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Diamond Blackfan Anemia: A Nonclassical Patient With Diagnosis Assisted by Genomic Analysis

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Diamond Blackfan anemia – an evasive diagnosis in a non-classical patient

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List of abbreviations:

ADA	Adenosine Deaminase
CBC	Complete Blood Count
DBA	Diamond Blackfan Anemia
MCV	Mean Corpuscular Volume
NGS	Next Generation Sequencing

Abstract

Diamond Blackfan anemia (DBA) is an inherited syndrome usually presenting with severe macrocytic anemia in infancy. We describe a toddler with mild normocytic anemia that did not require treatment, who was diagnosed with DBA. This case demonstrates the wide clinical spectrum of DBA, mandating high clinical suspicion of the disease.

Introduction

Diamond Blackfan anemia (DBA) is a disorder of ribosomal production, which is characterized by reduced erythroid precursors in the bone marrow and macrocytic anemia. Erythrocyte adenosine deaminase (ADA) and hemoglobin F levels are usually elevated[1,2]. Extra hematopoietic manifestations include growth retardation, thumb abnormalities, facial dysmorphism and congenital heart defects. Most patients are diagnosed before the age of 1 year. However, recently there has been increasing awareness of atypical milder presentation, termed "non-classical"[1]. We describe a toddler with DBA who presented with mild normocytic anemia and neutropenia, in which the only clue for the diagnosis was a triphalengeal thumb. This case emphasizes the need to consider DBA in the differential diagnosis in patients with non-classical presentation.

Case report

A 15-month old female patient was referred to our hematology clinic for evaluation of anemia. The patient was born at term and her birth weight was 2200 grams. Family history was unremarkable. She was diagnosed with a single umbilical artery and a right

triphalangeal thumb. Echocardiography at the age of 4 months demonstrated a small patent foramen ovale. At the age of 8 months the patient underwent correction of her thumb abnormality.

At the age of 6 months her hemoglobin level was 8.6g/dL with a mean corpuscular volume (MCV) of 86.4 fl. Ferritin, serum iron, vitamin B12 levels and thyroid function tests were normal. Osmotic fragility test was also normal. Hemoglobin electrophoresis at the age of 15 months showed hemoglobin F of 2.4% (normal < 1%) and hemoglobin A2 of 2.7% (normal < 3.5%).

On her first visit in our hematology clinic, the physical examination revealed a systolic murmur of 2-3/6, and a surgical scar on the right thumb. Her complete blood count (CBC) disclosed an hemoglobin of 10.9g/dL, MCV-81fL, reticulocyte count-1%, white blood cell count- 6830/uL, absolute neutrophil count-900/uL, and platelets count of 344,000/uL. Peripheral blood smear revealed a normal morphology of all lineages. Bone marrow aspiration showed normal cellularity, normal maturation of the myeloid cells, and mildly decreased erythroid precursors with a Myeloid:Erythroid ratio of 5:1 (Figure 1). The patient underwent a workup for suspected bone marrow failure that ~~suggested~~ included normal telomere length and chromosomal breakage tests. Due to mild neutropenia, the patient underwent a workup for cyclic neutropenia that was excluded by ~~repeat~~ serial blood counts. Genetic testing was normal for *SBDS* and *ELANE*.

During her follow up, failure to thrive and mild hypotonia were noted. Hemoglobin levels were between 9.9-11.4g/dL (except for a single test of 9.2 during an acute illness), with MCV levels between 79.6-84.3fl. Neutrophil counts normalized. The patient never required blood transfusions.

Eventually, targeted next generation high throughput sequencing was performed. This method involves enrichment of a DNA sample for a pre-determined group of genes, followed by high throughput sequencing[3]. It enables a rapid sequencing of all relevant genes with a deep coverage[4]. This method revealed the change c.527 (+1) G>A (IVS 5+1) in the *RPL5* gene (OMIM 603634) in the patient, and not in family members. Sanger sequencing confirmed the splice site mutation that has not been previously described. It disrupts the splice donor consensus sequence, and is predicted to be deleterious for splicing. The erythrocyte ADA level was 4.5 U/gr of hemoglobin (normal value 0.8-1.2U/gr). Ribosomal RNA analyses were also performed. A 32S rRNA species (a precursor of the 28S rRNA) was evident (Fig. 2). Accumulation of t~~T~~his rRNA precursor ~~specie~~ ~~can be found in~~is characteristic of patients with abnormalities in the large ribosomal subunit[5]. Thus, the ADA levels and rRNA analysis confirmed the pathogenicity of the splice site mutation in *RPL5*.

Methods

Blood samples were collected from the patient and her family members after informed consent was obtained. Genomic DNA was isolated from white blood cells using a DNA isolation kit for mammalian blood (Roche Diagnostics, Mannheim, Germany), according to the manufacturer's instructions. Sanger sequencing was performed as described[6]. Targeted gene enrichment, sequencing and analysis were performed as previously described[3]. Ribosomal RNA species were evaluated as previously reported[5].

Discussion

The classical presentation of DBA includes severe macrocytic anemia that is diagnosed before the age of 1 year, no other significant cytopenias, paucity of the erythroid

precursors in the bone marrow and congenital anomalies. We describe a patient, born small for gestational age, with mild normocytic anemia and transient neutropenia who had a few congenital anomalies: a single umbilical artery, patent foramen ovale, and a triphalengeal thumb. Albeit the atypical clinical presentation, targeted next generation sequencing revealed a mutation in the *RPL5* gene. Elevated levels of ADA and an abnormal ribosomal RNA analysis confirmed the diagnosis of DBA.

The case emphasizes the growing evidence of non-classial presentation of DBA. In this case, the main clue for the diagnosis of DBA was the thumb abnormality. Triphalangeal thumb may occur as an isolated congenital anomaly~~,~~ in association with other hand abnormalities or as a part of a syndrome[7]. Thumb abnormalities are found in 9-19% of patients with DBA[1]. However, the very mild anemia and the lack of macrocytosis ~~did not support~~ reduced suspicion for the diagnosis of DBA and led to a delay in the identification of the syndrome in this patient.

Previous reports include a few patients with DBA who~~that~~ had a late onset anemia or a relatively mild clinical course. Farruggia et al. describe a patient with *RPL5* mutation who~~that~~ was anemic from infancy but became transfusion-dependent at the age of 12 years[8]. An adult onset of anemia was described in a patient with congenital thumb abnormalities and a genetic diagnosis of DBA[9]. A mother of two DBA patients had macrocytosis with no anemia, although carrying the same mutation as her affected children[10]. In a number of cohorts, a few patients were diagnosed at an age older than 12 months or with a mild anemia that required no therapy[11,12,13,14].

The case we describe, along with previously published cases, emphasize the wide clinical variability of DBA and the need to maintain high clinical suspicion of the disorder

in patients with thumb abnormalities, even in the presence of mild anemia and only a moderatte reduction of red cell precursors in the marrow. Incorporation of the targeted next generation sequencing method will not only facilitate an accurate and rapid diagnosis of patients with DBA, but may also broaden our understanding of the clinical heterogeneity of the disease.

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Figure Legends

Figure 1. Bone marrow aspiration of the patient. Left – a representative field. A scale bar is located in the right lower corner. Right – a higher magnification of the marked field. Bone marrow aspiration smears (X 50) were stained with Hematek (Siemens) and photos were taken with a light microscope (BX51, Olympus).

Figure 2. Ribosomal rRNA electropherogram confirms the pathogenicity of the mutation. Total RNA of peripheral blood mononuclear cells of the patient (left panel) compared with a healthy control (right panel). A 32S rRNA specie (black arrow) is evident in the patient's sample.

Figure 1

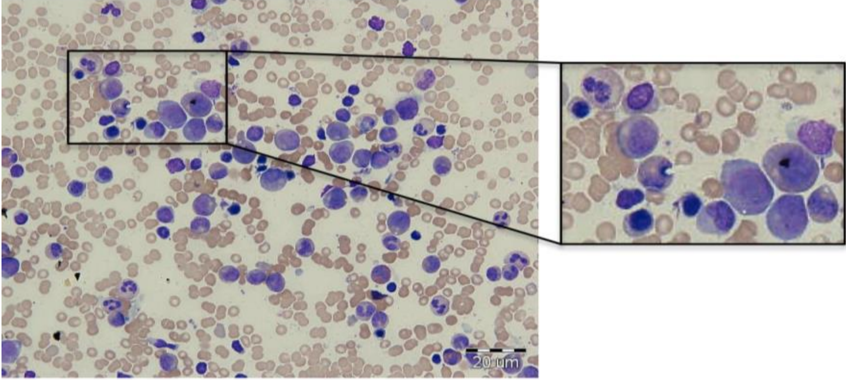

Figure 2