TCF3 dominant negative variant causes an early block in B-lymphopoiesis and agammaglobulinemia

Ebtehal Al Shiekh1,4, Peter D Arkwright1,2, Archana Herwadkar3, Tracy Hussell1, Tracy A. Briggs1,4

1Lydia Becker Institute of Immunology & Inflammation, University of Manchester, Manchester, M13 9XX, UK

2Department of Paediatric Allergy & Immunology, Royal Manchester Children’s Hospital, Manchester, M13 9WL, UK

3Department of Immunology, Salford Royal Foundation NHS Trust, Manchester, M6 8HD, UK

4Manchester Centre for Genomic Medicine, St Mary’s Hospital, Manchester, M13 9WL, UK

Corresponding author: Dr Peter Arkwright, Department of Paediatric Allergy & Immunology, Royal Manchester Children’s Hospital. Oxford Rd., Manchester, M13 9WL, UK Tel: + 44 161 701 0678, email peter.arkwright@manchester.ac.uk

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To the Editor, Inborn Errors in Immunity (IEI) causing agamaglobulinemia may involve blockade of one of several stages of B-lymphocyte maturation, from development of pro-B cells, pre-B cells, immature B cells, to immunoglobulin class switching. X-linked agammaglobulinemia due to BTK gene variants is the most common genetic cause resulting in arrest at the pre-B-cell stage of differentiation in the bone marrow. Homozygous and heterozygous transcription factor 3 (TCF3/E2A) gene variants are a rare cause of agammaglobulinemia. The TCF3 gene codes for two basic helix-loop-helix transcription factor isoforms: E12 and E47. These E proteins bind to regulatory “E-box” CANNTG sequences on target genes as heterodimers or homodimers and play a critical role in Igλ transcription and VJ rearrangement [1]. Homozygous TCF3 variants have been reported in only two patients, a nine-year-old girl with agammaglobulinemia who harboured a homozygous deletion of TCF3 exons 5 through 11 [2], and a 10 year old boy with a homozygous nonsense variant TCF3 (c.808C>T; p.Gln270X) associated with agammaglobulinemia and also with B-cell acute lymphoblastic leukaemia [3]. Heterozygous TCF3 variants have been described in a mother with CVID and SLE, and her son with selective IgA deficiency and type 1 diabetes (c.168insT) [4], as well as in four unrelated patients with agammaglobulinemia caused by the same de novo heterozygous TCF3 (c.1663G>A; p.Glu555Lys) [5]. In view of the rarity of this IEI, as well as its phenotypic and genotypic variation, in this report we describe the clinical, peripheral blood and bone marrow immune phenotype of an additional patient with a heterozygous TCF3 c.1663G>A; p.Glu555Lys to further highlight this condition to clinicians.

Case Presentation

A 35-year-old white British woman with no significant family history, presented to the immunodeficiency service at the age of six-years-old. She was well until 11-months-old, when she developed a Streptococcus pneumoniae pneumonia and empyema, treated with
intravenous antibiotics, pleural drainage and decortication. Immune investigations at the time
demonstrated an agammaglobulinemia, with a complete absence of CD19 and CD20 positive
B-lymphocytes (Table I). A high-resolution chest CT scan showed no bronchiectasis. From
diagnosis at 12 months old she was treated with regular intravenous immunoglobulin
replacement infusions, maintaining a trough serum IgG concentration of >8.0g/L. The route
of administration was changed to subcutaneous when she was 9 years old. She remains well,
except for intermittent sinusitis treated with oral azithromycin and *Haemophilus influenzae*
conjunctivitis responding to topical chloramphenicol eye drops. She did not suffer from any
skin, liver or autoimmune problems.

Whole exome sequencing identified a heterozygous TCF3 c.1663G>A; p.Glu555Lys
variant (Fig. 1A), not present in over 120,000 control individuals in the gnomAD dataset,
predicted to be damaging *in silico* and previously described in four patients with functional
data supporting a dominant negative pathogenic effect in the E47 isoform [5]. Genetic testing
of the parents confirmed the variant was *de novo*. To characterize where the block in B-cell development occurred, detailed flow
cytometry was performed on a bone marrow aspirate sample, part of which was sent to our
Manchester laboratory, and another aliquot sent to the Immunology Flow Cytometry Service,
Rotterdam, The Netherlands. A multicolour immunophenotyping panel was used to
differentiate pro-B, pre-BI, pre-BII-large, immature, and mature B cellular subsets in the
CD3, CD16, CD33 negative lymphocyte population. Data were analyzed using FlowJo™
Software (Version10.7. Ashland, US) (Fig 1C-J, Table I). The CD19*+* B-cell fraction made up
2% of 820,000 recorded events. 97% of B-cell precursors were PreBI-cells
(CD19*Lin*—*sIgM*—*VpreB*—*(CD179a)*), with very few PreBII, immature or mature B-cells (Fig
1H, I, J). There were no lymphoblasts to suggest neoplastic transformation. These results were
compared with the bone marrow phenotype of eight 46 – 71-year-old controls from the
Rotterdam laboratory, where 6 – 29% were PreBI and 70% were more mature B-cell precursors [6].

**Discussion**

Non-**BTK** causes of agammaglobulinemia are very uncommon, and most clinical immunologists rarely come across these IEI in clinical practice. Furthermore, most e.g. **BLNK** or μ heavy chain deficiency are inherited in an autosomal recessive manner. **TCF3** deficiency is currently the only known heterozygous cause of IEI causing profound reduction in all serum immunoglobulin isotypes without clinical dysmorphism. This is important to note in the diagnostic setting and because it infers a 50% risk of recurrence to the proband’s offspring. Our report also illustrates the fact that bone marrow B-cell precursor phenotyping can provide clues as to where to look for the genetic defect: very early in B-cell maturation in the case of **TCF3** deficiency [1,7].

The p.Glu555Lys E47, but not the E12 isoform has been shown to act in a dominant negative manner altering the binding of this transcription factor with the DNA and other isoforms, leading to the early block in B-lymphopoiesis [5]. E47 expression during B-cell development regulates rearrangement of Ig light chains and the quality assurance of non-self-reactivity [1,5].

Although neoplasia has not been described patients with heterozygous **TCF3** gene variants, one patient with a homozygous nonsense variant developed acute lymphoblastic leukaemia, as well as agammaglobulinaemia [3]. The oncogenic trigger is unclear, but one possibility is that TCF3 also acts as a tumour suppressor gene in relation to quality control and self-reactivity. Additional studies looking at the functional interaction between E47 and
other B-cell transcription factors may shed further light on this, particularly as the E family
proteins are known to interact with several repressor and transcription activators.

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Conflict of Interests

None of the authors declare any conflict of interests in relation to this research article.
References


Figure legends

Figure 1 Schematic representation of E47 & E12 isoforms, as well as immunophenotyping of B-cell precursors in bone marrow from the patient with the TCF3 negative dominant variant. A. Pedigree demonstrating patient and parent genotype; B. E47 and E12 isoforms highlighting the Glu555 residue. Functional domains: Activation Domains (AD1, AD2), checked area represents the basic Helix-Loop-Helix (bHLH) DNA binding domain which is 74% homologous between the two isoforms; C. CD3-CD16-CD33- B-cell precursors selected for further analysis. D. Live CD3-CD16-CD33- cells (Zombie UV stain negative); E. Single cells selection (forward scatter height (FSC-H) vs forward scatter area (FSC-A)); F. CD19/CD34 plot: <2% of total population were CD19+; G. ProB cells (CD34+CD22+CD19-Lin-) were 3% of the CD19- population. H. 97% of CD19+ B cells were sIgM negative; I. The majority of CD19+/sIgM- B-cell precursors were PreB1 (CD19+Lin-sIgM-VpreB-(CD179a)), with only 0.03% PreBII-large (CD19+Lin-sIgM-VpreB+); J. Immature (CD19+Lin-sIgM+sIgD-) and mature (CD19+Lin-sIgM+sIgD+) B-cells were almost completely absent.
Table I Laboratory parameters

<table>
<thead>
<tr>
<th>LABORATORY TEST</th>
<th>PATIENT</th>
<th>NORMAL RANGE</th>
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</thead>
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<tr>
<td><strong>HAEMATOLOGY</strong></td>
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</tr>
<tr>
<td>Haemoglobin (g/L)</td>
<td>134</td>
<td>(121 – 151)</td>
</tr>
<tr>
<td>WBC (x 10^9/L)</td>
<td>6.0</td>
<td>(4.5 – 13.0)</td>
</tr>
<tr>
<td>Neutrophils (x 10^9/L)</td>
<td>4.1</td>
<td>(1.5 – 6.0)</td>
</tr>
<tr>
<td>Lymphocytes (x 10^9/L)</td>
<td>1.3</td>
<td>(1.5 – 4.5)</td>
</tr>
<tr>
<td>Platelets (x 10^9/L)</td>
<td>269</td>
<td>(150 – 430)</td>
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<tr>
<td><strong>PERIPHERAL BLOOD LYMPHOCYTE SUBSETS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD3 (x 10^9/L)</td>
<td>1034</td>
<td>(700 – 2100)</td>
</tr>
<tr>
<td>CD4 (x 10^9/L)</td>
<td>475</td>
<td>(300 – 1400)</td>
</tr>
<tr>
<td>CD8 (x 10^9/L)</td>
<td>315</td>
<td>(200 – 900)</td>
</tr>
<tr>
<td>CD19/CD20 (x 10^9/L)</td>
<td>2</td>
<td>(100 – 500)</td>
</tr>
<tr>
<td>CD16/56 (x 10^9/L)</td>
<td>126</td>
<td>(90 – 600)</td>
</tr>
<tr>
<td><strong>SERUM IMMUNOGLOBULINS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgM (g/L)</td>
<td>&lt;0.1</td>
<td>(0.6 – 2.1)</td>
</tr>
<tr>
<td>IgG (g/L)</td>
<td>&lt;0.5</td>
<td>(2.4 – 8.8)</td>
</tr>
<tr>
<td>IgA (g/L)</td>
<td>&lt;0.07</td>
<td>(0.2 – 0.7)</td>
</tr>
<tr>
<td>IgE (kU/L)</td>
<td>&lt;2.0</td>
<td>(0 – 114)</td>
</tr>
<tr>
<td><strong>BONE MARROW B-CELL PRECURSORS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pro B-cells (%)</td>
<td>3%</td>
<td>7%</td>
</tr>
<tr>
<td>Pre BI-cells (%)</td>
<td>97%</td>
<td>23%</td>
</tr>
<tr>
<td>Pre BII cells (%)</td>
<td>0.03%</td>
<td>39%</td>
</tr>
<tr>
<td>Immature B-cells (%)</td>
<td>0%</td>
<td>31%</td>
</tr>
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</table>
Laboratory parameters are all taken when the patient was 34 years old, except for the serum immunoglobulins, which were taken when the patient was 11 months old. *control values from Immunology Laboratory, Erasmus MC, Rotterdam [6]