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CASE REPORT

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Mutation in BTK Gene Causing an Atypical Presentation of XLA

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ABSTRACT

Patients with X-linked agammaglobulinemia (XLA) are susceptible to bacterial infections particularly due to encapsulated pyogenic bacteria, organisms for which opsonization by antibody is a primary host defense. Additionally, enteroviral encephalitis and chronic diarrhea due to Giardia are seen in XLA. However, fungal infections are rarely present. In this report, we describe the case of a 17 month-old boy with absent BTK protein expression caused by a novel *de-novo* variant of the BTK gene, who presented with neutropenic fever and sepsis caused by Candida and *Pseudomonas aeruginosa.*

Keywords: XLA, BTK, Fungal infection, Candida

INTRODUCTION

X-linked agammaglobulinemia (XLA) was first identified in 1952 by Ogden Bruton as a primary immunodeficiency disorder (PID) (XLA OMIM 300755) (1). It is one of the most common PIDs in childhood, with an estimated prevalence ranging from 1:100,000 to 1:200,000 live births (2, 3). Bruton tyrosine kinase (BTK), the gene mutated in XLA, is located on the X chromosome (4, 5). The associated B cell deficiency (<2%), along with the absence of precursor B cell differentiation in the bone marrow, supports a critical role of BTK in B cell development and maturation. In XLA, plasma cells fail to form immunoglobulin classes and specific antibody responses are insufficient (5).

Patients with XLA are susceptible to bacterial infections (mainly sinopulmonary infections, skin infections, sepsis, lymphadenitis), particularly those due to encapsulated pyogenic bacteria, organisms for which opsonization by antibody is a primary host defense (6). Additionally, enteroviral encephalitis and chronic diarrhea due to Giardia are seen in XLA, are usually difficult to treat, and may be associated with delay in diagnosis (7). Fungal infections are rarely present in these patients. *Ex vivo* nail infection with *C. albicans* and *T. mentagrophytes* and invasive pulmonary aspergillosis after chronic sinusitis have been reported in XLA patients in previous studies (8, 9).

Here, we present an XLA patient who presented with neutropenic fever and sepsis caused by *Candida* and *Pseudomonas aeruginosa*.

CASE REPORT

The patient was 17 months of age when he presented with neutropenic fever, acute necrotizing tonsillitis, and sepsis. The blood culture was positive for *Candida* and *Pseudomonas aeruginosa*. There was no history of intravenous catheter use or admission to the critical care unit, which are risk factors. There was a previous history of recurrent bronchiolitis during infancy. His physical

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examination revealed growth retardation (body weight <3rd percentile). Laboratory tests on admission revealed agammaglobulinemia, absence of anti-rubella antibodies, and 1% CD19 lymphocytes (Table I). Immunoglobulin replacement therapy and trimethoprim-sulphametaxole (TMP-SMX) prophylaxis were initiated. Informed consent was obtained from the patient for sending the blood sample to the NIH. Whole exome sequencing (WES) of the patient that was performed at the NIH (protocol #06-I-0015) revealed a *de-novo* variant of the BTK gene in the patient (chrX:g.100614313G>A, NM_000061.2:c.862C>T, p.(Arg288Trp) (Figure 1). Flow cytometric analysis of BTK protein expression revealed severely decreased levels in the patient's CD14 monocytes compared to healthy control cells. Figure 2 shows the expression levels of BTK protein in the patient and the healthy control. Our patient improved, without any subsequent neutropenia or infections, with monthly intravenous immunoglobulin infusions and TMP-SMX prophylaxis.

Flow cytometric analysis of BTK protein expression

Blood samples were collected from the patients and control subjects after signing the informed consent form approved by the Ethics Boards and Commissions of Hacettepe University. Peripheral blood mononuclear cells were separated by density gradient centrifugation over Biocoll separation solution following centrifugation at 2000 rpm for 20 minutes (Biochrom, USA). Following collection of the buffy coat to a fresh tube, cells were washed 1 time in 1X PBS (Phospphate Buffer Saline) and centrifuged. The cell pellet was resolved in 1 ml 1X PBS and 100 ul of cell suspension was transferred to a flow cytomery tube. Cells were stained with Allophycocyanin (APC) tagged anti-CD14 antibodies. Following the washing step, fixation was performed with 100 ul fixation buffer for 15 min. Then the cells were permeabilized with permeabilization buffer for 15 min and washed (BD, USA). Phycoerythrin (PE)-tagged anti- BTK antibody was applied to the cells for 30 min at room temperature (BD, USA). Then, cells were washed and analysed using the FACSDiva programme in the FACS CANTO II flow cytometer (BD, USA).

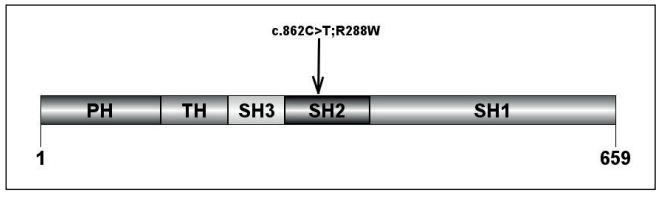

Figure 1. Mutation of the patient and the affected domain.

DNA extraction, library preparation, whole exome sequencing, and data analysis

Genomic DNA was extracted by standard protocols from the peripheral blood of the index case after informed consent. The concentration and quantity of the DNA samples were measured and the samples were then prepared for whole exome sequencing. The raw sequence file was processed for generating mapped reads, analyzing coverage data, and variant calling. The variants that passed the quality score filters were annotated. Variants were selected according to the criteria at the variant level, including minor allele frequency (MAF), variant annotation, and potential functional effects. We selected rare and possibly causative variants with a MAF<0.01 using genetic variation databases (e.g., dbSNP, 1000 Genomes Project, and Genome Aggregation Database) and databases of germline mutations (HGMD and ClinVar). We removed variants that were already found in our in-house database. We focused on the genes (485) accounting for inborn errors of immunity. Furthermore, we kept missense, nonsense, splice site, frameshift, start and stop codon variants, and in-frame indels that were predicted to be deleterious.

DISCUSSION

In this report, we describe the case of a 17 month-old boy with absent BTK protein expression caused by a *de-novo* variant of the BTK gene. The age of diagnosis of the patient was quite earlier than the usual age of diagnosis (median 3.5 years) reported in the study including 32 patients with XLA from our department (10). Patients are

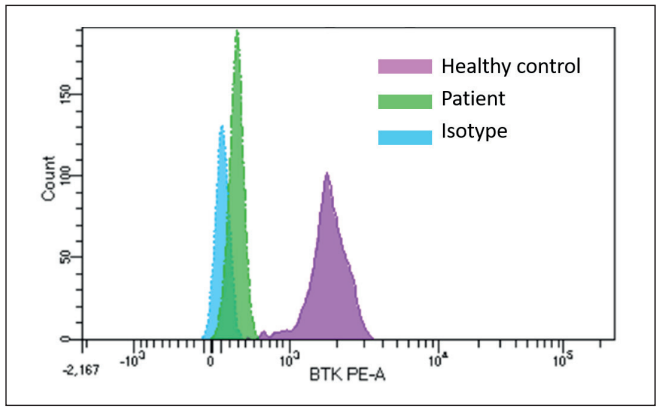

Figure 2. BTK protein expression levels in the patient and the healthy control subject. After gating the CD14+ monocytes, the BTK protein expression levels were investigated using MFI median values.

Table I. Laboratory findings of the patient

Tests	On admission 17 months	Reference values
Complete blood count		
Hemoglobin (g/dl)	11.8	
Leukocyte (/mm³)	4400	6400-12000
Platelet (/mm³)	397.000	150.000-450.000
Absolute lymphocyte count (/mm³)	**2500**	3600-8900
Absolute neutrophil count (/mm³)	**1200**	>1500
Total protein(g/dL)	6.32	5.6-7.5
Albumin(g/dL)	4.55	3.5-5.2
Serum immunoglobulins		
IgA (mg/dl)	**<6.67**	26-296
IgG (mg/dl)	**69**	604-1941
IgM (mg/dl)	**20**	71–235
Total IgE (IU/ml)	74.3	
Lymphocyte subset (% / number) (/µl)		
CD3	90 % 2250	43-76 200-2100
CD4	58 % 1450	24-48 500-2400
CD8	33 % 825	14-33 300-1600
CD16-56	16 % 400	4-23 100-1000
CD19	**1 %** **25**	14-44 1700-6900
CD20	**0.3 %**	
T Lymphocyte subgroups (%)		
CD4+ Cell Ratio	57	29-59
Naive CD4+ CCR7+/CD45RA+	76	57.1-84.9
Central memory CD4+ CCR7+/CD45RA-	13.5	11.3-26.7
Effector Memory CD4+ CCR7-/CD45RA-	5.9	3.3-15.2
TEMRA CD4+ CCR7-/CD45RA+	4.2	0.4-2.6
TREC CD4+/CD45RA+/CD31+	53	31-81
CD8 + Cell Ratio	35	19-29
Naive CD8+ CCR7+/CD45RA+	78	28.4-80.6
Central memory CD8+ CCR7+/CD45RA-	1.6	1.0-4.5
Effector Memory CD8+ CCR7-/CD45RA-	5.5	6.2-29.3
TEMRA CD8+ CCR7-/CD45RA+	14.5	9.1-49.1
T lymphocyte activation		
CD3+ CD25+	85	43.3-97.4
CD4+ CD25+	32	
CD3+ CD69+	84	44.8-99.6
CD4+ CD69+	37	
CD3	96	50.1-80.7
CD4	43	
CD25	87	77.1-97.9
CD69	87	77.3-99.2

being diagnosed earlier as a result of increased awareness of PIDs and the increased use of genetic diagnostic tools in our country.

Our patient had a serious fungal infection, which is unusual in XLA patients. Patients with antibody deficiencies, including XLA, are more susceptible to bacterial infections than fungal infections. Fungal infections are particularly common in primary immunodeficiencies affecting innate or cellular immunity. Fungal infections, particularly invasive mycoses, are related to BTK inhibitors. According to some authors, this is because BTK is expressed in neutrophils as well as B cells (11). However, individuals who are BTK deficient do not typically develop fungal infections, in contrast to those who receive BTK inhibitors. This might be due to impaired T cell activity in individuals with chronic lymphocytic leukemia who are often treated with BTK inhibitors, or it could be because growing without BTK favors alternate antifungal immune pathways.

Neutropenia may occur at any time during follow-up in 15%–25% of patients with XLA (12). The patient's neutropenia at the time of diagnosis may have aided the progression of sepsis caused by the fungal pathogen. As was the case with our patient, neutropenia has been found to improve following antibacterial and immunoglobulin therapy.

Although rare, infections with *Pseudomonas* species, and neutropaenia also exhibit XLA clinical manifestations. In the presence of XLA, soft tissue infections caused by *Pseudomonas* species (called ecthyma gangrenosum) and pseudomonal sepsis may be fatal. The morbidity and mortality of the patients can be reduced by using proper antibiotics for *Pseudomonas* as soon as possible. It is recommended, particularly in boys, to measure serum immunoglobulins and CD19+, CD20+ lymphocytes when evaluating otherwise unexplained neutropaenia with soft tissue infections and sepsis (13, 14).

Other common clinical manifestations and complications during the follow-up of XLA patients have been reported as autoimmunity, chronic atrophic gastritis, Crohn's disease, malnutrition and less frequently obesity, malignancy, neutropenia, polyarthritis nodosa, juvenile idiopathic arthritis, and bronchiectasis (3). Since our patient is still young, he is at risk of developing pulmonary and gastrointestinal complications, as well as autoimmune diseases, and is being closely monitored. Increased awareness of PID means earlier diagnosis for patients with XLA, less complications, and improved quality of life.

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Authorship Contributions

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