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Article:
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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.
William T. Shearer, MD, PhD
Associate Editor, Journal of Allergy and Clinical Immunology

25th Jan 2015

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
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

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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. 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 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 (Fig 2). Intercurrently, she had an isolated episode of autoimmune hemolytic anemia and thrombocytopenia that was treated with immunomodulation including high dose IV
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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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Table E1. Results of immunological investigations

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**Immunoglobulins**

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**Other investigations**

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* Double negative T cells
Table E2 Homozygous variants identified by exome sequencing in Family x, individual V:5.

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<td>NP_848515.1:p.Cys32Ter</td>
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<td>EFNA4</td>
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<td>CD5L</td>
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<td>OR6K6</td>
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<td>FRAS1</td>
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<td>LIN54</td>
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<td>XP_005262806.1:p.Arg252Gly</td>
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<td>FLNC</td>
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<td>NP_001449.3:p.Arg526Gln</td>
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<td>FADD</td>
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<td>NP_003815.1:p.Cys105Trp</td>
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<td>RELT</td>
<td>NM_152222.1:c.995C&gt;T</td>
<td>NP_689408.1:p.Ala332Val</td>
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### Table E3 Clinical information related to HSCT outcomes

<table>
<thead>
<tr>
<th></th>
<th>Patient 1</th>
<th>Patient 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type of HSCT</strong></td>
<td>Related (sibling) Bone Marrow&lt;br&gt;<strong>Fully matched CD34+ 4.8 x 10^6/kg</strong></td>
<td>Related (Grandfather) PBSC&lt;br&gt;<strong>Fully matched CD34+ 10.6 x 10^6/kg</strong></td>
</tr>
<tr>
<td><strong>Conditioning</strong></td>
<td>Treosulphan 42 g/m²&lt;br&gt;Fludarabine 150 mg/m²&lt;br&gt;Thiotepa 5 mg/kg</td>
<td>Treosulphan 42 g/m²&lt;br&gt;Fludarabine 150 mg/m²&lt;br&gt;Alemtuzumab day -8 → day -4 (Total 1 mg/kg)</td>
</tr>
<tr>
<td><strong>Engraftment</strong></td>
<td>Neutrophils &gt; 0.5 x 10⁹/L day +13&lt;br&gt;Platelets &gt; 50 x 10⁹/L day +25</td>
<td>Neutrophils &gt; 0.5 x 10⁹/L day +14&lt;br&gt;Platelets &gt; 50 x 10⁹/L day +8</td>
</tr>
<tr>
<td><strong>Chimerism</strong></td>
<td>100% donor day +28</td>
<td>CD15+ 12%  CD19+ 7%  CD3+ 27%&lt;br&gt;(Stable 4 years post HSCT)</td>
</tr>
<tr>
<td><strong>Infective</strong></td>
<td>Norovirus enteritis day -8&lt;br&gt;CMV viremia day -8 to day +56</td>
<td>E. coli UTI day +25</td>
</tr>
<tr>
<td><strong>GvHD</strong></td>
<td>Skin grade II day +28&lt;br&gt;Resolved by day +42.</td>
<td>None</td>
</tr>
<tr>
<td><strong>Other complication related to HSCT</strong></td>
<td>None</td>
<td>Autoimmune hemolytic anemia day +240 → day +300&lt;br&gt;Top up HSCT +310 days&lt;br&gt;Recurrent GI bleeding, small bowel perforation &amp; strictures from day +330&lt;br&gt;Non-inflammatory vasculopathy, improved with Sirolimus &amp; Amlodipine.</td>
</tr>
<tr>
<td><strong>Outcome</strong></td>
<td>Alive and well, no infection or GvHD, thriving, no encephalopathy or hepatitis 6 months post HSCT.&lt;br&gt;On ScIg &amp; Cotrimoxazole.</td>
<td>Long term TPN&lt;br&gt;Skin ulcer due to Vasculopathy&lt;br&gt;Cause of vasculopathy unclear.</td>
</tr>
</tbody>
</table>

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