A Novel RPL35A Mutation Associated with Diamond-Blackfan Anemia

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Introduction

Diamond Blackfan anemia (DBA) is a heterogeneous disorder characterized by macrocytic anemia, reticulocytopenia, congenital anomalies, and predisposition to cancer. The hematological symptoms of DBA typically occur within the first year of life. Congenital malformations are present in approximately half of individuals with DBA, including craniofacial, heart, and genitourinary malformations as well as small or malformed thumbs and other upper-limb malformations. Growth retardation also occurs in thirty percent of affected individuals. DBA is typically treated with steroids, red blood cell transfusions, and hematopoietic stem cell transplantation.

The underlying defect of DBA is hypothesized to be faulty ribosomal biogenesis, resulting in pro-apoptotic erythropoiesis and erythroid failure. Mutations are identified in approximately 60% of DBA patients by sequencing fifteen genes, which all encode ribosomal proteins associated with the small or large subunit [Figure 1]. Ribosomal Protein L35a (RPL35a) is a protein in humans encoded by the RPL35a gene, which is located on chromosome 3q29-pter [Figure 2]. Five DBA-associated pathogenic mutations have been identified within this gene.

In our study, a novel RPL35a gene mutation was identified in a 4-month-old Asian male who presented with macrocytic anemia, neutropenia, genitourinary malformations, and growth retardation.

Clinical Presentation

The patient was a non-identical male twin born at 33 weeks gestation. At 4 months of age, he was first evaluated for growth swelling, predominantly on the right. Physical examination showed he was small for his age (2nd percentile by WHO growth charts), with multiple genitourinary malformations, including small penile glans, preocrotal hypospadias, congenital chordee, and hooded foreskin. Laboratory tests showed WBC 3.46, HGB 7.2, HCT 20.6, MCV 102.1, PLT 570, and ANC 0.2. He had no significant family history, and his twin brother was normal in size. His anemia did not improve with 3-month iron supplementation. Bone marrow evaluations revealed relative lymphocytic and megakaryocytic hyperplasia, relative granulocytic hypoplasia, and decreased marrow iron stores. However, an accurate assessment of the marrow cellularity was not possible due to the subcortical nature of this biopsy. Adenosine deaminase was elevated (2.4 U/g Hb) and tests for Swlczman Diamond syndrome and Fanconi anemia were negative.

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Results

Genomic DNA was isolated from the patient’s blood specimen using standardized methodology and quantified. All the analyzed regions of the gene were amplified through polymerase chain reaction (PCR) and sequence alterations were identified by double-stranded sequencing from sense and anti-sense directions. Nine RP genes were sequenced, including RPL11, RPL35A, RPL5, RPS10, RPS17, RPS19, RPS24, RPS26, and RPS7.

Sequence analysis revealed a novel c.227G>C (p.R76P) variant, located in coding exon 3 of the RPL35A gene, resulting from a G to C substitution at nucleotide position 227 [Figure 3]. A highly conserved arginine residue at codon 76 was replaced by a proline residue, an amino acid with dissimilar properties (Grantham distance score=103) [Figure 4]. Evolutionary conservation analysis shows this amino acid position is highly conserved in available vertebrate species [Figure 5]. This variant was not reported in population based cohorts in the following databases: Database of Single Nucleotide Polymorphisms, NHLBI Exome Sequencing Project, and 1000 Genomes Project. In addition, this alteration is predicted to be possibly damaging and deleterious by PolyPhen (0.886) and SIFT in silico analyses (0.000), respectively. No pathogenic mutations or variants of unknown significance were detected in the other nine genes mentioned above. Based on this finding and the patient’s clinical presentation, a diagnosis of DBA was postulated and steroid therapy was initiated (prednisolone 1mg/Kg/day, later reduced to 0.8mg/Kg/day). One year later, his anemia had improved and laboratory tests showed WBC 3.77, HGB 11.5, HCT 33.0, MCV 103.1, PLT 545, and ANC 0.4.

Conclusions

- We report a novel variant of unknown significance in the RPL35A gene (p.R76P) in a DBA patient.
- We suspect that this variant is likely pathogenic and contributing to the patient’s DBA phenotype based on its change of a highly conserved amino acid, and the excellent clinical response of the patient’s anemia to steroid treatment.

References