −4 (total 1 mg/kg)
Engraftment	Neutrophils > 0.5 × 10 ⁹ /L day +13 Platelets > 50 × 10 ⁹ /L day +25	Neutrophils > 0.5 × 10 ⁹ /L day +14 Platelets > 50 × 10 ⁹ /L day +8
Chimerism	100% donor day +28	CD15 ⁺ 12% CD19 ⁺ 7% CD3 ⁺ 27% (stable 4 y post-HSCT)
Infective complications	Norovirus enteritis day −8 CMV viremia day −8 to day +56	<i>Escherichia coli</i> UTI day +25
GvHD	Skin grade II day +28 Resolved by day +42	None
Other complications related to HSCT	None	Autoimmune hemolytic anemia day +240 → day +300 Top up HSCT +310 d Recurrent GI bleeding, small-bowel perforation, and strictures from day +330 Noninflammatory vasculopathy, improved with sirolimus and amlodipine
Outcome	Alive and well, no infection or GvHD, thriving, no encephalopathy or hepatitis 6 mo post-HSCT On ScIg and cotrimoxazole	Long-term TPN Skin ulcer due to vasculopathy Cause of vasculopathy unclear

CMV, Cytomegalovirus; GI, gastrointestinal; GvHD, graft versus host disease; PBSC, peripheral blood stem cells; ScIg, subcutaneous immunoglobulin; TPN, total parenteral nutrition; UTI, urinary tract infection.