JIG Journal of Interdisciplinary Genomics

https://doi.org/10.22742/JIG.2024.6.2.25
Journal of Interdisciplinary Genomics 2024;6(2):25-32
eISSN 2671-6771

Exploring the Genetic Mechanisms Underlying Diamond-Blackfan Anemia

Ye Jee Shim

Department of Pediatrics, Keimyung University School of Medicine, Keimyung University Dongsan Hospital, Daegu, Korea

Diamond-Blackfan Anemia (DBA) is a rare congenital bone marrow failure syndrome primarily characterized by erythroblastopenia and macrocytic anemia. This disorder results from mutations in ribosomal protein (RP) genes, which lead to defective ribosomal RNA maturation, nucleolar stress, and impaired erythropoiesis. Mutations in RP genes have been identified, with *RPS19* being the most commonly affected gene, accounting for approximately 25% of all cases. Other frequently mutated genes include *RPL5, RPL11*, and *RPS26*. These mutations are mostly heterozygous and cause defective ribosome assembly and biogenesis, which activates the p53 pathway, resulting in cell cycle arrest and apoptosis. In addition, non-RP gene mutations, such as those in *GATA1, TSR2,* or *HEATR3*, have been linked to DBA-like phenotypes, further complicating the genetic landscape. Congenital malformations, particularly craniofacial anomalies, thumb abnormalities, and cardiac defects, are common in patients with specific RP gene mutations, such as *RPL5* and *RPL11*. Advances in next-generation sequencing have improved the identification of novel mutations; however, approximately 20–25% of DBA cases remain genetically unexplained. In this review, we explore the genetic landscape of DBA and provide insights into the underlying mutations and their contributions to disease pathophysiology.

Key words: Diamond-Blackfan Anemia, Erythropoiesis, Ribosomal protein genes, p53 pathway

REVIEW ARTICLE

Received: September 12, 2024
Revised: October 5, 2024
Accepted: October 21, 2024

Correspondence to: Ye Jee Shim, MD, PhD
Department of Pediatrics, Keimyung University School of Medicine, 1095 Dalgubeol-daero, Dalseo-gu, Daegu 42601, Korea
Tel: +82-53-258-7824
Fax: +82-53-258-4875
E-mail: yejeeshim@dsmc.or.kr

ORCID
https://orcid.org/0000-0002-5047-3493

INTRODUCTION

Diamond-Blackfan Anemia (DBA) is a congenital bone marrow (BM) failure syndrome characterized by increased susceptibility of erythroid progenitors and precursors to apoptosis, resulting in impaired erythropoiesis and subsequent erythropoietic failure [1]. The incidence of DBA is estimated to be approximately 7 cases per million live births, with most diagnoses occurring within the first year of life [1]. Clinically, DBA presents with congenital malformations in approximately 50% of patients, including craniofacial, skeletal, genitourinary, cardiac, and ophthalmologic anomalies [2]. Laboratory findings typically reveal normochromic macrocytic anemia, reticulocytopenia, and reduced levels of erythroid precursors in the BM, whereas other hematopoietic lineages are usually unaffected [2].

This disorder is primarily caused by heterozygous mutations in ribosomal protein (RP) genes, leading to defective ribosome biogenesis and ribosomal RNA (rRNA) processing [3]. Recent studies using next-generation sequencing have identified additional RP and non-RP genes, such as *GATA1* [4], *TSR2* [5], and *HEATR3* [6]. RP gene mutations explain 50–60% of DBA cases, and mutations in non-RP genes account for less than 1% [3]. Approximately 20%–25% of DBA cases remain genetically unexplained [3]. The most commonly affected gene, *RPS19*, accounts for approximately 25% of DBA cases [7]. These findings have significantly expanded our understanding of the genetic heterogeneity and molecular pathophysiology of DBA, paving the way for more accurate diagnosis and potential new

therapeutic strategies. In this review, we focus on the genetic framework of DBA and offer insights into the associated mutations and their roles in the pathogenesis of the disease. The genes are listed in the order of their discovery.

RP genes and p53 pathway activation

Approximately 75% of DBA cases are caused by loss-of-function mutations in RP genes, typically affecting only one allele [3]. Ribosomes, which are composed of the 40S small subunit (containing RPS proteins) and the 60S large subunit (containing RPL proteins), are essential for translating mRNA into proteins, a process crucial for cell function and growth [8]. Mutations in *RPS* or *RPL* genes compromise functional ribosome production, resulting in defective rRNA processing and impaired subunit assembly [9,10]. *RPS* gene mutations in the 40S subunit disrupt 18S rRNA processing, leading to incomplete or nonfunctional small ribosomal subunits [10]. In the 60S subunit, *RPL* gene mutations impair the maturation of 28S and 5.8S rRNA, thus disrupting the assembly of the large subunit [9]. Although ribosomes are essential for all cell types, erythroid progenitor cells are particularly sensitive to defects in ribosome biogenesis [11]. This heightened sensitivity is due to the increased demand for protein synthesis during red blood cell (RBC) production [11]. Impaired ribosome production triggers a cellular stress response, particularly through the activation of the p53 pathway [11]. In DBA, RP mutations lead to the accumulation of free RPs that bind to and inhibit MDM2, a negative regulator of p53 [11]. This inhibition stabilizes and activates p53, resulting in increased apoptosis of erythroid progenitor cells in the BM [11].

40S Small Ribosomal Subunit (RPS genes)

RPS19 (Autosomal dominant [AD])

The *RPS19* gene is the first gene identified in relation to DBA and remains the most commonly mutated gene among affected individuals [12]. Draptchinskaia et al. [13] reported that in a female patient with DBA and a *de novo* balanced translocation t(X;19)(p21;q13), the *RPS19* gene was disrupted within its third intron. In a study screening for *RPS19* mutations in 40 unrelated individuals with DBA, Draptchinskaia et al. [13] identified nine distinct mutations in 10 patients. All individuals with mutations were heterozygous for these alterations, and no additional sequence variations were observed in the protein-coding region [13]. Willig et al. [14] examined 190 patients with DBA and discovered *RPS19* sequence alterations in approximately 24% of cases. Mutations in *RPS19* critically disrupt the maturation of the 3′ end of 18S rRNA, which is essential for the formation of the 40S ribosomal subunit [10]. This disruption leads to defects in ribosome assembly and ultimately activates the p53 pathway, resulting in apoptosis and impaired differentiation of erythroid progenitor cells [15].

RPS24 (AD)

Gazda et al. [16] conducted whole-exome sequencing (WES) and genetic linkage analysis on a cohort of patients with DBA and identified multiple mutations in *RPS24*, which interfered with its function and ultimately resulted in the failure of RBC production. This study emphasized the importance of ribosomal dysfunction in DBA, positioning *RPS24* alongside other RP genes involved in the disease [16]. The clinical features of patients with *RPS24* mutations typically include macrocytic anemia, with some cases presenting with congenital anomalies, such as growth retardation and craniofacial malformations [16]. Additionally, Choesmel et al. [17] demonstrated that *RPS24* mutations impair the maturation of 18S rRNA, a critical step in the formation of functional ribosomes, leading to defects in ribosome biogenesis and the disruption of erythropoiesis. Their experiments further revealed that *RPS24* mutations cause delayed pre-rRNA processing and failure to properly assemble the small ribosomal subunit [17]. Furthermore, this study highlights the functional interaction between *RPS19* and *RPS24*, suggesting that defects in multiple RPs may contribute to the disease's pathogenesis [17].

RPS17 (AD)

Cmejla et al. [18] discovered a *de novo* mutation in the *RPS17* gene in patients with DBA, macrocytic anemia, craniofacial dysmorphism, thumb/neck anomalies, congenital heart defects, urogenital malformations, and short stature [18]. Functional analyses have demonstrated that these mutations interfere with proper ribosomal assembly and prevent efficient translation, ultimately leading to the failure of RBC production [18]. The study further highlighted that a mutation in the *RPS17* gene, particularly affecting the start codon, significantly disrupts protein translation, thereby impacting ribosomal biogenesis [18].

RPS7 (AD)

Watkins-Chow et al. [19] demonstrated that *RPS7* mutations in mouse models caused developmental abnormalities, including reduced body size and neuroanatomical defects. Interestingly, unlike human *RPS7* mutations linked to anemia in

DBA, mouse models do not exhibit blood cell defects, highlighting potential species-specific differences in RP function [19]. Akram et al [20]. recently reported a novel *RPS7* variant that causes aberrant splicing, leading to DBA in mother and daughter. The mother experienced spontaneous remission of transfusion-dependent anemia at the age of 13 years, while her daughter was born with an occipital meningocele and later diagnosed with DBA [20]. This study expands the genotype-phenotype correlations in DBA, linking *RPS7* haploinsufficiency to hematologic abnormalities as well as neuroanatomical defects, such as meningocele [20].

RPS10 (AD)

Doherty et al. [21] identified three distinct mutations in *RPS10* in five patients: missense, nonsense, and frameshift mutations. These mutations affect the production of rRNA, particularly 18S rRNA, which is essential for the assembly of ribosomal small subunits [21]. Further analysis using pre-rRNA processing in patient-derived cells revealed that mutations in *RPS10* led to an abnormal accumulation of 18S-E pre-rRNA, impairing ribosomal biogenesis [21]. This is consistent with observations from knockdown studies in HeLa cells, in which reduced expression of *RPS10* caused similar defects in rRNA maturation, ultimately disrupting normal ribosomal function [21]. This study also used high-throughput sequencing to confirm that loss-of-function mutations in *RPS10* are major factors in DBA pathogenesis [22].

RPS26 (AD)

Doherty et al. [21] identified two unrelated DBA families with *RPS26* mutations, one affecting mRNA splicing and the other causing protein truncation. These mutations impair ribosome biogenesis by accumulating 18S-E pre-rRNA, leading to defective ribosome function and disrupted erythropoiesis, which contributes directly to the pathogenesis of DBA [21]. Gripp et al. [5] also reported two unrelated DBA families that carried *RPS26* mutations, one of which affected mRNA splicing, leading to improper gene expression, whereas the other caused protein truncation, disrupting ribosome biogenesis. These mutations are linked to defective ribosome assembly, a hallmark of DBA pathophysiology that impairs RBC production [5].

RPS29 (AD)

Mirabello et al. [23] identified two novel non-synonymous mutations in *RPS29* in two large families affected by DBA. These mutations result in the haploinsufficiency of *RPS29*, leading to defects in pre-rRNA processing and impaired ribosome biogenesis [23]. Functional studies using a zebrafish model showed that mutant *RPS29* failed to rescue defective erythropoiesis, confirming the pathogenic role of these mutations in DBA [23].

RPS28 (AD)

Gripp et al. [5] identified *RPS28* as a novel gene implicated in DBA and mandibulofacial dysostosis (MFD), including microtia or cleft palate, through WES. Two unrelated probands carried a *de novo* mutation affecting the start codon of the *RPS28* gene [5]. This mutation severely disrupts protein translation, leading to haploinsufficiency and impaired ribosome biogenesis [5]. This study also noted that experimental depletion of *RPS28* using siRNA resulted in reduced levels of other RPs, similar to the knockdown of *RPS19* [5].

RPS15A (AD)

Ikeda et al. [24] identified a splicing mutation in *RPS15A* (c.213G>A, p.K71K) in a DBA-affected family that caused aberrant splicing and production of a truncated transcript. The proband was diagnosed with DBA at 3 months of age, and BM study revealed severe erythroid hypoplasia (0%) with otherwise normal cellularity [24]. She had a total anomalous pulmonary venous connection and bilateral acetabular dysplasia [24]. There was a family history of anemia, as both her mother and older sister had experienced anemia during childhood, although there were no physical abnormalities [24]. The proband responded to corticosteroid therapy and eventually became steroid-independent [24]. Functional studies using CRISPR/Cas9 in human erythroid cells have shown that *RPS15A* haploinsufficiency disrupts 18S-E pre-rRNA processing and impairs 40S subunit assembly [24]. Zebrafish models further confirmed that *rps15a* knockdown leads to developmental defects, particularly impaired erythropoiesis, highlighting its role in DBA pathogenesis [24].

RPS27 (AD)

Wang et al. [25] identified loss-of-function mutations in the *RPS27* gene through WES in patients with DBA. A single nucleotide deletion in the *RPS27* gene led to a frameshift mutation, producing a premature stop codon, which causes defective ribosome biogenesis and impairs the pre-rRNA processing necessary for normal RBC production [25]. Functional studies using zebrafish models with *rps27* knockdown revealed devel-

opmental defects, including impaired erythropoiesis, further confirming the role of *RPS27* mutations in DBA pathogenesis [25].

60S Large Ribosomal Subunit (RPL genes)

RPL5 (AD)

Gazda et al. [26] found that *RPL5* mutations disrupt the maturation of pre-rRNA, particularly affecting the processing of 28S and 5.8S rRNA, which are essential for the assembly of the large ribosomal subunit. This disruption of ribosome biogenesis impairs erythropoiesis and contributes to the clinical manifestations of DBA [26]. *RPL5* mutations are frequently associated with distinct craniofacial and skeletal abnormalities, including cleft palate, thumb abnormalities, and congenital heart defects [26]. In a study of 196 patients with DBA, *RPL5* mutations were found in a subset of patients, many of whom exhibited these malformations, highlighting a strong genotype-phenotype correlation [26]. Furthermore, *RPL5* mutations tend to result in a more severe phenotype than mutations in other RP genes, such as *RPS19* [3]. Approximately 70% of patients with DBA with *RPL5* mutations present with congenital malformations, and cleft lip or palate is significantly more common in these patients than in those with other mutations [3].

RPL11 (AD)

Gazda et al. [26] have reported that individuals with *RPL11* mutations display a characteristic phenotype that includes both congenital abnormalities and DBA. Notably, *RPL11* mutations are predominantly associated with isolated thumb malformations, whereas *RPL5* mutations lead to a broader spectrum of physical abnormalities, including cleft palate and heart anomalies [26].

RPL35A (AD)

Farrar et al. [27] found that *RPL35A* is associated with DBA through high-resolution genomic mapping and gene expression microarray analyses. Researchers have identified *RPL35A* as a candidate gene in patients with DBA with chromosome 3q deletions and confirmed mutations in additional patients through sequence analysis [27]. Patients with *RPL35A* mutations exhibit both hematological and congenital features typical of DBA, including anemia in infancy and congenital anomalies affecting the craniofacial region, heart, genitourinary system, and upper limbs [27]. Some patients also display neutropenia, thrombocytopenia, and a heightened risk of developing hematological malignancies and osteosarcoma [27].

RPL26 (AD)

Gazda et al. [28] identified *RPL26* as a gene associated with DBA through largescale screening of RP genes in a cohort of 96 patients. Researchers have reported that a patient with an *RPL26* frameshift mutation exhibited DBA and multiple physical abnormalities, including craniofacial, upper limb, and cardiac malformations [28]. Interestingly, this mutation leads to defective ribosome biogenesis, affecting both small and large ribosomal subunits and disrupting the maturation of 18S and 28S rRNAs [28]. Additionally, the patient presented with macrocytic anemia and elevated erythrocyte adenosine deaminase levels [28].

RPL15 (AD)

Landowski et al. [29] identified a novel *RPL15* deletion in a patient with DBA that disrupted the formation of the 60S subunit and impaired pre-rRNA processing, particularly affecting the maturation of the 28S and 5.8S rRNAs. This leads to reduced ribosome production and impaired erythropoiesis [29]. Similarly, Wlodarski et al. [30] reported truncating mutations in *RPL15* in patients with severe hydrops fetalis, who later achieved spontaneous remission [30]. These mutations cause defects in ribosome assembly, resulting in decreased cell proliferation, delayed erythroid differentiation, and TP53-mediated apoptosis of hematopoietic cells [30]. Overall, *RPL15* mutations are linked not only to the development of DBA but also to unique clinical outcomes, such as spontaneous remission and treatment independence [30].

RPL27 (AD)

Using WES, Wang et al. [25] identified a splicing mutation in *RPL27* in a patient with DBA having an atrial septal defect and pulmonary stenosis, leading to defective ribosome biogenesis. Functional analysis using knockdown experiments in human erythroid cells revealed that depletion of *RPL27* impaired pre-rRNA processing, specifically affecting the maturation of 28S rRNA, which is essential for the proper formation of the 60S ribosomal subunit [25]. Moreover, zebrafish models with *RPL27* knockdown exhibited abnormal development, including reduced erythropoiesis, mimicking the anemia observed in patients with DBA [25].

RPL18 (AD)

Mirabello et al. [31] identified a non-synonymous *RPL18*

variant in two DBA-affected family members. One proband presented with anemia at 8 months of age and was steroid-responsive [31]. Another patient, the proband's child, exhibited mild anemia at birth, which later recurred at the age of one and also responded to steroid therapy [31]. None of the patients exhibited dysmorphic features, but both had intermittent neutropenia from birth [31]. This mutation causes the accumulation of 36S pre-rRNA, disrupts the maturation of 28S rRNA, and impairs the assembly of the 60S subunit, which hinders ribosome biogenesis and leads to defective erythropoiesis, contributing to the clinical symptoms of DBA [31].

RPL35 (AD)

Mirabello et al. [31] also identified a non-synonymous variant of *RPL35* in a family with DBA. The proband developed anemia at 2 months of age, which spontaneously resolved by 18 years of age without any known relapse [31]. BM evaluation at 3 months revealed erythroid hypoplasia, leading to the diagnosis of DBA [31]. The proband's daughter presented with anemia at 1 month of age, which was responsive to steroid therapy [31]. However, at age 15, she developed ulcerative colitis, and during treatment, her blood count dropped, leaving her dependent on RBC transfusions [31]. This mutation impairs pre-rRNA processing, leading to the accumulation of 32S pre-rRNA and defective 28S rRNA maturation, which disrupts the 60S subunit assembly and contributes to the clinical features of DBA [31].

Non-RP genes

GATA1 (X-linked recessive)

GATA1 plays a crucial role in regulating gene expression and the maturation of erythroid cells; in its absence, erythroid progenitors are unable to differentiate properly and ultimately undergo apoptosis [32]. Although important insights into *GATA1* function have been derived from animal models, the discovery that rare red cell disorders, such as DBA, are associated with *GATA1* mutations has provided a deeper understanding [32]. *GATA1* was recognized as the first non-RP mutation in DBA using WES [4]. A recent study in human cells revealed that the reduced translation of *GATA1* due to RP haploinsufficiency, a common cause of DBA, plays a key role in the erythroid abnormalities observed in this disorder [33,34]. Ludwig et al. demonstrated that *GATA1* mRNA has a high threshold for translation initiation, making it particularly susceptible to defects in RP levels [34]. In patients with DBA having *RPS19* mutations, despite unchanged *GATA1* mRNA levels, the activity of *GATA1* target genes is significantly diminished, indicating a translational defect [34]. The study also examined the effect of reducing *RPL11*, *RPL5*, and *RPS24* and found that these reductions similarly decreased GATA1 protein levels, suggesting a general mechanism [34]. This study offers strong evidence that impaired translation of *GATA1* mRNA, resulting from RP haploinsufficiency, plays a crucial role in the erythroid defects observed in DBA [33]. This aligns with the discovery that rare mutations in the *GATA1* gene itself can lead to disease, effectively linking the two mechanisms [33]. These insights could potentially be harnessed therapeutically, possibly by focusing on enhancing GATA1 protein production to alleviate anemia associated with DBA [33]. Additionally, Rio et al. [35] demonstrated that decreased HSP70 levels lead to a reduction in GATA1, causing an imbalance between globin and heme synthesis in DBA. This imbalance results in excess free heme, increased reactive oxygen species, and enhanced apoptosis of erythroid cells. The study shows that restoring HSP70 expression can rebalance globin and heme synthesis, reduce free heme toxicity, and improve erythropoiesis in DBA [35].

TSR2 (X-linked recessive)

Gripp et al. [5] explored the genetic basis of DBA combined with MFD in seven individuals from six unrelated families using WES of these individuals and their family members. They identified mutations in known DBA genes, such as *RPS26*, along with novel mutations in *TSR2* and *RPS28* [5]. Specifically, the *TSR2* hemizygous mutation has been analyzed for its effect on RP interactions and RNA processing, with researchers confirming that the mutation impairs the ability of the protein to bind to RPS26, a key step in ribosome assembly [5]. This study highlights the genetic heterogeneity of the combined DBA and MFD phenotypes, suggesting that disrupted ribosomal function may underlie the diverse clinical manifestations observed across different ribosomopathies [5]. A recent study by Yang and Karbstein [36] demonstrated that the chaperone TSR2 plays a crucial role in managing the release and reintegration of RPS26 from mature ribosomes, facilitating a reversible response to stress. Under stressful conditions, RPS26 dissociates from fully assembled ribosomes and triggers a targeted translational response [36]. TSR2 is essential for this process, aiding the release of RPS26 during stress and its reintegration into ribosomes once normal conditions are restored [36]. This mechanism enables ribosomes to swiftly adapt to environmental changes with minimal energy use, without compromising quality control [36]. Moreover, this study identified

a specific residue in RPS26 linked to DBA that influences the sodium stress response, highlighting the significance of this ribosome remodeling mechanism in ribosomopathies [36]. Together, these findings enhance our understanding of ribosomal heterogeneity and its role in stress responses and provide valuable insights into the development of DBA and related disorders [36].

HEATR3 (Autosomal recessive)

A recent study by O'Donohue et al. [6] showed that DBA can also be caused by biallelic mutations in the *HEATR3* gene. Six individuals from four families with biallelic mutations in *HEATR3* showed BM failure with selective erythroid hypoplasia, short stature, facial dysmorphism, limb deformities, cardiac defects, and intellectual disability [6]. *HEATR3* mutations destabilize a protein that is crucial for importing the RPs uL5 (RPL11) and uL18 (RPL5) into the nucleus, which are essential for ribosome assembly and p53 stabilization [6]. This study demonstrated that *HEATR3* mutations or reduced HEATR3 expression led to impaired cell growth, differentiation, and ribosome subunit formation, mimicking the effects of mutations in large subunit RP genes associated with DBA [6]. Furthermore, HEATR3-deficient cells exhibit decreased nuclear accumulation of RPL5 and abnormal erythrocyte maturation, independent of p53 activation [6]. Appropriate ribosome biogenesis is essential for the proliferation and differentiation of erythroid progenitors into RBCs [37]. In normal erythroid progenitors within the BM, HEATR3 functions as a transport factor moving RPL5 from the cytoplasm to the nucleus [37]. After entering the nucleus, RPL5 binds with RPL11 and 5S rRNA to form the 5S ribonucleoprotein complex, which is subsequently incorporated into the assembly of large ribosomal subunits, contributing to the formation of the central protuberance [37]. However, biallelic *HEATR3* mutations disrupt this process, leading to defects in pre-RNA processing, reduced 60S ribosomal subunits, and failure in erythropoiesis, which clinically manifests as DBA [37].

CONLCLUSION

In summary, this review highlights significant advances in understanding the genetic underpinnings of DBA, particularly the role of mutations in RP genes. These findings underscore the critical role of ribosomal dysfunction in DBA pathogenesis, which contributes to defective ribosomal biogenesis, p53 pathway activation, and impaired erythropoiesis. In addition, the identification of non-RP gene mutations broadens the genetic landscape of DBA and suggests that ribosomal stress and erythroid-specific defects may arise from a wider array of genetic abnormalities. This reinforces the complexity of DBA as a ribosomopathy and highlights the need for further exploration of non-RP gene mutations.

ACKNOWLEDGEMENT

None.

REFERENCES

1. Vlachos A, Ball S, Dahl N, Alter BP, Sheth S, Ramenghi U, et al. Diagnosing and treating Diamond Blackfan anaemia: results of an international clinical consensus conference. Br J Haematol 2008;142(6):859-76. doi: 10.1111/j.1365-2141.2008.07269.x.
2. Vlachos A, Muir E. How I treat Diamond-Blackfan anemia. Blood 2010;116(19):3715-23. doi: 10.1182/blood-2010-02-251090.
3. Ulirsch JC, Verboon JM, Kazerounian S, Guo MH, Yuan D, Ludwig LS, et al. The genetic landscape of Diamond-Blackfan Anemia. Am J Hum Genet 2018;103(6):930-47. doi: 10.1016/j.ajhg.2018.10.027.
4. Sankaran VG, Ghazvinian R, Do R, Thiru P, Vergilio JA, Beggs AH, et al. Exome sequencing identifies GATA1 mutations resulting in Diamond-Blackfan anemia. J Clin Invest 2012;122(7):2439-43. doi: 10.1172/jci63597.
5. Gripp KW, Curry C, Olney AH, Sandoval C, Fisher J, Chong JX, et al. Diamond-Blackfan anemia with mandibulofacial dystostosis is heterogeneous, including the novel DBA genes TSR2 and RPS28. Am J Med Genet A 2014;164a(9):2240-9. doi: 10.1002/ajmg.a.36633.
6. O'Donohue MF, Da Costa L, Lezzerini M, Unal S, Joret C, Bartels M, et al. HEATR3 variants impair nuclear import of uL18 (RPL5) and drive Diamond-Blackfan anemia. Blood 2022;139(21):3111-26. doi: 10.1182/blood.2021011846.
7. Martinez Barrio A, Eriksson O, Badhai J, Fröjmark AS, Bongcam-Rudloff E, Dahl N, et al. Targeted resequencing and analysis of the Diamond-Blackfan anemia disease locus RPS19. PLoS One 2009;4(7):e6172. doi: 10.1371/journal.pone.0006172.
8. Fox JM, Rashford RL, Lindahl L. Co-assembly of 40S and 60S ribosomal proteins in early steps of eukaryotic ribosome assembly. Int J Mol Sci 2019;20(11). doi: 10.3390/ijms20112806.
9. Dörner K, Badertscher L, Horváth B, Hollandi R, Molnár C, Fuhrer T, et al. Genome-wide RNAi screen identifies novel players in human 60S subunit biogenesis including key enzymes of polyamine metabolism. Nucleic Acids Res 2022;50(5):2872-88. doi: 10.1093/nar/gkac072.
10. Flygare J, Aspesi A, Bailey JC, Miyake K, Caffrey JM, Karlsson S, et al. Human RPS19, the gene mutated in Diamond-Blackfan anemia, encodes a ribosomal protein required for the maturation of 40S ribosomal subunits. Blood 2007;109(3):980-6. doi:

10.1182/blood-2006-07-038232.
11. Le Goff S, Boussaid I, Floquet C, Raimbault A, Hatin I, Andrieu-Soler C, et al. p53 activation during ribosome biogenesis regulates normal erythroid differentiation. Blood 2021;137(1):89-102. doi: 10.1182/blood.2019003439.
12. 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):1269-79. doi: 10.1002/humu.21383.
13. Draptchinskaia N, Gustavsson P, Andersson B, Pettersson M, Willig TN, Dianzani I, et al. The gene encoding ribosomal protein S19 is mutated in Diamond-Blackfan anaemia. Nat Genet 1999;21(2):169-75. doi: 10.1038/5951.
14. Willig TN, Draptchinskaia N, Dianzani I, Ball S, Niemeyer C, Ramenghi U, et al. Mutations in ribosomal protein S19 gene and diamond blackfan anemia: wide variations in phenotypic expression. Blood 1999;94(12):4294-306.
15. Campagnoli MF, Ramenghi U, Armiraglio M, Quarello P, Garelli E, Carando A, et al. RPS19 mutations in patients with Diamond-Blackfan anemia. Hum Mutat 2008;29(7):911-20. doi: 10.1002/humu.20752.
16. 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):1110-8. doi: 10.1086/510020.
17. Choesmel V, Fribourg S, Aguissa-Touré AH, Pinaud N, Legrand P, Gazda HT, et al. Mutation of ribosomal protein RPS24 in Diamond-Blackfan anemia results in a ribosome biogenesis disorder. Hum Mol Genet 2008;17(9):1253-63. doi: 10.1093/hmg/ddn015.
18. 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):1178-82. doi: 10.1002/humu.20608.
19. Watkins-Chow DE, Cooke J, Pidsley R, Edwards A, Slotkin R, Leeds KE, et al. Mutation of the diamond-blackfan anemia gene Rps7 in mouse results in morphological and neuroanatomical phenotypes. PLoS Genet 2013;9(1):e1003094. doi: 10.1371/journal.pgen.1003094.
20. Akram T, Fatima A, Klar J, Hoeber J, Zakaria M, Tariq M, et al. Aberrant splicing due to a novel RPS7 variant causes Diamond-Blackfan Anemia associated with spontaneous remission and meningocele. Int J Hematol 2020;112(6):894-9. doi: 10.1007/s12185-020-02950-6.
21. 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):222-8. doi: 10.1016/j.ajhg.2009.12.015.
22. Gerrard G, Valgañón M, Foong HE, Kasperaviciute D, Iskander D, Game L, et al. Target enrichment and high-throughput sequencing of 80 ribosomal protein genes to identify mutations associated with Diamond-Blackfan anaemia. Br J Haematol 2013;162(4):530-6. doi: 10.1111/bjh.12397.
23. Mirabello L, Macari ER, Jessop L, Ellis SR, Myers T, Giri N, et al. Whole-exome sequencing and functional studies identify RPS29 as a novel gene mutated in multicase Diamond-Blackfan anemia families. Blood 2014;124(1):24-32. doi: 10.1182/blood-2013-11-540278.
24. Ikeda F, Yoshida K, Toki T, Uechi T, Ishida S, Nakajima Y, et al. Exome sequencing identified RPS15A as a novel causative gene for Diamond-Blackfan anemia. Haematologica 2017;102(3):e93-e6. doi: 10.3324/haematol.2016.153932.
25. Wang R, Yoshida K, Toki T, Sawada T, Uechi T, Okuno Y, et al. Loss of function mutations in RPL27 and RPS27 identified by whole-exome sequencing in Diamond-Blackfan anaemia. Br J Haematol 2015;168(6):854-64. doi: 10.1111/bjh.13229.
26. 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):769-80. doi: 10.1016/j.ajhg.2008.11.004.
27. 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):1582-92. doi: 10.1182/blood-2008-02-140012.
28. 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):1037-44. doi: 10.1002/humu.22081.
29. 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):1265-74. doi: 10.1007/s00439-013-1326-z.
30. Wlodarski MW, Da Costa L, O'Donohue MF, Gastou M, Karboul N, Montel-Lehry N, et al. Recurring mutations in RPL15 are linked to hydrops fetalis and treatment independence in Diamond-Blackfan anemia. Haematologica 2018;103(6):949-58. doi: 10.3324/haematol.2017.177980.
31. Mirabello L, Khincha PP, Ellis SR, Giri N, Brodie S, Chandrasekharappa SC, et al. Novel and known ribosomal causes of Diamond-Blackfan anaemia identified through comprehensive genomic characterisation. J Med Genet 2017;54(6):417-25. doi: 10.1136/jmedgenet-2016-104346.
32. Ling T, Crispino JD. GATA1 mutations in red cell disorders. IUBMB Life 2020;72(1):106-18. doi: 10.1002/iub.2177.
33. Boultwood J, Pellagatti A. Reduced translation of GATA1 in Diamond-Blackfan anemia. Nat Med 2014;20(7):703-4. doi: 10.1038/nm.3630.
34. Ludwig LS, Gazda HT, Eng JC, Eichhorn SW, Thiru P, Ghazvinian R, et al. Altered translation of GATA1 in Diamond-Blackfan anemia. Nat Med 2014;20(7):748-53. doi: 10.1038/nm.3557.
35. Rio S, Gastou M, Karboul N, Derman R, Suriyun T, Manceau H, et al. Regulation of globin-heme balance in Diamond-Blackfan anemia by HSP70/GATA1. Blood 2019;133(12):1358-70. doi: 10.1182/blood-2018-09-875674.
36. Yang YM, Karbstein K. The chaperone Tsr2 regulates Rps26 re-

lease and reincorporation from mature ribosomes to enable a reversible, ribosome-mediated response to stress. Sci Adv 2022; 8(8):eabl4386. doi: 10.1126/sciadv.abl4386.

37. Iskander D, Warren AJ. Turning up the HEAT(R3) in Diamond-Blackfan anemia. Blood 2022;139(21):3101-2. doi: 10.1182/blood.2022015881.