ORIGINAL ARTICLE

A HETEROZYGOUS *BCL11B* GENE VARIANT INDUCES SCID AND HYPO-IMMUNOGLOBULINEMIA IN A PAKISTANI FAMILY

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Background: The gene B-cell lymphoma/leukaemia 11B (*BCL11B*) which encodes the zinc finger transcription factor plays a crucial role in the development of the central nervous system and T cell differentiation by regulating the expression of numerous genes in early development. *BCL11B* gene defects lead to neurological and immunologic disease manifestations. **Methods:** In this study, we enrolled a 2-month-old male patient from a highly consanguine Pakistani family segregating autosomal dominant type of Primary Atopic Disorders (PADs) along with low immunoglobulin levels. Detailed clinical evaluations were performed by expert paediatrician. Whole blood samples were collected in EDTA tubes for genetic analysis. Detailed laboratory tests including complete blood count, and serum immunoglobulin levels, were performed. **Results:** Genetic analysis revealed four mutations in different genes. A heterozygous missense mutation [(c.2035C>T; p.Pro679Ser), ENST00000357195.8] in the gene *BCL11B* gene was found segregating (parents and healthy siblings were homozygous normal while patient was heterozygous) in the family. Flow cytometery and serum immunoglobulin estimation revealed Elevated Total Leukocyte Counts (TLC) and low serum immunoglobulins. **Conclusion:** The current study describes a novel heterozygous missense mutation in gene BCL11B. Clinical investigations revealed novel findings with novel clinical manifestations including short-term neurological seizures and recurrent pneumonia.

Keywords: DNA Sanger sequencing, *BCL11B* gene, Hypo-immunoglobulinemia, Complete Blood Count, CBC

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INTRODUCTION

Severe Combined Immune Deficiency (SCID) and Neuro-Developmental Disorders (NDD) are a group of monogenic disorders characterized by dysregulated allergic effector responses, leading to heightened susceptibility to atopic diseases such as asthma, eczema, food allergies, allergic rhinitis, intellectual disability and language disorders.[1] Major clinical features include severe atopic dermatitis, food allergies, allergic asthma, urticaria, eosinophilia, and elevated IgE. PAD manifestations include comorbid immunodeficiency and immune dysregulation such as systemic lupus erythematosus (SLE) and autoimmune vasculitis as is the case in some patients with dominant-negative STAT3 variants.[1] Due to the heterogeneous presentation of PADs, it can often be difficult to differentiate monogenic from polygenic aetiologies. In recent years, the rapid development of genome sequencing technology has provided an effective solution in the diagnosis of such heterogeneous disorders with varied clinical presentations.

The gene B-cell lymphoma/leukaemia 11B (*BCL11B*, OMIM 617237) is localized on chromosome 14q32 which encodes a zinc finger transcription factor protein essential for development of T-cell, skin, and teeth. Alternate BCL11B splicing yields four isoforms with variable length of protein. The isoform 1 (NM_138576.4; NP_612808.1), consist of four exons and encodes 894 six zinc finger C2H2-type domains.[2,3]

The protein is involved in proliferation, apoptosis, and T-cell receptor modulation by controlling DNA-binding transcription and directing haematopoietic progenitor migration.[4] Heterozygous mutations of *BCL11B* have been identified in some patients with developmental disorders and severe immunodeficiency (SCID, IMD49) with dysmorphic features, skin abnormalities, and global developmental delay.[5,6] *BCL11B* knockout mice (without functional BCL11B) showed lacking the functional T-cell lineage by affecting the differentiation and function of thymic lymphocytes.[7]

In the current study, we report a 2-months-old male patient belonging to a family of Pakistani origin with severe combined immunodeficiency and mild neurological seizures. Genetic analysis of the patient revealed a missense mutation in the gene *BCL11B*.

METHODOLOGY

To conduct this research study ethical approval was obtained from the Institutional Review Board (IRB) of HBS Medical College (EC20/4), Islamabad, Pakistan. Informed written consent was obtained from the parents and healthy siblings.

The current research study was a 'family-based genetic association study'. To find the inheritance pattern of the disease considering the shared genetic background among family members, the DNA sequencing data was analysed using BioEdit —a biological sequence alignment editor.

In the current genetic study, a three-generation highly consanguineous Pakistani family was selected with a positive family history of SCID. A 2-year-old male child was first hospitalized at age 2 months with birth weight 5.1 Kg with symptoms of cough, chest congestion, and recurrent pneumonia along with his healthy parents and siblings were enrolled in this study (Figure-1A). The patient was admitted to Department of Paediatrics, Combined Military Hospital, Rawalpindi. An expert team of paediatricians performed detailed clinical investigations. Laboratory tests were ordered including blood complete picture (CP), serum immunoglobulin levels, and flow cytometry. While taking family history mother of the patient disclosed that a male child with similar disease symptoms died at the age of two months. Based on this crucial information physician suggested a positive family history and there might be involvement of genetics. To rule out immune deficiency or related genetic anomalies, genetic testing was ordered.

Patient's peripheral blood sample (5 mL) was acquired in EDTA tubes (BD Vacutainer® EDTA Tubes, Becton Dickinson, UK). Lymphocyte subset analysis was performed in the Department of Immunology, Armed Forces Institute of Pathology, using flow cytometry with a standard protocol BD FACSCanto™ II Clinical Flow Cytometry System (Becton Dickinson, San Jose, USA). To estimate serum immunoglobulin levels 2 mL blood samples were acquired in a serum separator tube (SST) BD FACSCanto™ II Clinical Flow Cytometry System (Becton Dickinson, San Jose, USA).

To perform genetic analysis 3 mL blood samples were obtained from patients in EDTA tubes. DNA extraction and genetic analysis was performed at Alpha Genomic Islamabad. The identified pathogenic variant was tested in the healthy family members using exon-specific primers DNA Sanger sequencing.

RESULTS

We enrolled one 2-month-old male patient. The family history was positive with one patient who died at two months of age with similar disease manifestations. According to pedigree, the patient belonged to a four-generation family where the parents are first cousins with a recessive mode of inheritance of disease (Figure-1A). A patient with a severe productive cough at the age of two months and 10 days was admitted to CMH Rawalpindi, Pakistan. He was suffering from chest congestion for 14 days and fever (101.5 °F). For a few minutes, he suffered focal seizures.

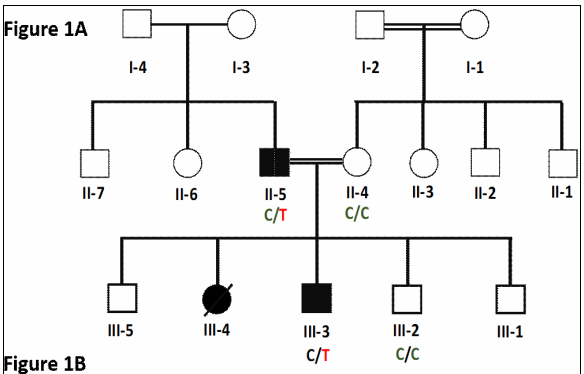

Figure 1B

Mother (II-4)	Father (II-5)	Patient (III-3)	Healthy SonIII-2
C/C	C/T	C/T	C/C
Healthy	Genotype Patient	Patient	Healthy

Figure-1: A=Family Pedigree and B=Genotype

Figure-1A: Family Pedigree. Circles represent females while squares represent males. Filled squares and circles characterize patients. Parents with double lines display cousin marriages. Figure-1B presents *BCL11B* SNP analysis. C/C genotypes are homozygous and denote healthy while the C/T genotype is heterozygous and represents patient (III-3). Mother II-4 is homozygous healthy with (C/C) genotype while Father (II-5) is genotypically heterozygous (C/T) which is a disease genotype with low disease manifestations due to low penetrance. III-3 is a heterozygous (C/T) patient. Male sibling III-2 with genotype C/C is healthy.

Blood complete picture test revealed persistent raised total leukocyte count (TLC, 88.7 counts/µL). Platelet counts were also elevated (1,099 cells/µL). Monocytes (4.9 cells/µL), Basophils (0.2 cells/µL), and Haemoglobin levels (11.8 g/dL) were within the reference range. (Table-1). Serum Immunoglobulin levels were very low (Hypogammaglobulinemia) including (IgA<0.15; IgM 0.3, IgG 2.26, and IgE 4.0). (Table-2). Due to persistent high levels of TLC lymphocyte subset analysis could not be performed.

Family history, disease symptoms, and low serum immunoglobulin levels showed possible involvement of genetics in this family. Next Generation Sequencing (NGS) revealed multiple mutations as shown in Table 3. Genetic analysis showed interesting variants in multiple genes. A missense variant (c.593C>T; p.Thr198Met) in the gene *C2orf81* on chromosome 2p23.2 was identified which

was initially considered a pathogenic. A second missense variant (c.593C>T, p.Thr198Met) is identified in the gene *VSIR* located on chromosome 10q22.1. Both of these variants were non segregating with disease manifestations within the family. Another interesting heterozygous missense variant (c.2035C>T; p.Pro679Ser) is observed in gene *BCL11B* located on chromosome 14q32.2. SNP-based segregation studies confirmed the involvement of the *BCL11B* variant (c.2035C>T; p.Pro679Ser) in this family. Father was with the patient's heterozygous (C/T) genotype while the mother and one of the siblings were homozygous (T/T). Due to low disease penetrance father showed only a few disease symptoms while the patient (III-3) showed full disease symptoms. The healthy sibling had homozygous health (C/C) (Figure-1B).

Table-1: Blood count of the patients

Test	Patient	Reference Range
Total Leukocyte Count (TLC)	**88.7**	5.0–19.0 Cells/µL
Absolute Neutrophil Count (ANC)		
Monocyte	4.9	5–9 Cells/µL
Basophil	0.2	0–1 Cells/µL
Eosinophils	**0.5**	1–6 Cells/µL
Absolute Lymphocyte Count (ALC)		
Haemoglobin	11.8	11.5–16.5 g/dL
Platelets	**1,099**	210–500 cells/µL

Table-2: Serum immunoglobulin levels

Test	Patient	Reference Range
Serum IgM	**0.3**	0.5–3.0 mg/dL
Serum IgG	**2.26**	6.5–16 mg/dL
Serum IgA	**<0.15**	0.4–3.5 mg/dL
Serum IgE	4.0	≤13 IU/mL

Table-2: Patient gene variants

Gene Name	Chromosome	c.DNA Change	Amino Acid Change	READS	CAAD
C2orf81	2p23.2.	c.451G>T	p.Glu151Ter	242	33
VSIR	10q22.1	c.593C>T	p.Thr198Met	169	26,2
IFIH1	2q24	c.1943del	p.Gly648ValfsTer16	111 22	111 22
BCL11B	**14q32.2**	**c.2035C>T**	**p.Pro679Ser**	**170**	**18,75**

c.DNA=Complementary DNA, CAAD=Combined Annotation Dependent Depletion

DISCUSSION

In the current research study, we enrolled a highly consanguineous Pakistani family with low immunoglobulin levels and probably low levels of circulatory T-cells. The gene *BCL11B* encodes a transcription factor called C2H2-type zinc finger protein, which plays an important role in T-cell development, especially during the early stages and development of the central nervous system. The protein functions both as a gene activator and repressor which affects cell differentiation and apoptosis.[8]

The B-cell leukaemia/lymphoma 11B (*BCL11B*) is located on chromosome 14q32.2. The gene *BCL11B* (isoform-1) encodes 894 amino acids through four functional exons which compose six Zinc finger C2H2-type domains (OMIM, 617237).[2] To date, there are 17 variants of the *BCL11B* gene identified in 19 patients through different studies. The major types of *BCL11B* genetic variation include frameshift, non-sense, missense, and complex chromosome rearrangement. However, there is still no report on splicing variation.[8,9] Mutations in *BCL11B* are associated with ($T^-B^+NK^+$) type of Severe Combined Immune Deficiency and neurological seizures regulating the expression of numerous genes.[8,10]

In the current study, we identified a novel mutation in the gene *BCL11B* (c.2035C>T; p.Pro679Ser). This mutation lies in exon four of the gene which encodes all six zinc finger domains, which are crucial for the protein's DNA binding function. The missense mutation possibly affects the zinc finger domains which lead to affect the protein function.

The current study revealed raised TLC levels along with low levels of immunoglobulin levels. These findings are in contrast to those already reported in the previous reports where immunoglobulin levels and TLC levels were within the reference range.[8,10,11] Due to elevated total leukocyte count, Flow-cytometery was not performed. Based on previously published findings we can predict the possible T-cell deficiency. As for the disease symptoms are a severe cough and recurrent episodes of pneumonia and seizure attacks match with the findings reported earlier.[8,10–12]

CONCLUSION

SCID, with or without low immunoglobulin levels, is a rare genetic disorder characterized by a severe deficiency of both B cells and T lymphocytes, while natural killer (NK) cells may remain present, and immunoglobulin levels can vary. Genetic analysis in patients exhibiting T-cell or B-cell deficiency in association with or without low immunoglobulin levels can confirm the disease diagnosis.

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