Polymorphism

RESEARCH

Exome sequencing reveals a novel splice site variant in the *RPS26* gene in a patient with suspected Diamond-Blackfan anemia

Deepak Panwar, Kumar Gautam Singh, Vandana Lal, Atul Thatai[#]

Molecular Diagnostic Division, National Reference Laboratory, Dr. Lal Pathlabs Ltd, Block E, Sector 18, Rohini, New Delhi, India

*Corresponding author e-mail: atul.thatai@lalpathlabs.com

ABSTRACT

Background: Diamond Blackfan anemia (DBA) is an autosomal dominantly inherited congenital disease of bone marrow failure, characterized by anemia and malformations. It is mainly associated with mutations in the ribosomal protein genes that lead to an imbalance in rRNA biosynthesis. *RPS26* was identified to be the disease-causing gene in Diamond-Blackfan anemia. The objective of the present study was to screen likely pathogenic mutations in a patient with severe red cell aplasia.

Methods: Whole exome sequencing (WES) was performed in combination with Sanger sequencing to identify the causative mutation in a patient with severe red cell aplasia and a clinical phenotype resembling Diamond-Blackfan Anemia.

Results: WES led to the identification of a novel heterozygous splice site mutation, c.182-1G>A, in the *RPS26* gene, that resulted in an aberrant transcript in the patient. The identified splice site mutation was not identified in the proband's parents.

Conclusions: A novel heterozygous *RPS26* splicing variant c.182-1G>A was identified in co-segregation with the DBA phenotype after comprehensive consideration of the clinical manifestations, genetic analysis, and whole-exome sequencing data. The current study would play a significant role in better understanding of DBA genetics.

KEYWORDS: Diamond-Blackfan anemia (DBA); Whole exome sequencing (WES); *RPS26*; Splicing variant; Sanger Sequencing

Citation: Panwar et al. Exome sequencing reveals a novel splice site variant in the *RPS26* gene in a patient with suspected Diamond-Blackfan anemia. Polymorphism 2021; 7: 102-107.

Received: December 13, 2021; revised: January 22, 2022; Accepted: January 24, 2022

INTRODUCTION

Diamond-Blackfan anemia (OMIM:613309) is an inherited pure red cell aplasia (PRCA) and bone marrow failure disorder that usually occurs in children under one year of age. While the central phenotype is pure red cell aplasia, it is characterized by normochromic macrocytic anemia and reticulocytopenia. Most Diamond-Blackfan anemia (DBA) patients have growth retardation and several physical malformations are also associated (Vlachos et al., 2008). A total of 30-50% of the patients present malformations in craniofacial features, upper limbs, hearts defects, as well as urogenital defects. The symptoms of this disease are diverse; they can differ even among the affected family members who share the same mutation. The majority of the patients (>90%) are diagnosed within the first year of life, with a minority presenting with anaemia at birth (Ball et al., 1996; Lipton et al., 2006).

Almost all mutations linked to DBA have been found in genes coding for ribosomal proteins (RPs), which include RPS19, RPL5, RPS10, RPL11, PRL35A, RPL26, RPS24, RPS17, RPS7, GATA1 and *RPS26* (MIM 603701) (Boria et al., 2010). Each of these genes encodes the cell's ribosomes, which are important in the process of protein production. Genetic changes or mutations in any one of these genes are thought to interfere with this process, thereby adversely affecting the protein production. Approximately 60% of individuals with DBA have an identifiable mutation in one of these genes. There has been a paradigm shift in the search paremeters of DBA causing genes from the classic cytogenetic and extended linkage analysis to targeted resequencing of the known RP protein genes. Such studies have identified both large and small subunit r-protein gene abnormalities in several families (Gazda et al., 2006, 2008; Cmejla et al., 2007; Farrar et al., 2008; Doherty et al., 2010). Although, the significance of these rare RP-protein gene alterations is not yet clear, it is conceivable that many of them are pathogenic. However, despite the large number of potential DBA genes, sequencing studies evaluating the RP-protein gene complement in DBA patients have failed to find the causative mutation in 40-45% of patients (Doherty et al., 2010). The *RPS26* gene, which is located on chromosome 12, has four exons spanning 2.32 kb and encodes a protein that belongs to the ribosomal protein family. The *RPS26* is a crucial component of the 40S and can regulate its own expression by binding its pre-mRNA and suppressing its splicing (Ivanov et al., 2005; Min et al., 2013). Mutations in the *RPS26* gene could significantly affect the production of the small subunit of ribosome and cause disease (Doherty et al., 2010).

In a cohort study in the year 2010, participants from 117 DBA families aimed to perform a large-scale screening of ribosomal protein mutations in the suspected patients. Five of the 117 patients had mutations in the RPS10 gene and 12 had mutations in the *RPS26* gene. RPS10 gene was found to have the most nonsense mutations, while *RPS26* gene primarily had mutations in the translation start codon. To date, 30 disease-causing *RPS26* variants have been identified, with only three of them being small insertion mutations (Doherty et al., 2010).

Here, we report this experience in the diagnosis of a 17 months old girl having DBA. This pateint had severe red blood cell aplasia with no malformations and no relevant family history. A novel splice site mutation in the *RPS26* gene was identified. Our finding broaden the *RPS26* variant diversity and provide more clinical information on patients with Diamond-Blackfan anemia 10 (DBA10).

Methods and results

Ethics and Consent

The study design and protocol was conducted in accordance with the American College of Medical Genetics and Genomics (ACMG) guidelines, and was approved by the Ethical Review Committee (ERC) of Dr. Lal Pathlabs.

Written informed consent has been taken from parents of child included in the study for publishing the data.

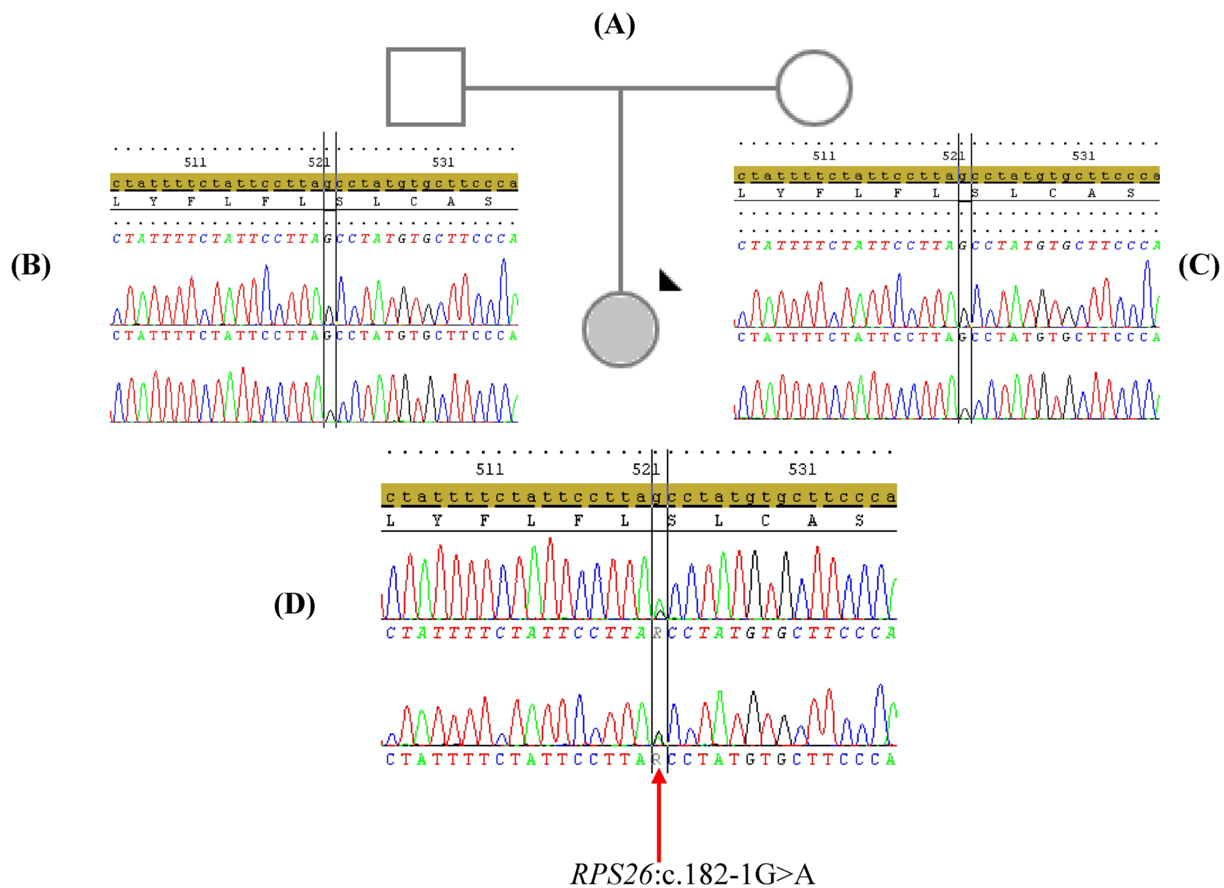

Figure 1. Family pedigree and Sanger sequencing results for the patient and her parents identifying a mutation in the *RPS26* gene. (A) Family pedigree of individual affected by DBA, (B & C) Sanger electropherogram for PCR of her parents (B for the proband's father, C for the proband's mother), neither of her parents carried this mutation, (D) Sanger electropheorgram for the patient identified a heterozygous splice site mutation (c.182-1G>A) in the *RPS26* gene.

Case presentation

The proband, 17 months old girl presented with severe red cell aplasia and low Hb. She was assigned a differential diagnosis of Diamond-Blackfan anemia. To identify the mutation responsible for the disorder, we employed Next generation sequencing (NGS) based Whole Exome Sequencing (WES). WES analysis identified one novel variant (NM_001029.5:c.182-1G>A) in

the *RPS26* gene, which affected a splice site at the junction of exons 2 and 3. The mutation is predicted to disrupt splicing using three different splice prediction tools: MutationTaster (http://www.mutationtaster.org/), FATHMM-MKL (http://fathmm.biocompute.org.uk/fathmmMKL.htm) and DANN (Quang et al., 2015) (Table 1). The identified variant was confirmed by Sanger sequencing in proband and her parents. The partial DNA sequences involving the splicing mutation site were amplified by PCR using primers: Frd 5′-TGTGCTCAGGTATTGGGCTG-3′ and Rev 5′-TCACCGCAGGTCTAAATCGG-3′. PCR products were analyzed by 1% agarose gel electrophoresis and then purified and sequenced on an ABI 3500Dx automated Genetic Analyzer (Applied Biosystems, Thermo Fisher Scientific). Sanger sequencing confirmed that this variant was a *de novo* and showed that none of the patient's parents carried it (Figure 1).

Table 1. Whole exome sequencing analysis identified the *RPS26* gene mutation in the proband.

Locus	Gene	Exon	Variant	DANN	Mutation Taster	DANN	EIGEN	FATHMM-MKL	BayesDel addAF
chr12: 56437146	*RPS26*	3	c.182-1G>A	D	D	D	P	D	D

In silico tools provide the functional prediction scores in which increasing values indicate a more damaging effect. Abbreviations: D-damaging or deleterious; P-Pathogenic

This variant was classified as "pathogenic" according to the ACMG, 2015 guidelines, following the criteria that: (1) it is predicted as null variant due to introns within ±2 of splice site (PVS1), (2) this mutation is a *de novo* variant in a family without disease history (PS2), (3) this mutant is absent in control (PM2) and PP3 (computational predictions based on 6 tools from MutationTaster, DANN, FATHMM-MKL, BayesDel_addAF, and scSNV-Splicing) (Richards et al., 2015). This *RPS26* variant is a novel variant, which has not been previously reported in genomAD (https://gnomad.broadinstitute.org/) and 1000 Genomes (https://www.internationalgenome.org/). Therefore, we conclude that this heterozygous null variant in *RPS26* gene likely involved to DBA10 in this patient.

DISCUSSION

Diamond-Blackfan anemia (DBA) is a congenital pure red cell aplasia with an estimated incidence ranging from 1:100,000 to 1:200,000 live births (Vlachos et al., 2008). Bone marrow shows almost no erythroid progenitors and insignificant megakaryocyte and neutrophil lineages. Clinically, anemia manifests as macrocytic anemia with high mean blood cell volume, ADA erythrocytes, HbF, and a reduced number of reticulocytes (Vlachos et al., 2008). Approximately, 30% to 50% of patients with DBA typically show growth retardation and congenital malformations that affect the craniofacial structures, upper extremities, heart, and the urinary tract (Landowski et al., 2013). Currently, DBA is treated with transfusions, corticosteroid therapy, or stem cell transplants. The diagnostic principles have extended intensely as a result of detailed insight into genes and updated data on the epidemiology of DBA (Vlachos et al., 2010; Lipton et al., 2009). Mutations causing DBA are from known

15 ribosomal genes. Mostly inherited as an autosomal dominant trait, with the exception of two ribosomal genes located on the X chromosome, in which mutations are inherited in an X-linked recessive manner. Both incomplete penetrance and variable expressivity have been reported for DBA (Vlachos et al., 2008). In several studies, mutations in the *RPS26* gene are responsible for 2.6% to 6.4% of patients with DBA (Doherty et al., 2010; Gazda et al., 2012). Therefore, detection of the causative gene and mutations is essential for an accurate diagnosis. It also offers an opportunity in gene therapy in the future. The mutation identified in this study is c.182-1G>A in the *RPS26* gene. Sanger sequencing revealed that none of the child's parents is a carrier of this mutation; therefore, the patient carried a *de novo* variant in the *RPS26* gene (Figure 1).

According to the latest ACMG guidelines, this variant (loss of function mutation) has strong three supporting evidence in favour of its pathogenicity, namely, parental segregation, in-silico scores, and patient-specific phenotype) (Richards et al., 2015). In addition, this variant was absent in gnomAD and 1000 genome browser databases with no reported homozygotes. Furthermore, the (c.182-1G>A) variant is highly predicted to cause skipping of exon 2 and 3 during mRNA maturation. Splicing alteration leads to skipping of an exon or retention of an intron, both significantly altering protein structure and function (Ward et al., 2010). Therefore, this variant can be currently classified as "pathogenic".

Conclusion

In summary, we identified a novel splice site mutation *RPS26* (c.182-1G>A) in a patient presented with severe anemia that is associated with autosomal dominant Diamond-Blackfan anemia. However, the precise effect of this mutation on protein structure and function remains unknown. The identification of the affected *RPS26* gene in DBA would be the key to understand the molecular pathogenesis and development of novel therapies. Further, investigations on the related effects of this mutation and the associated pathology in DBA still needs to be carried out.

Acknowledgements

Authors are grateful to the patient and their family to support the case study. We would also like to thank the management of Dr. Lal PathLabs for providing excellent facilities.

Author's contribution

DP and AT conceived and designed the experiments. DPand KGS processed the data, conceptualized and conceived the analytical methods. DP drafted the manuscript. AT and VL supervised the study and was in charge of overall professional scientific direction and planning. All authors discussed the results, provided critical feedback, helped shape the research and analysis, and finalized the manuscript.

Conflict of interest

Authors have no conflict of interest.

Source of Funding

The authors declare that this research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of originality

The authors have declared that the data/text presented in this manuscript is original and no text, figure or data has been copied from any other source without appropriate citation.

Jurisdiction and maps

Polymorphism and Peer Publishers remain neutral to the jurisdictional claims, maps, boundaries and

institutional affiliations shown or claimed in any of the articles published.

REFERENCES

Ball SE, McGuckin CP, Jenkins G, Gordon-Smith EC. Diamond-Blackfan anaemia in the U.K.: analysis of 80 cases from a 20-year birth cohort. Br J Haematol. 1996;94(4):645-653.

Boria I, Garelli E, Gazda HT, Aspesi A, Quarello P, Pavesi E, et al. The ribosomal basis of Diamond-Blackfan Anemia: mutation and database update. Hum Mutat. 2010;31(12): 1269-1279.

Campagnoli MF, Garelli E, Quarello P, Carando A, Varotto S, Nobili B, et al. Molecular basis of Diamond-Blackfan anemia: new findings from the Italian registry and a review of the literature. Haematologica. 2004;89(4):480-489.

Cmejla R, Cmejlova J, Handrkova H, Petrak J, Pospisilova D. Ribosomal protein S17 gene (RPS17) is mutated in Diamond-Blackfan anemia. Hum Mutat. 2007;28(12):1178-1182.

Doherty L, Sheen MR, Vlachos A, Choesmel V, O'Donohue MF, Clinton C, et al. Ribosomal protein genes RPS10 and *RPS26* are commonly mutated in Diamond-Blackfan anemia. Am J Hum Genet. 2010;86(2):222-228.

Farrar JE, Nater M, Caywood E, McDevitt MA, Kowalski J, Takemoto CM, et al. Abnormalities of the large ribosomal subunit protein, Rpl35a, in Diamond-Blackfan anemia. Blood. 2008; 112(5): 1582-1592.

Gazda HT, Grabowska A, Merida-Long LB, Latawiec E, Schneider HE, Lipton JM, et al. Ribosomal protein S24 gene is mutated in Diamond-Blackfan anemia. Am J Hum Genet. 2006; 79(6):1110-1118.

Gazda HT, Preti M, Sheen MR, O'Donohue MF, Vlachos A, Davies SM, et al. Frameshift mutation in p53 regulator RPL26 is associated with multiple physical abnormalities and a specific pre-ribosomal RNA processing defect in diamond-blackfan anemia. Hum Mutat. 2012; 33(7): 1037-1044.

Gazda HT, Sheen MR, Vlachos A, Choesmel V, O'Donohue MF, Schneider H, et al. Ribosomal protein L5 and L11 mutations are associated with cleft palate and abnormal thumbs in Diamond-Blackfan anemia patients. Am J Hum Genet. 2008;83(6):769-780.

Ivanov AV, Malygin AA, Karpova GG. Human ribosomal protein S26 suppresses the splicing of its pre-mRNA. Biochim Biophys Acta. 2005;1727(2):134-140.

Landowski M, O'Donohue MF, Buros C, Ghazvinian R, Montel-Lehry N, Vlachos A, et al. Novel deletion of RPL15 identified by array-comparative genomic hybridization in Diamond-Blackfan anemia. Hum Genet. 2013; 132(11): 1265-1274.

Lipton JM, Atsidaftos E, Zyskind I,Vlachos A. Improving clinical care and elucidating the pathophysiology of Diamond Blackfan anemia: an update from the Diamond Blackfan Anemia Registry. Pediatr Blood Cancer. 2006; 46(5):558-564.

Lipton JM, Ellis SR. Diamond Blackfan anemia 2008-2009: broadening the scope of ribosome biogenesis disorders. Curr Opin Pediatr. 2010; 22(1):12-19.

Lipton JM, Ellis SR. Diamond-Blackfan anemia: diagnosis, treatment, and molecular pathogenesis. Hematol Oncol Clin North Am. 2009;23(2):261-282.

Min EE, Roy B, Amrani N, He F, Jacobson A. Yeast Upf1 CH domain interacts with *RPS26* of the 40S ribosomal subunit. RNA. 2013;19(8):1105-1115.

Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405-424.

Vlachos A, Ball S, Dahl N, Alter BP, Sheth S, Ramenghi U, et al. Participants of Sixth Annual Daniella Maria Arturi International Consensus conference. Diagnosing and treating Diamond Blackfan anaemia: results of an international clinical consensus conference. Br J Haematol. 2008;142(6):859-876.

Vlachos A, Klein GW, Lipton JM. The Diamond Blackfan Anemia Registry: tool for investigating the epidemiology and biology of Diamond-Blackfan anemia. J Pediatr Hematol Oncol. 2001;23(6):377-382.

Vlachos A, Muir E. How I treat Diamond-Blackfan anemia. Blood. 2010;116(19):3715-3723.

Ward AJ, Cooper TA. The pathobiology of splicing. J Pathol. 2010;220(2):152-163.