Elsevier Editorial System(tm) for Journal of Allergy and Clinical Immunology
Manuscript Draft

Manuscript Number: JACI-D-14-01353R3

Title: A new case of FADD deficiency and update on treatment outcomes

Article Type: Letter to the Editor

Section/Category: Immune Deficiencies, Infection, and Systemic Immune Disorders

Keywords: FADD; pneumococcus; functional hyposlenism; encephalopathy; bone marrow transplant

Corresponding Author: Dr. Sinisa Savic, MD,PhD

Corresponding Author's Institution: St James's University Hospital

First Author: Sinisa Savic, MD,PhD

Order of Authors: Sinisa Savic, MD,PhD; David Perry, PhD; Clive Carter, PhD; Colin Johnson, PhD; Julian E Thomas, MD; Beatriz Morillo Gutierrez, MD; Chris M Bacon, MD,PhD; Andrew Cant , MD; Sophie Hambleton, MD, PhD

Manuscript Region of Origin: UNITED KINGDOM

Abstract: We describe a new case of FADD deficiency which confirms the specific clinical phenotype associated with this genetic mutation. This is only the second such case world-wide. We also describe clinical outcomes of bone marrow transplant in the two surviving patients.

Sinisa Savic MD PhD
Department of Immunology and Allergy
St James's University Hospital
Leeds LS9 7TF
UK
☎: +44 1132065567
Email: sinisa.savic@nhs.net

William T. Shearer, MD, PhD 25th Jan 2015
Associate Editor, Journal of Allergy and Clinical Immunology

Dear Professor Shearer

Re: Letter entitled 'New case of FADD deficiency and update on treatment outcomes'

We have endeavoured to address all the reviewers' comments following the third round of revisions. We hope that our manuscript is now of the required standard for publication in your journal

Kind regards

Dr Sinisa Savic
Consultant Clinical Immunologist
Honorary Clinical Associate Professor

***Responses to Comments**

We thank reviewers for their comments.

COMMENTS FROM REVIEWER #1:
The authors have satisfactorily addressed the responses according to the reviewers' comments. Just a very minor comment: please change Table E3 by Table E2, after the sentence "we identified a list of 12 candidate homozygous variants".

Reply:
Changes to the text made according to the comment

COMMENTS FROM REVIEWER #2:
Revised Figure S1 was not included in this revision.

I disagree with considering criteria of protective antibodies if the Binding Site Test is >30ug/ml.
It is possible, and we have seen actual patients showing a response over 30 for only one or few serotypes, while titers for other serotypes remain at low levels. Conversely, there could have been a 4-fold increase in every one of the 13 serotypes included in the vaccine, however not reaching 30 ug/ml levels adding all together. Actually, from the previous figure submitted, it does appear that a response was observed, even though it did not reach 30, the particular serotype might have provided with the observed increase.

Reply:
The patient had received Prevenar 13 on 3 separate occasions and also had a documented pneumococcal infection, and yet despite all of this did not manage to sustain adequate levels of anti-pneumococcal antibodies. We accept that there is a theoretical possibility whereby the patient might have responded adequately to each individual serotype, but that the total amount of anti-pneumococcal Ab was still within inadequate range. To highlight this point we will change the text of the article to say that impairment of the specific antibody response to pneumococcus was not confirmed by a serotype specific assay.

A new case of FADD deficiency and update on treatment outcomes

*Savic S, MD PhD[a,b], Parry DA, PhD[c], Carter C, PhD[a], Johnson C, PhD[c], Logan CV, PhD[c], Gutierrez BM, MD[d], Thomas JE, MD[d], Bacon CM, MD PhD[e], Cant A, MD[d,f], *Hambleton S, MD DPhil[d,f]

[a] Department of Clinical Immunology and Allergy, St James's University Hospital, Leeds, UK
[b] Leeds Institute of Rheumatic and Musculoskeletal Medicine, University of Leeds, UK
[c] Leeds Institute of Biomedical and Clinical Sciences, University of Leeds, UK
[d] Great North Children's Hospital, Royal Victoria Infirmary, Newcastle upon Tyne, UK
[e] Northern Institute for Cancer Research, Newcastle University, Newcastle upon Tyne, UK
[f] Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, UK

*Corresponding authors:
Sinisa Savic MD, PhD,
Department of Clinical Immunology and Allergy,
Leeds Institute of Rheumatic and Musculoskeletal Medicine,
University of Leeds,
Wellcome Trust Brenner Building,
St James' University Hospital,
Leeds LS9 7TF.
UK
Email: s.savic@leeds.ac.uk
Tel: +441132065567, Fax: +441132067250

Sophie Hambleton, MD DPhil,
Institute of Cellular Medicine,
4th floor Catherine Cookson Building,
Newcastle University Medical School,
Framlington Place,
Newcastle upon Tyne NE2 4HH,
UK.
Email: sophie.hambleton@newcastle.ac.uk
Tel +441912087139, Fax: +441912085545

Capsule Summary

FADD deficiency is associated with susceptibility to invasive pneumococcal disease, encephalopathy, hepatic dysfunction and cardiovascular malformations. HSCT should be considered early since the condition is usually fatal.

Key words:

FADD
Whole exom sequencing
Encephalopathy
Pneumococcus
HST

To the editor:

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

Her immunological work up showed a normal immunoglobulin profile and complement studies, but suboptimal anti-pneumococcal antibody (anti-PnAb) titers despite repeated Prevenar-13 vaccination (Fig E1). This was determined by measuring the total anti-PnAb levels, rather then serotype specific responses. Lymphocyte numbers were grossly normal except for marginal elevation of CD4-CD8- (double negative) T cells (DNT's) which measured 3% on one occasion (Table E1). T-cell proliferative responses to phytohemagglutinin (PHA) and anti-CD3 were within normal limits and lipopolysaccharide-induced CD62L shedding was normal; an autoimmune screen was negative. Since 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) followed by processing and variant calling using Picard[2] and GATK.[3] Variant consequences were annotated using the Ensembl Variant Effect Predictor[4] and bespoke scripts used to identify rare and potentially damaging variants. We identified a list of 12 candidate homozygous variants (Table E2~~3~~) 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 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 (e.g Varicella Zoster virus, Epstein-Barr Virus, 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 DNT's and relative lymphocytosis.

The pedigrees of both affected families are remarkable for the death in early childhood of multiple affected or putatively affected individuals. 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 potentially life-saving procedure in both cases and we now report initial outcome.

The previously described child with FADD deficiency[1] 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 the age of 2 that 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 one 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 two life-threatening episodes. This began with severe abdominal pain and diarrhoea that evolved into peri-rectal 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 patients[5] (Fig 2). Intercurrently, she had an isolated episode of autoimmune hemolytic anemia and thrombocytopenia that was treated with immunomodulation including high dose IV

immunoglobulin and Rituximab. Following a second episode of gastrointestinal bleeding, repeat biopsies confirmed an obliterative arteriopathy and the patient was commenced on sirolimus and amlodopine, 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 four years post-transplant 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: six months post-transplant 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.

Sinisa Savic PhD[a,b]
David Parry PhD[c]
Clive Carter PhD[a]
Colin Johnson PhD[c]
Clare Logan PhD[c]
Beatriz Morillo Gutierrez MD[d]
Julian E Thomas MD[d]
Chris M Bacon PhD[e]
Andrew Cant MD[d,f]
Sophie Hambleton DPhil[d,f]

From [a] Department of Clinical Immunology and Allergy, St James's University Hospital, Leeds. UK; [b] Institute of Rheumatic and Musculoskeletal Medicine University of Leeds; [c] Leeds Institute of Biomedical and Clinical Sciences, section of genetics, University of Leeds, Wellcome Trust Brenner Building, St James's University Hospital, Leeds; [d] Great North Children's Hospital, Royal Victoria Infirmary,

Newcastle upon Tyne; [e] Northern Institute for Cancer Research, Newcastle University, UK; [f] Institute of Cellular Medicine Newcastle University Medical School. Email: s.savic@leeds.ac.uk or sophie.hambleton@newcastle.ac.uk
Authors have no potential conflict of interest to disclose

References:

1. Bolze A, Byun M, McDonald D, Morgan NV, Abhyankar A, Premkumar L, et al. Whole-exome-sequencing-based discovery of human FADD deficiency. American journal of human genetics. 2010;87:873-81
2. Available from: http://picard.sourceforge.net/.
3. DePristo MA, Banks E, Poplin R, Garimella KV, Maguire JR, Hartl C, et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nature genetics. 2011;43(5:491-8
4. McLaren W, Pritchard B, Rios D, Chen Y, Flicek P, Cunningham F. Deriving the consequences of genomic variants with the Ensembl API and SNP Effect Predictor. Bioinformatics. 2010;26:2069-70
5. Selby DM, Rudzki JR, Bayever ES, Chandra RS. Vasculopathy of small muscular arteries in pediatric patients after bone marrow transplantation. Hum Pathol. 1999; 30:734-740
6. Robinson JT, Thorvaldsdottir H, Winckler W, Guttman M, Lander ES, Getz G, et al. Integrative Genomics Viewer. Nat Biotechnol 2011;29:24–6.

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 Viewer[6] is shown for the mutation FADD mutation

FIG 2. Gastrointestinal pathology post-HSCT in a previous case of FADD deficiency. The small intestine showed marked mucosal and submucosal haemorrhage and oedema (A) with multiple ischaemic lesions. Acute lesions (A) showed mucosal necrosis with ischaemic damage to the muscularis propria, leading to multiple perforations. Some ischaemic lesions healed with granulation tissue (B) and some more chronic/mild lesions characterised 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 characterised 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 which 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 microhaemorrhage on the gradient echo sequence.

FIG E1. Anti-pneumococcal antibody levels before and after vaccination with Prevnar 13. Arrow indicates the time of vaccination. The response to vaccination was determined using the Binding Site^{TM} 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).

A new case of FADD deficiency and update on treatment outcomes

*Savic S, MD PhD[a,b], Parry DA, PhD[c], Carter C, PhD[a], Johnson C, PhD[c], Logan CV, PhD[c], Gutierrez BM, MD[d], Thomas JE, MD[d], Bacon CM, MD PhD[e], Cant A, MD[d,f], *Hambleton S, MD DPhil[d,f]

[a] Department of Clinical Immunology and Allergy, St James's University Hospital, Leeds, UK
[b] Leeds Institute of Rheumatic and Musculoskeletal Medicine, University of Leeds, UK
[c] Leeds Institute of Biomedical and Clinical Sciences, University of Leeds, UK
[d] Great North Children's Hospital, Royal Victoria Infirmary, Newcastle upon Tyne, UK
[e] Northern Institute for Cancer Research, Newcastle University, Newcastle upon Tyne, UK
[f] Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, UK

*Corresponding authors:
Sinisa Savic MD, PhD,
Department of Clinical Immunology and Allergy,
Leeds Institute of Rheumatic and Musculoskeletal Medicine,
University of Leeds,
Wellcome Trust Brenner Building,
St James' University Hospital,
Leeds LS9 7TF.
UK
Email: s.savic@leeds.ac.uk
Tel: +441132065567, Fax: +441132067250

Sophie Hambleton, MD DPhil,
Institute of Cellular Medicine,
4th floor Catherine Cookson Building,
Newcastle University Medical School,
Framlington Place,
Newcastle upon Tyne NE2 4HH,
UK.
Email: sophie.hambleton@newcastle.ac.uk
Tel +441912087139, Fax: +441912085545

Capsule Summary

FADD deficiency is associated with susceptibility to invasive pneumococcal disease, encephalopathy, hepatic dysfunction and cardiovascular malformations. HSCT should be considered early since the condition is usually fatal.

Key words:

FADD
Whole exom sequencing
Encephalopathy
Pneumococcus
HST

To the editor:

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

Her immunological work up showed a normal immunoglobulin profile and complement studies, but suboptimal anti-pneumococcal antibody (anti-PnAb) titers despite repeated Prevenar-13 vaccination (Fig E1). This was determined by measuring the total anti-PnAb levels, rather then serotype specific responses. Lymphocyte numbers were grossly normal except for marginal elevation of CD4-CD8- (double negative) T cells (DNT's) which measured 3% on one occasion (Table E1). T-cell proliferative responses to phytohemagglutinin (PHA) and anti-CD3 were within normal limits and lipopolysaccharide-induced CD62L shedding was normal; an autoimmune screen was negative. Since 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) followed by processing and variant calling using Picard[2] and GATK.[3] Variant consequences were annotated using the Ensembl Variant Effect Predictor[4] and bespoke scripts used to identify rare and potentially damaging variants. We identified a list of 12 candidate homozygous variants (Table E2) 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 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 (e.g Varicella Zoster virus, Epstein-Barr Virus, 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 DNT's and relative lymphocytosis.

The pedigrees of both affected families are remarkable for the death in early childhood of multiple affected or putatively affected individuals. 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 potentially life-saving procedure in both cases and we now report initial outcome.

The previously described child with FADD deficiency[1] 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 the age of 2 that 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 one 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 two life-threatening episodes. This began with severe abdominal pain and diarrhoea that evolved into peri-rectal 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 patients[5] (Fig 2). Intercurrently, she had an isolated episode of autoimmune hemolytic anemia and thrombocytopenia that was treated with immunomodulation including high dose IV

immunoglobulin and Rituximab. Following a second episode of gastrointestinal bleeding, repeat biopsies confirmed an obliterative arteriopathy and the patient was commenced on sirolimus and amlodopine, 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 four years post-transplant 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: six months post-transplant 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.

Sinisa Savic PhD[a,b]
David Parry PhD[c]
Clive Carter PhD[a]
Colin Johnson PhD[c]
Clare Logan PhD[c]
Beatriz Morillo Gutierrez MD[d]
Julian E Thomas MD[d]
Chris M Bacon PhD[e]
Andrew Cant MD[d,f]
Sophie Hambleton DPhil[d,f]

From [a] Department of Clinical Immunology and Allergy, St James's University Hospital, Leeds. UK; [b] Institute of Rheumatic and Musculoskeletal Medicine University of Leeds; [c] Leeds Institute of Biomedical and Clinical Sciences, section of genetics, University of Leeds, Wellcome Trust Brenner Building, St James's University Hospital, Leeds; [d] Great North Children's Hospital, Royal Victoria Infirmary,

Newcastle upon Tyne; [e] Northern Institute for Cancer Research, Newcastle University, UK; [f] Institute of Cellular Medicine Newcastle University Medical School. Email: s.savic@leeds.ac.uk or sophie.hambleton@newcastle.ac.uk
Authors have no potential conflict of interest to disclose

References:

1. Bolze A, Byun M, McDonald D, Morgan NV, Abhyankar A, Premkumar L, et al. Whole-exome-sequencing-based discovery of human FADD deficiency. American journal of human genetics. 2010;87:873-81
2. Available from: http://picard.sourceforge.net/.
3. DePristo MA, Banks E, Poplin R, Garimella KV, Maguire JR, Hartl C, et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nature genetics. 2011;43(5:491-8
4. McLaren W, Pritchard B, Rios D, Chen Y, Flicek P, Cunningham F. Deriving the consequences of genomic variants with the Ensembl API and SNP Effect Predictor. Bioinformatics. 2010;26:2069-70
5. Selby DM, Rudzki JR, Bayever ES, Chandra RS. Vasculopathy of small muscular arteries in pediatric patients after bone marrow transplantation. Hum Pathol. 1999; 30:734-740
6. Robinson JT, Thorvaldsdottir H, Winckler W, Guttman M, Lander ES, Getz G, et al. Integrative Genomics Viewer. Nat Biotechnol 2011;29:24–6.

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 Viewer[6] is shown for the mutation FADD mutation

FIG 2. Gastrointestinal pathology post-HSCT in a previous case of FADD deficiency. The small intestine showed marked mucosal and submucosal haemorrhage and oedema (A) with multiple ischaemic lesions. Acute lesions (A) showed mucosal necrosis with ischaemic damage to the muscularis propria, leading to multiple perforations. Some ischaemic lesions healed with granulation tissue (B) and some more chronic/mild lesions characterised 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 characterised 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 which 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 microhaemorrhage on the gradient echo sequence.

FIG E1. Anti-pneumococcal antibody levels before and after vaccination with Prevnar 13. Arrow indicates the time of vaccination. The response to vaccination was determined using the Binding Site^{TM} 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).

Figure No.1 - Unmarked

Click here to download high resolution image

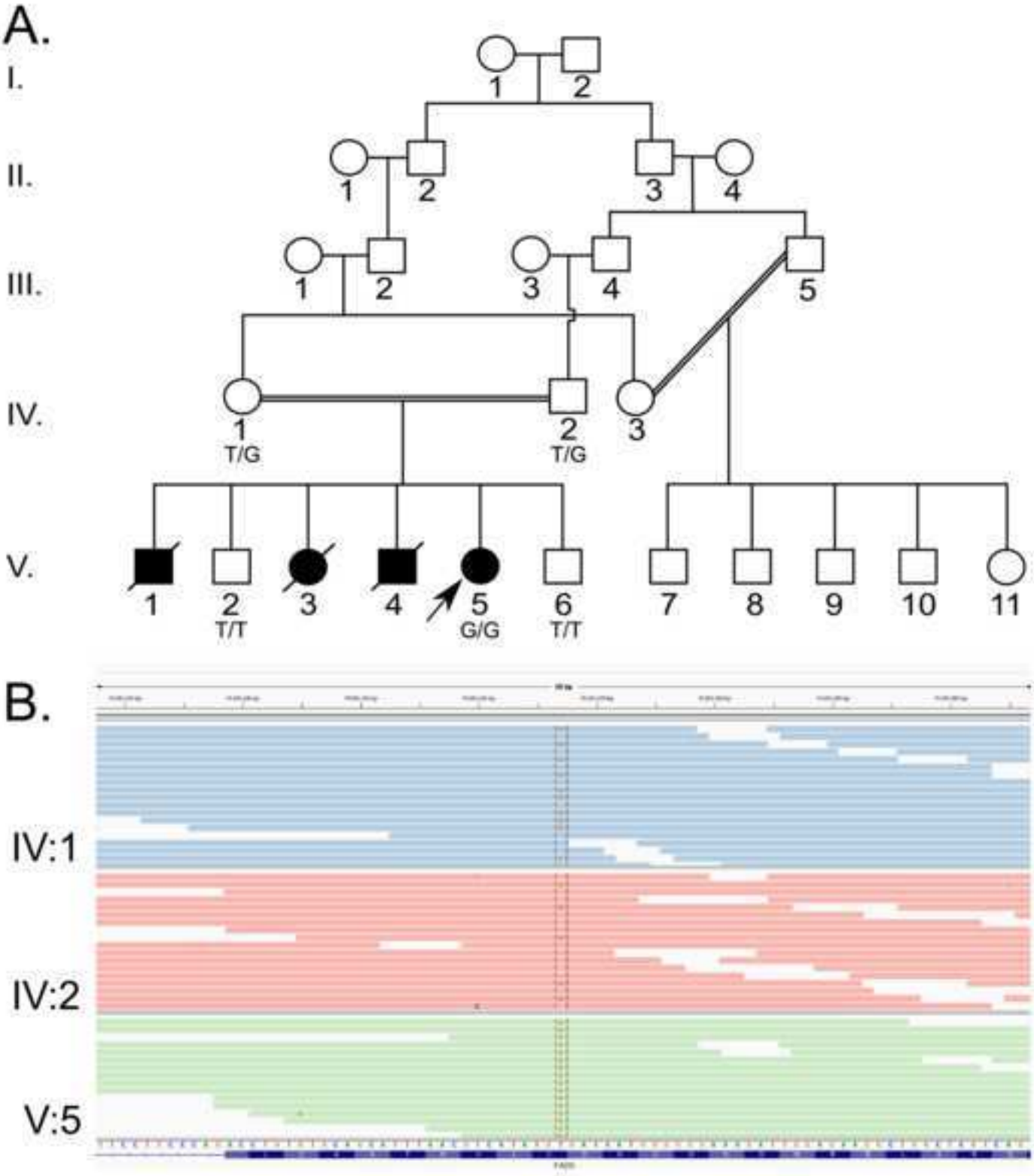
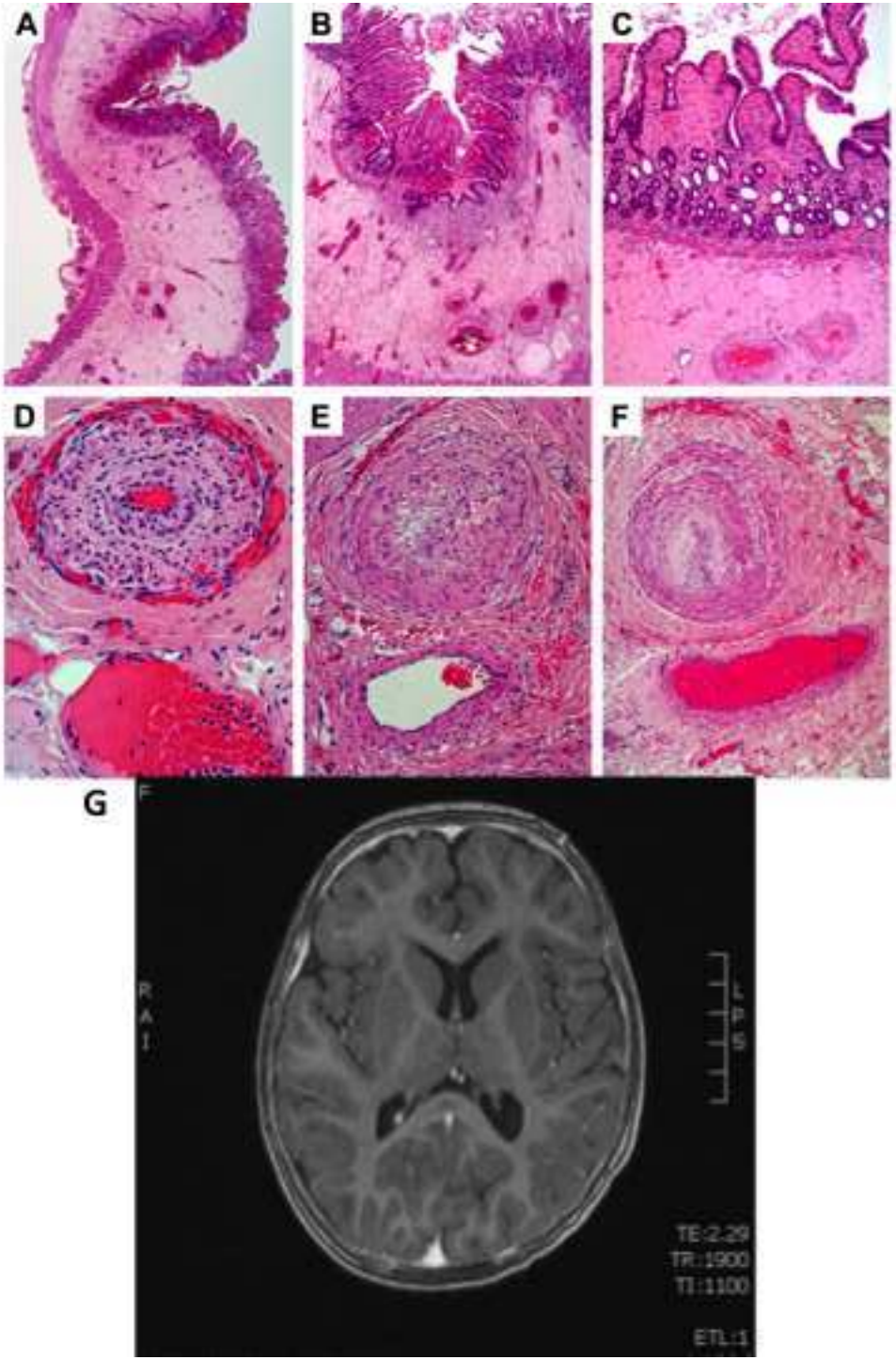


Figure No.2 - Unmarked

Click here to download high resolution image



Repository - Unmarked E Figure No.1
Click here to download high resolution image

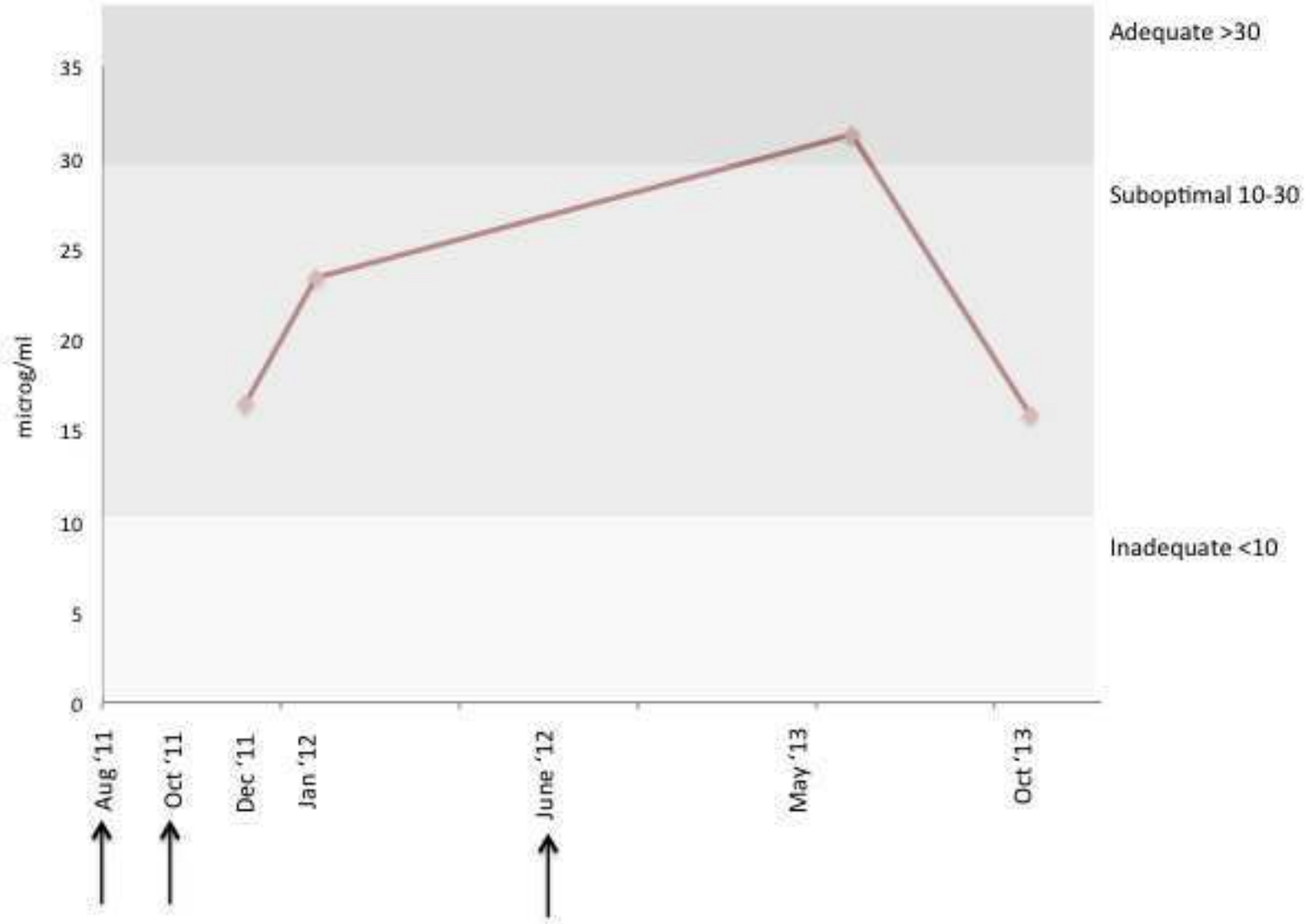


Table E1. Results of immunological investigations

Lymphocyte subsets				
	21/12/2011	10/12/2012	30/01/2013	Ref. Range (10x9/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
NK	0.234	0.403	0.185	0.200-1.200
*DNT's	<2%	3%	<2%	<2%
CD4/CD8	2.45	2.29	3.23	1.07-1.87
Immunoglobulins				
	07/12/2012	08/10/2013	Ref 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	
IgG1	3.49	5.39	1.51-7.92	
IgG2	0.53	0.8	0.26-1.36	
IgG3	0.172	0.339	0.093-0.920	
IgG4	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		

* Double negative T cells

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

Table E3 Clinical information related to HSCT outcomes

	Patient 1	Patient 2
Type of HSCT	Related (sibling) Bone Marrow Fully matched CD34+ 4.8 x 10^6/kg	Related (Grandfather) PBSC Fully matched CD34+ 10.6 x 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 x 10^9/L day +13 Platelets > 50 x 10^9/L day +25	Neutrophils > 0.5 x 10^9/L day +14 Platelets > 50 x 10^9/L day +8
Chimerism	100% donor day +28	CD15+ 12% CD19+ 7% CD3+ 27% (Stable 4 years post HSCT)
Infective complications	Norovirus enteritis day -8 CMV viremia day -8 to day +56	E. coli UTI day +25
GvHD	Skin grade II day +28. Resolved by day +42.	None
Other complication related to HSCT	None	Autoimmune hemolytic anemia day +240 → day +300 Top up HSCT +310 days Recurrent GI bleeding, small bowel perforation & strictures from day +330 Non-inflammatory vasculopathy, improved with Sirolimus & Amlodipine.
Outcome	Alive and well, no infection or GvHD, thriving, no encephalopathy or hepatitis 6 months post HSCT. On ScIg & Cotrimoxazole.	Long term TPN Skin ulcer due to Vasculopathy Cause of vasculopathy unclear.

HSCT, hematopoietic stem cell transplant; CMV, cytomegalovirus; UTI, urinary tract infection; GvHD, graft versus host disease; TPN, total parenteral nutrition; GI, gastrointestinal: ScIG, subcutaneous immunoglobulin