

Enteroviral Infection in a Patient with BLNK Adaptor Protein Deficiency

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Abstract B-cell linker (BLNK) protein is a non-redundant adaptor molecule in the signaling pathway activated by (pre) B-cell antigen receptor signals. We present two siblings with a homozygous deleterious frameshift mutation in *BLNK*, resulting in a block of B cell development in the bone marrow at the preB1 to preB2 stage, absence of circulating B cells and agammaglobulinemia. This is the first description of an enteroviral infection associated arthritis and dermatitis in a patient with *BLNK* deficiency.

Keywords BLNK · B cell linker · arthritis · dermatitis · enterovirus · agammaglobulinemia

Introduction

Congenital agammaglobulinemia is caused by inborn errors in early B cell development abrogating differentiation, maturation and antibody production. X-linked agammaglobulinemia (XLA), which results from mutations in Bruton tyrosine kinase (*BTK*), constitutes 85 % of the congenital agammaglobulinemia. Other defects include mutations in μ heavy chain, $\lambda 5$, *Ig α* , *Ig β* , *BLNK*, *PIK3R1* and others [1].

B-cell linker (BLNK) adaptor protein is a key molecule in the signaling pathways of B cells activated by pre- and B-cell antigen receptor (BCR) signals [2]. Activation of pre-BCR

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assembles through phosphorylation induced protein-protein interactions BTK, phospholipase C γ 2 (PLC γ 2) and others to the *BLNK* adaptor protein and thereby facilitates the phosphorylation of PLC γ 2 by BTK. This enables downstream signals setting up for recombination-activating gene (RAG) protein expression, light chain recombination and further differentiation of pre-BII cells [3].

Here, we report a child with a homozygous mutation in the *BLNK* gene with arthritis and dermatitis associated with persistent enteroviral infection.

Case Reports

Patient 1 (P1) is a boy born in 2004 to consanguineous Arab parents and was diagnosed at the age of 6 month with congenital agammaglobulinemia based on his history of recurrent otitis media, diarrhea and a positive family history. Primary

care was provided in Queen Rania Children’s Hospital at Amman, Jordan. He has been maintained on a regular intravenous immunoglobulins (IVIG) therapy. Despite trough IgG serum levels between 300 and 400 mg/dl he did not have serious infections till the age of 8 years when he developed diarrhea. Endoscopy and histopathology were unremarkable. At the age of 9 years a polyarthritis of the wrists, ankles, and knees was diagnosed. Seven months later, he had an exacerbation of his diarrhea (with negative cultures) and hypoalbuminemia. The dose of IVIG was increased to 600 mg/kg every 3 weeks. Colonoscopy was performed and demonstrated signs of enteritis with intraepithelial lymphocytes and was suggestive of virally induced colitis or celiac disease. A trial of gluten free diet was unsuccessful. At the age of 10 years he developed generalized edema and dermatitis of the abdomen, upper and lower extremities (Fig. 1a). Skin biopsy demonstrated skin hyperkeratosis and infiltration of lymphocytes into the epidermis compatible with chronic dermatitis. IVIG dose was

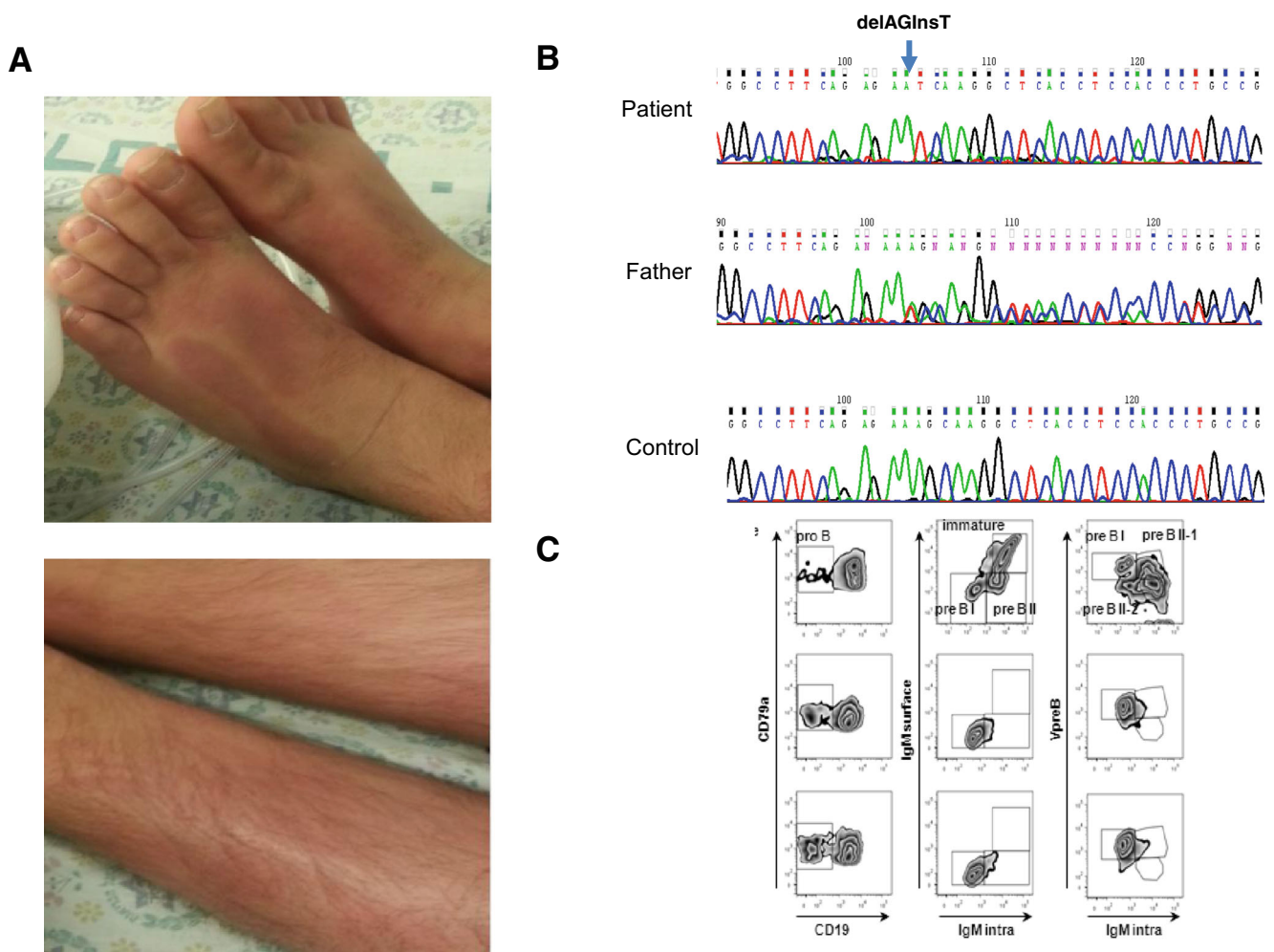


Fig. 1 a Dermatitis of patient P1; note edema and induration of the skin. b c.438_439delinsT in the DNA sequence of exon 6 of the human *BLNK* gene in patients (upper row), obligate heterozygote (middle row), and a healthy control (lower row). The mutation is indicated by the arrow. c B

cell precursor populations in a normal bone marrow (control) and bone marrow of patient P1 and P2. Immature and pre B cell stainings are depicted after gating on CD10^{pos} B cell precursors and pro B cells are gated on CD79a^{pos}/TdT^{pos}/CD3^{neg}/CD16^{neg}/CD33^{neg} cells

increased to IgG trough level of above 700 mg/dl and a treatment with antibiotics, prednisone and methotrexate was started. His skin disease and edema however did not improve and he presented with an induration of the skin of the calf affecting knee joint flexion and normal activity. At that time, microbiological workup revealed a positive polymerase chain reaction (PCR) for enterovirus in his peripheral blood. Enterovirus or ureaplasma were not detected on skin biopsy or from synovial fluid. Despite increased IVIG dose and frequency his enteroviral PCR continues to be positive.

His older sister (P2) was born as the 4th sibling in 2000. She was diagnosed as congenital agammaglobulinemia at the age of 12 months due to the history of recurrent otitis media, sino-pulmonary infections and absent B lymphocytes. IVIG replacement was started. She was treated with rotating antibiotics, steroids and inhaled steroids for obstructive lung disease. Several episodes of culture negative diarrhea were observed. She suffered from chronic cough and arthralgia. Due to bronchiectatic changes on computed tomography, she was maintained on antibiotics and IVIG. Of note, enterovirus PCR of the blood was negative.

Autosomal recessive agammaglobulinemia was suspected and parental consent was obtained for genetic investigations.

For whole exome analysis, exonic sequences were enriched in the DNA samples of P1 using SureSelect Human All Exon 50 Mb Kit (Agilent Technologies, Santa Clara, California, USA). Sequences were determined by HiSeq2000 (Illumina, San Diego, California, USA) and 100-bp were read paired-end. Reads alignment and variant calling were performed with DNANexus software (Palo Alto, California, USA) using the default parameters with the human genome assembly hg19 (GRCh37) as a reference. This analysis yielded 47.05 million confidently mapped reads with a mean coverage of X55. We removed variants which were called less than X8, were off-target, heterozygous, synonymous, MAF>0.1 % at dbSNP138 and MAF>1 % in the Hadassah in-house dbSNP. 15 variants survived this filtering but only one affected a gene with immune relevance. This was a homozygous frameshift mutation in the *BLNK* gene, Hg19:Chr10:97983671delTC/InsA, NM_001114094 c.435_436delTC/InsA, p.E145fs25* (Fig. 1b). Genotyping the family members by Sanger sequencing confirmed that the sister (P2) is homozygous for the same mutation, whereas the parents and four healthy siblings are heterozygous.

Further immunological examinations were performed as previously described [4] and demonstrated no peripheral B cells in both siblings. A severe block at the pre B I to pre B II cell stage was observed by flow cytometric analysis of bone marrow aspirates of P1 with nearly absent immature B cells (Fig. 1c).

The T cell phenotype did not demonstrate a common deviation between both patients. In P1 especially absolute numbers of terminally differentiated and effector memory CD8 T

cells were oligoclonally expanded, probably in reaction to the enteroviral infection. Both patients had a low percentage of circulating CXCR5⁺ CD4 memory T cells as previously reported for BTK deficiency (P1: 13.1 %, P2: 17.4 %, norm: 18.2–31.3 % CXCR5⁺ of memory CD4 T cells), [5] which was also confirmed for bona-fide circulating T follicular helper (TFH) like CXCR5⁺CXCR3⁻PD1⁺ CD4 memory CD4 T cells (data not shown).

Discussion

We present two siblings with a homozygous deleterious frameshift mutation in *BLNK*, resulting in a developmental block of B cells at the preB1 to preB2 stage, absence of circulating B cells and agammaglobulinemia. *BLNK*^{-/-} mice also have a block in pre-B cell development in the bone marrow and reduced numbers of splenic mature B cells [6]. But compensatory mechanisms may allow for a leaky block at the pre B cell stage in *BLNK*^{-/-} mice, while the function of *BLNK* in human B cells seems non redundant [3]. This finding resembles the presentation of BTK and λ 5 deficiencies, which are also more severe in humans than in the murine model [1].

Only five patients with *BLNK* deficiency have been reported (Table 1) [1–3, 7]. All reported patients presented with bacterial respiratory tract infections, nearly absent circulating B cells and agammaglobulinemia. Systemic enteroviral infections (especially CNS) were previously reported in patients with XLA and common variable immune deficiencies [8–10]. However, to the best of our knowledge this infection has not been reported in the context of *BLNK* deficiency. Interestingly, the patient presented with skin and joint manifestations, as had been described in *BTK*-deficient patients. Thus, corroborating the special role of antibody-mediated mechanisms in the control of systemic enteroviral disease [8, 11]. However, as enteroviral infection was not isolated from skin biopsy or synovial fluid, its role in the etiology of P1 dermatitis and arthritis remains unclear [12–14].

With the improved diagnosis and the prompt wide use of IVIG fewer patients develop enteroviral meningoencephalitis [14, 15]. However, once infection occurs, IVIG treatment often fails to achieve clinical remission [16–18]. High doses IVIG and Pleconaril were suggested for the treatment of enteroviral meningoencephalitis, although more reports need to be awaited [18, 19]. The two cases described here provide support for the notion that in *BLNK* patients agammaglobulinemia, absent B cells, low TFH cells and the infection profile closely resemble findings in BTK deficiency and so far no abnormalities due to the absent expression of the protein in cells other than B cells have been identified.

Table 1 Reported patients with *BLNK* adaptor protein deficiency

Patient * (reference)	Age at onset (years), gender, ethnicity	Infection profile/outcome	Additional manifestations	Ig levels at diagnosis (mg/dL)	Circulating CD19 ⁺ B cells (% of lymphocytes)	<i>BLNK</i> mutation
Pat 1.1 (P1 in current report)	0.5, M, Arab	Recurrent otitis media, chronic diarrhea, enteroviral viremia/ currently 10 years old, persistent enteroviral infection	Chronic polyarthritis, dermatitis and sensorineural hearing loss	Undetectable	0	Homozygous c.435_436delTCInsA p. E145fs25*
Pat 1.2 (P2 in current report)	1, F, Arab	Recurrent diarrhea, otitis media and sino-pulmonary infections/ currently 15 years old, doing well on IVIG	Arthritis, bronchiectasis	Undetectable	0	Homozygous c.435_436delTCInsA p. E145fs25*
Pat 2 (Lagresle-Peyrou et al.[3])	6, M, NA	Recurrent otitis and pneumonia/ NA	None	Undetectable	0	Homozygous c.844C>T p.R282X NA
Pat 3.1 (Minegishi et al.[2])	6 months, M, NA	Recurrent otitis/ death due to Pseudomonas sepsis at 16 months.	neutropenia	NA	NA	NA
Pat 3.2 (Minegishi et al.[2])	8 months, M, Caucasian of Scot/Irish ancestry.	Recurrent otitis and pneumonia Hepatitis C/ currently 35 years old, intermittent protein losing enteropathy.	Intermittent protein losing enteropathy	Undetectable	0.05	Homozygous c.30C>A, p.P10P/c.47+3 A>T
Pat 4 (M.E. Conley, [1])	8, F, Turkish	Recurrent respiratory infections, diarrhea, otitis, Septic arthritis (proteus mirabilis in joint fluid) and conjunctivitis/ currently 20 years old, doing well on IVIG.	Resolved hepatitis with no clear diagnosis	IgG 111, IgA<6 IgM 10	0.01	Homozygous c.367 C>T, p.R123X

* Van Zelm et al. [7] reported of two additional patients. However, clinical, immunological and specific mutations details are not available //IG intravenous immunoglobulins, NA data is not available, M male, F female

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Conflict of Interest The authors declare that they have no conflict of interest.

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