Case Report

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A Novel Homozygous CGA > TGA Mutation at Codon 123 (Exon 6) of B-Linker Protein (BLNK) as a Potential Cause of Hepatopathy and Rickets: A Case Report

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ABSTRACT

BLNK deficiency is a subtype of autosomal recessive immune disorders that involves a lack of B cells, agammaglobulinemia, and recurrent infections. We present the case of a 29-year-old Turkish female with BLNK deficiency caused by a novel homozygous CGA > TGA mutation at codon 123 (exon 6) in the BLNK gene. She developed severe liver failure and rickets at the age of 12. Although BLNK mutations are a rare cause of agammaglobulinemia, it is important to consider them in patients with B-cell deficiency and non-immune involvement.

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INTRODUCTION

Agammaglobulinemia, a part of primary immunodeficiency disorder (PID), is caused by repeated infections that result in the absence of mature B cells in circulation and a severe deficiency of antibodies. X-linked agammaglobulinemia (XLA) or Bruton agammaglobulinemia is the most prevalent type of congenital agammaglobulinemia in patients (1). Most other patients with genetic abnormalities exhibit early blockage of B cell development, as shown. Mutations that cause autosomal recessive (AR) agammaglobulinemia (ARA) (2) occur in genes encoding parts of the pre-B cell receptor (BCR) complex (IGHM, IGLL1, CD79A, CD79B) and in the signal transduction genes of the BCR (BLNK, PIK3CD, PIK3R1, SLC39A7). Cases of agammaglobulinemia (2, 3) have also been identified with TOP2B and TCF3 mutations.

The BLNK gene is encoded by the 18 exons of the SLP-65 or BASH gene found on chromosome 10q24.1, which serves as a critical molecule in B cell lymphopoiesis (1). The BCR cell signaling cascade begins with BLNK binding to Ig and Syk's phosphorylation of tyrosine residues, leading to the binding and

activation of downstream signaling molecules. The phosphorylation process activates the BTK and phospholipase C gamma-2 protein and determines their ability to bind to the BLNK/SLP65 adapter protein. This complex's signaling causes RAG proteins to be expressed, resulting in IgG recombination and pre-B cellular differentiation. The levels of mature B lymphocytes in peripheral blood significantly decrease due to a developmental failure of B cells (4). The inability of B cells to produce plasmocytes leads to a marked reduction or complete absence of all immunoglobulin isotypes. BLNK deficiencies (4-10) have been reported in a few patients with a rare form of agammaglobulinemia. We present the first patient with BLNK deficiency with hepatopathy and stage 3 rickets.

CASE PRESENTATION

A 3-year-old female patient whose parents are related was referred to us with complaints of severe recurrent lower respiratory tract infections and diarrhea.

Upon diagnosis, she was found to have agammaglobulinemia and severe B cell lymphopenia. The absolute counts of CD19+ B cells, class-switched memory B cells and transitional B cells were very low. Her detailed immunological findings can be found in Table 1.

A novel homozygous mutation in the BLNK gene (CGA to TGA codon 123, exon 6) was identified using Sanger sequencing with the generous assistance of M.E Conley's lab (USA). The patient had $CD19^+$ B cell levels at 0.01%. We analyzed nearly 500,000 events and found that a small subset of B cells exhibited a phenotype similar to those seen in patients with mutations in Bruton's tyrosine kinase (Btk). These B cells showed varying levels of CD19 that appeared slightly dimmer than usual, along with high surface levels of immunoglobulin M (IgM). Bone marrow samples from the patient revealed a cell distribution similar to that of individuals with Btk mutations and identified a distinct population of stalled pro-B cells. She has been receiving IVIG at a dosage of 400 mg/kg every 3 weeks regularly. At the age of 4, she was diagnosed with aseptic arthritis and responded well to high doses of Ig treatment. At the age of 12, the hepatic enzymes AST (aspartate aminotransferase) and ALT (alanine aminotransferase) levels elevated and remained high for six months.GGT (gamma-glutamyl transferase) and ALP (alkaline phosphatase) levels were also elevated. Hepatic viral markers and autoimmune antibodies were negative. PCR testing for CMV and hepatitis B and C viruses was also negative. The liver enzyme levels at the 1-year follow-up are shown in supplementary Figs. 1, 2, and 3.

Table 1. Immunologic laboratory values of the patient upon admission

	Patients' values at admission	Reference range levels
ALS	2210	1300-3800
IgG (mg/dl)	lgG<120	294-1165
IgM (mg/dl)	lgM:<28	33-154
IgA (mg/dl)	lgA:<17,8	13,5-72
$CD3^+$ T cells (10^9/L)	2090	(1500-3870)
$CD19^+$ B cells (10^9/L)	2	(31-1113)
$CD3^+CD4^+$ T cells (10^9/L)	1110	(88-2360)
$CD3^+CD8^+$ T cells (10^9/L)	9-928	(41-1280)
$CD3^-CD16^+CD56^+$ NK cells (10^9/L)	55	(15-81)
$CD19^+CD27^+IgD^-10^9$/L	6	(25-112)
$CD38^+$ IgM^+10^9/L	0	(150-328)
Anti-HBs (mIU/ml)	329	
Tetanous IgG(mIU/ml)	1,5	

$CD19^+CD27^+IgD^-10^9$/L: Class-switched memory B cells, $CD38^+$ IgM^+ transitional B cells

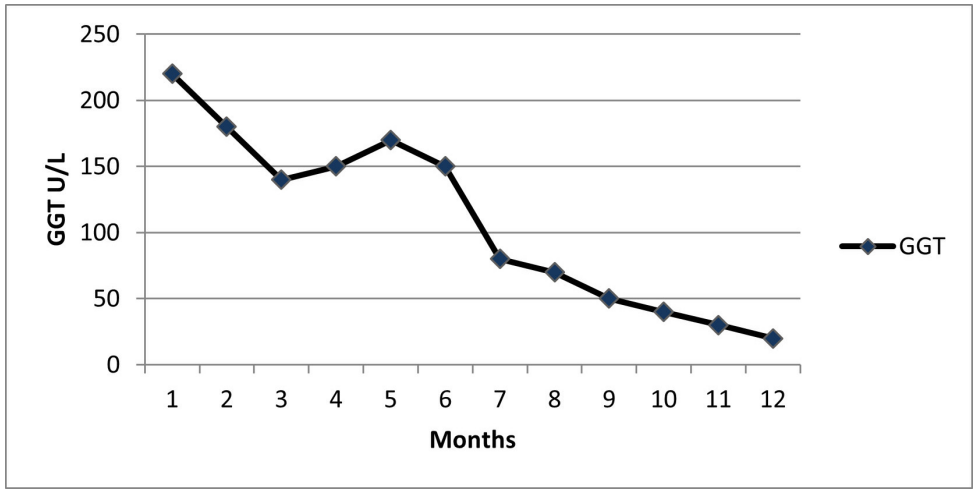

Fig. 1. GGT levels of the patient during the 1-year follow-up.

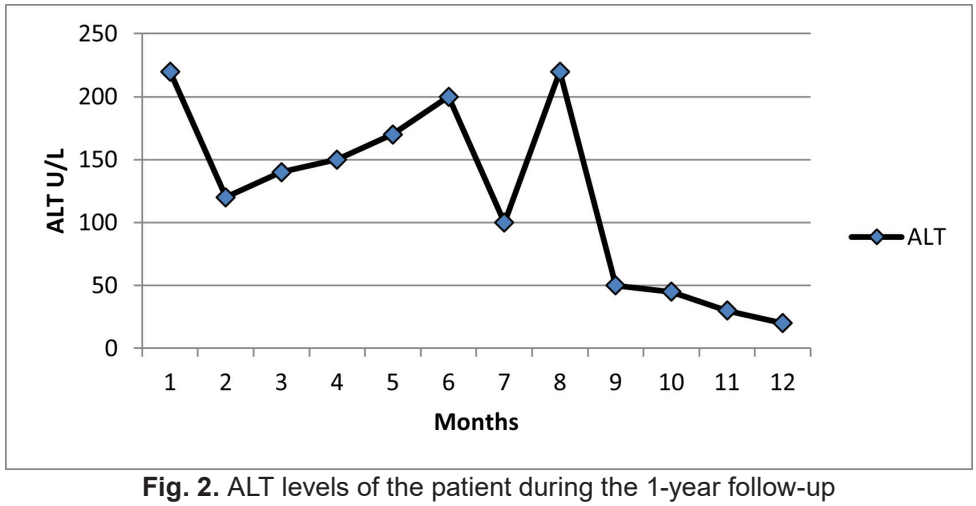

Fig. 2. ALT levels of the patient during the 1-year follow-up

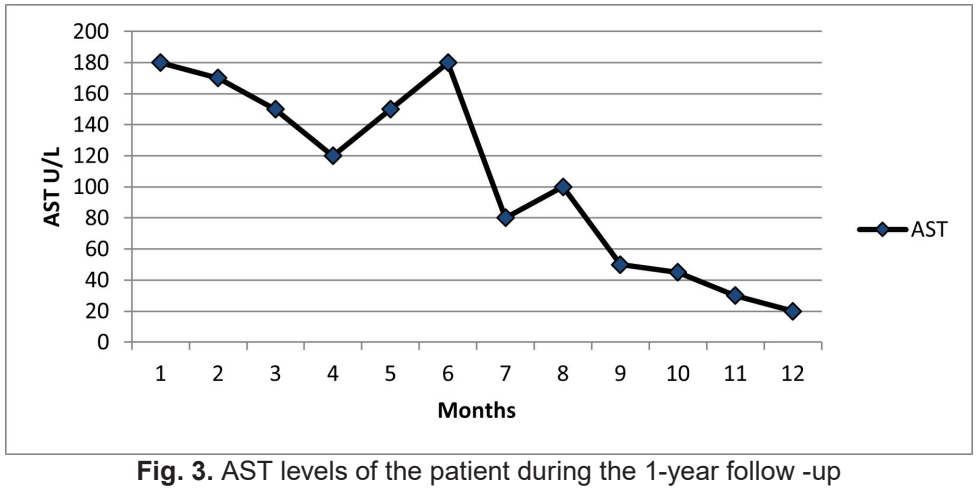

Fig. 3. AST levels of the patient during the 1-year follow -up

The abdominal ultrasound showed a slightly enlarged liver with a grade 2 appearance. The parenchyma echogenicity was coarser and granular, and the spleen size was slightly increased. Hepatomegaly and portal hypertension were also noted.

A liver biopsy indicated hydropic degeneration, mononuclear inflammatory cell infiltration in hepatocytes, and mild degenerative changes. Treatment for liver failure was started. Susequently, she developed ascites and bacterial peritonitis but responded well to antibiotics and ursodeoxycholic acid treatment. Plans were made for a liver

transplant, but her liver function tests improved and returned to normal within a year.

At the same time, she developed hypocalcemia, hypophosphatemia, and hyperparathyroidism (PTH:435ng/L, reference level (15-68)). Osteoporosis was confirmed by a DEXA bone density scan (dual-energy X-ray absorptiometry scan) with a z score of - 4.3. Her vitamin D level was 25ng/ml (reference level:20-50). She was diagnosed with stage III rickets and treated with calcuim lactate and bisphosphonate. Bone metabolism recovered during follow-up, and the z-score on the DEXA scan improved to - 1.2. The patient is currently undergoing IVIG treatment. Her liver enzyme levels remain within normal limits, and she is not showing any signs of infection.

Table 2. Patients with BLNK deficiencies published in the literature.

Patient	Age, gender	Year of diagnose	Clinical manifestations	Ig levels	CD19 $^{+}$B cell(%)	BLNK mutation
Our case	29, female,	1997	Aseptic arthritis, grade 3 rickets, hepatopathy Peritonitis Recurrent diarrhea	lgG:120 lgA:<17,8 lgM:<17,8	0,1	Homozygous CGA > TGA codon123, exon6
Patient 1 (4)	20, male,	1999	Recurrent upper and lower respiratory tract infections, hepatitis C protein-losing enteropathy.	Very low	0.05	Homozygous c.30°C>A (p.P10P)/ c.47 + 3 A> T
Patient 2 (5)	8, female,	2005	Recurrent bronchitis, diarrhea, septic arthritis	IgG: 111 IgA< 6 IgM 10	0.01	Homozygous c.367 C> T (p.R123X)
Patient 3 (6)	6, male,	2014	Recurrent upper and lower respiratory tract infections	Very low	0	Homozygous c.844 C> T (p.R282X)
Patient 4 (7)	0.5, male,	2015	Recurrent upper respiratory tract infections, chronic diarrhea, enteroviral infection, chronic polyarthritis, and sensorineural hearing loss	Very low	o	Homozygous c.435_436 del T CInsA (p.E145fs25∗)
Patient 5 (7)	1, female (older sister of (P4),	2015	Recurrent diarrhea, pneumonia, arthritis, bronchiectasis	Very low	o	Homozygous c.435_436 del T CInsA (p.E145fs25∗)
Patient 7 (8)	28, male,	2018	No infections but chronic renal failure	IgG: 903 IgA 791 IgM 27 (selective IgM deficiency)	14	Compound heterozygous c328 C > G (pPro110Ala)/c472 G > T (pAla158Ser)
Patient 8 (9)	5, female,	2020	Recurrent sinusitis, bronchitis, pneumonia	IgG: 135 IgA< 6 IgM < 18	3.5	Compound heterozygous c.676 + 1 G >A, exon 9 deletion, c.677_746del, p.R227Kfs ∗ 7
Patient 9 (9)	2, male,	2020	Recurrent bronchiolitis, pneumonia, and lymphadenitis	Very low	3	Heterozygous frameshift variant c.452_453dup CC, (p.T152Pfs ∗ 6), c. 525G >A
Patient 10 (10)	3.5, male,	2022	Recurrent pneumonia	IgG 81 IgA< 5 IgM 258	0,05	Homozygous mutation c.790 C> T (p.Gln264Ter)

DISCUSSION

We presented a patient with a new homozygous mutation in the BLNK gene that causes developmental arrest of B cells from the preB1 to preB2 stage, absence of circulating B cells, and agammaglobulinemia. Only ten patients with BLNK deficiency have ever been identified (Table 2). Our case was the first child patient diagnosed with BLNK deficiency in 2002 after the first adult case was described in 1999 by Minegishi et al (4). After IVIG treatment began, our patient did not suffer from recurrent infections. However, to our knowledge, hepatic failure and rickets have not been reported in patients with BLNK deficiency.

Most published cases exhibited recurrent infections in both the lower and upper respiratory tracts, with some cases also showing enteroviral infections and arthritis. B cell deficiency and agammaglobulinemia were significant features in all cases (4-10). BLNK is known for its expression in B cells, monocytes, and macrophages. A study in mice shows that both SLP-76 and BLNK are involved in FcγR signaling in macrophages (11).

Immune dysregulation, intestinal barrier disruption, infections, and malignancy are all potential factors leading to liver involvement in immunodeficiency. The intestinal barrier consists of physical, immunological, and microbial components (12, 13). Intestinal mucosal immunity and the maintenance of intestinal homeostasis are significantly impacted by IgA, the main component of the mucosal immunity. IgA can either kill or inhibit bacteria, neutralize viral toxins, and regulate the colonization and growth of intestinal flora (13). Barrier disruption increases intestinal permeability, leading to the movement of pathogen-associated molecular patterns (PAMPs) into the bloodstream, triggering the innate immune response. The liver and gut are connected through the portal circulation. Immune cells in the liver are the first to encounter PAMPs in the portal blood. Hepatic inflammation plays a role in the development of liver injury and disease (14). Our patient had been experiencing recurrent diarrhea, which, along with low IgA levels, may have caused liver damage by disrupting the intestinal barrier.

Hepatopathy may be seen in patients with primary immunodeficiency, more frequently in CVID. It is characterized by nodular regenerative hyperplasia (NRH), lymphocytic infiltration, and granulomatous disease. In CVID, nodular regenerative hyperplasia is the most frequent cause of liver damage (15). Although NRH appears similar to autoimmune hepatitis due to a specific pattern of hepatic nodules, it is believed to be caused by intrahepatic vasculopathy. This condition occurs in numerous hepatic diseases and causes both hepatocyte injury and regeneration (16). As expected in severe B-cell defect, autoantibodies are typically absent even when patients have significant autoimmune manifestations. Furthermore, NRH may itself be an immune-mediated condition. Moderate to severe inflammatory changes in the liver could indicate various pathogenic mechanisms. In our patient, liver failure resembling autoimmune hepatitis occurred, and liver biopsy results were consistent with NRH. The absence of autoantibodies was attributed to underlying B-cell deficiency.

During the same period, stage 3 rickets was detected at age 12. Although nutritional deficiency can cause rickets, the patient's age was not typical for this condition. Rickets is characterized by impaired bone growth and mineralization at the growth plate, leading to structural bone abnormalities (17). Osteoclasts, which play a key role in bone remodeling, are tightly regulated by hormones and local factors that control their differentiation and activity. BLNK may also impact bone metabolism. Within osteoclasts, the cytoskeleton forms a resorptive microenvironment delineated by an actin ring and houses the cell resorptive machinery. This polarization is mediated by αvβ3 integrin interaction with the M-CSF receptor (18). Upon activation, αvβ3 initiates

a canonical signaling cascade involving c-Src, Syk, Dap12, BLNK, Vav 3, and Rac, leading to cell spreading and actin ring formation. The absence of any component of this signaling pathway impairs osteoclast cytoskeletal organization and bone resorption. In BLNK and SLP-76-deficient mice, cytoskeletal organization and bone resorption were significantly inhibited in cultured osteoclasts, as observed in an experimental study (19). In our patient, we propose that combined BLNK deficiency and nutritional factors contributed to the bone metabolism dysregulation.

CONCLUSION

Our case, diagnosed at three years of age, reveals a novel autosomal recessive mutation in the BLNK gene with an atypical clinical presentation compared to previously reported cases. To our knowledge, this represents the first documented instance of combined hepatopathy and bone metabolism disorder in BLNK deficiency. The concurrent presentation of Hepatopathy and rickets in our patients suggests these clinical manifestations should be considered in the evaluation of BLNK deficiency. As a rare cause of B-cell deficiency, BLNK deficiency warrants further investigation into its potential multisystem effects, particularly regarding hepatic involvement and endocrine dysfunction.

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AUTHORS' CONTRIBUTION

SSK, YK, and HK contributed to the conception and design of this study. SSK critically reviewed the manuscript and supervised the entire study process. All authors have read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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