

# Diamond Blackfan Anemia: genetics, pathogenesis, diagnosis and treatment

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## ABSTRACT

Diamond Blackfan Anaemia (DBA) is a sporadic inherited anemia with broad spectrum of anomalies that are presented soon after delivery. It is inherited mainly in autosomal dominant inheritance manner and caused by mutations and deletions in either large or small ribosomal protein genes that results in an imbalance between the biosynthesis of rRNA and ribosomal proteins, eventually the activation and stabilization of p53. Diagnosing DBA is usually problematic due to a partial phenotype and its wide inconsistency in its clinical expression; however, molecular studies have identified a heterozygous mutated gene in up to 50% of the DBA cases and corticosteroid drugs are the backbone treatment options of DBA. Anomalies in bone marrow function in DBA cases are broadly associated with both congenital and acquired bone marrow failure syndromes in human. In this review different literatures were searched in Medline (eg. PubMed, PMC, Hinari, Google scholar), OMIM, EMBASE by using search engines (Google, Yahoo, Baidu Ask.com) and searching was performed by using search key words (DBA, ribosomopathies, Bone Marrow Failure Syndromes, pure red cell aplasia). Only human studies were included. This review is summarizing the current understandings of DBA.

## INTRODUCTION

Diamond Blackfan Anemia (DBA) is a sporadic heterogeneous genetic disorder characterized by red blood cell aplasia in association with skeletal anomalies and short stature that classically appear soon after birth (1-4). Although the prominent feature of DBA is anemia (5), clinically it is a broader disorder and is manifested by growth retardation and congenital malformations of the head, heart, neck, upper limbs, and urinary system, which are present in approximately 30% to 50% of the DBA patients (6-9).

As shown by many studies, the incidence of DBA was estimated to be 1-4 cases per 500,000 live births in a year and it seems to be constant over time. No seasonal disparity has been observed as a function of the date of conception. The tendency of the disorder across the ethnicity of the people and in both genders, is almost comparable (10,11).

Chronic macrocytic-normochromic anemia, low reticulocyte count, and decreased or totally absent erythroid precursors characterized by failure of erythropoiesis with normal production of leukocytes and platelets in the bone marrow, are the main hematological features of DBA (2,3,12). Furthermore, a majority of the patients have laboratory findings of increased mean corpuscular volume (MCV), elevated erythrocyte adenosine deaminase activity (eADA) and persistently elevated fetal hemoglobin (Hgb F). However, these laboratory findings may not be observed in some DBA cases; and even in the same families, signs and symptoms may vary among affected family siblings (13,14).

After a succession of studies, by now DBA has been shown to be associated with both ribosomal and non-ribosomal mutations in genes located on more than 11 chromosomes which are responsible for encoding the ribosomal proteins (RP) (12,15).

Molecular mechanisms underlying the causal consequence between RP haploinsufficiency and anemia have not yet been clearly elucidated. A generally documented pathogenetic hypothesis implies that a defective ribosome biosynthesis leads to apoptosis in erythroid progenitors which in turn is leading to erythroid failure. This mechanism has been named "ribosomal stress", and there are indications that it may be signaled through p53. All genes identified to be mutated in DBA encode ribosomal proteins which are involved in either the small (RPS) or large (RPL) subunits of these proteins and the scarcity of these proteins can cause the development of the disease (16). The disorders of ribosome synthesis or associated genes that lead to disrupted ribosomal biosynthesis (ribosomopathy) are more than one independent ribosomal protein mutation i.e., all patients of DBA may not show the same RP mutations (6). Corticosteroids, transfusion therapy and stem cell transplantation are the current options for the treatment of DBA (17,18).

The main aim of this narrative review is to provide the reader with a comprehensive knowledge on DBA genetics, pathogenesis, diagnosis and treatment. Different literatures were searched in Medline (e.g., PubMed, PMC, Hinari, Google scholar), OMIM, EMBASE by using search engines (Google, Yahoo, Baidu Ask.com) and searching was performed by using search key words (Diamond Blackfan Anemia, Ribosomopathies, Pure red cell aplasia, Ribosomal proteins, Bone marrow failure syndromes). Only human studies were included.

## MOLECULAR PATHOPHYSIOLOGY OF DIAMOND BLACKFAN ANEMIA

### *Impaired ribosomal biogenesis in DBA*

Recently, DBA is a well-recognized inherited bone marrow failure syndrome, frequently caused by alterations in ribosomal protein (RP)

genes. Otherwise it rarely results from the mutation of the hematopoietic transcription factor gene, GATA1. After the preliminary descriptions of heterozygous RPS19 mutations in a subset of DBA cases, a substantial progress has been made over the past decades for improved explanation of the genetic causes of DBA (13). With a growing emphasis on the RP genes, the search for DBA-related genes, which was initially based on classic genetics techniques including cloning of cytogenetic abnormalities and extended linkage analysis, has shifted to target resequencing of the known RP genes (19-21).

These advanced studies have recognized both large and small subunits of RP gene anomalies which currently includes but is not limited to RPL5, RPL11, RPL35A, RPS7, RPS10, RPS17, RPS19, RPS24, and RPS26 as a mutated gene in multiple families of DBA patients (8,22,23). Changes in larger number of RPs have also been identified in isolated patients or families, including RPL3, RPL7, RPL9, RPL14, RPL19, RPL23A, RPL26, RPL35, RPL36, RPS8, RPS15, RPS27A, RPL18 and RPL35 (24-26).

Mutations in RP genes have been confirmed to be the direct cause of faulty erythropoiesis and consequently anemia, and are found in more than half of DBA cases (27). The RPS19 gene was the formerly known mutated small ribosomal protein and is still the most frequently mutated gene in DBA patients which accounts about 25% of total DBA patients (13). More than 50% of RPS19 mutations are either deletions of one RPS19 allele, or insertional, frame shift, splice site, or nonsense mutations, which lead to an untimely termination of RPS19 protein synthesis. This results in a deficiency of RPS19 protein which is termed as "haploinsufficiency" in human cells (28,29). Again, greater than half of the missense mutations termed as "class I" which cause the RPS19 protein to be folded improperly and rapidly aimed at premature degradation

of ribosomal proteins consequently leading to RPS19 haploinsufficiency (30).

RPS19 protein plays an important role in 18S rRNA maturation and 40S synthesis in human cells (29,31,32). Mutations associated with RPS19 can disrupt the pre-rRNA processing of the 18S rRNA and pre-40S subunits, leading to reduced production of 40S ribosomal subunits. Finally, the decreased expression of RPS19 is able to imitate many aspects of the DBA phenotype (21,35). As a result, this knockdown of RPS19 by RNA interference can cause a severe defect that alters the normal primary human hematopoietic progenitor differentiation and proliferation of the erythroid progenitor (EP) cells (33). Furthermore, the defective erythropoiesis in DBA caused by RPS19 deficiency can be rescued by ectopic overexpression of exogenous RPS19. All of these strongly support the notion that RPS19 haploinsufficiency resulted from RPS19 modifications could be the central pathogenic mechanism in the underlying DBA pathology suggesting the feasibility of RPS19 gene augmentation to treat DBA cases (31).

Thus, RPS19-lacking cells suffer from a relatively shortage of 40S rRNA and has a reduced ability for translation initiation. RPS19 deficiency can lead to increased apoptosis in hematopoietic cell lines and bone marrow cells. Suppression of RPS19 prevents cell proliferation and early erythroid differentiation, but not late erythroid maturation in RPS19-deficient DBA cell lines. Haploinsufficiency of RPS19 has been shown in a subset of patients and appears to be sufficient to cause DBA (20,22).

Advanced studies showed that the RPS19Dsk3 mouse recapped the human DBA phenotype insofar as a hypoproliferative, pro-apoptotic anemia with growth hindrance. Given existing data, it is now generally believed that DBA resulted from an intrinsic cellular flaw in which erythroid progenitors and precursors are greatly sensitive to demise

by apoptosis (36,37). The phenotypes observed in zebrafish and mouse DBA models DBA can be partly or entirely rescued by alterations in p53, robustly proposed that p53 stabilization and activation plays a noteworthy role in the pro-apoptotic phenotype of cells with RP haploinsufficiency (38).

DBA have led to the formulation of “ribosomal stress” hypothesis in which reduced RP synthesis activates p53 that induces the downstream events and leads to cell cycle termination or apoptosis. Finally, this phenomenon results in the DBA phenotype of anemia, deprived growth and results in congenital abnormalities (16).

There are numerous potential mechanisms by which a faulty ribosome assembly or nucleolar stress might signal to p53 stimulation. One interesting mechanism in the pathophysiology of DBA is the interaction of ribosomal proteins with Murine Double Minute (MDM2) which is a powerful controller of p53 level and its activity. MDM2 is a ring finger ubiquitin ligase that interacts with and enhances the degradation of p53. In this interaction, the large proteins of the 60S subunit namely RPL5, RPL11, and RPL23 have been presented to bind to MDM2 and reduces the activities of MDM2 which in turn results in p53 stabilization (39).

The heterozygous DBA mutations result in loss of function in a single copy of ribosomal protein gene. Thus, the pathophysiology of DBA is now attributed to ribosomal malfunction and these mutations in ribosomal genes have been identified in approximately 50% of DBA patients (40). The large number of ribosomal proteins mutated in DBA fail to cluster in any specific region of the ribosome. Haploinsufficiency or reduced expression of a ribosomal protein results in decreased levels of the cognate 40S or 60S subunit and a defect in processing of the ribosomal RNA precursors (33,41,42).

Although DBA is considered as a ribosomopathy, it can be also caused by non-RP gene mutations.

GATA1 encodes a transcription factor which is essential for erythroid differentiation and therefore it is not surprising to state that this gene is involved in DBA pathogenesis (43). The mutation in the GATA1 gene is a Guanine-Cytosine (G→C) transversion at location 48,649,736 on the X chromosome. By applying whole-exome sequencing, aberration in the zinc-finger transcription factor gene “GATA1”, has been identified to be one of the possible causative agents for the development of DBA. This G→C transversion is associated with X-linked form of DBA, leading to the substitution of leucine to valine at amino acid 74 of GATA1. This aberration affects GATA1 splicing processes and leads to termination of the full-length GATA1 protein level and synthesis of a short isoform what we call GATA1 short (GATA1s) (11). While comparing the two isoforms, there is no exon 2 in GATA1s consequently the absence of a transactivation domain. Full-length GATA1 has a crucial role in humans for the differentiation of erythroid cells. During erythroid differentiation, full-length GATA1 enhances the synthesis of erythroid genes by silencing megakaryocytic or other hematopoietic lineage-specific genes. This up and down regulation of GATA1 is vital during the megakaryocyte erythroid progenitor (MEP) commitment program to megakaryocytic and erythroid differentiation (44,45).

While the specific role of GATA1s in DBA pathophysiology remains unclear, studies suggest that full-length GATA1 protein level could be an important driver of DBA pathophysiology. Researchers used short ribonucleic acids (shRNA) against several RPs in normal CD34+ cells to induce RP deficiency to confirm the role of GATA1 in DBA patients.

Thereafter they observed a reduction of full-length GATA1 protein level and erythroid maturation defects. On the other hand, increasing the GATA1 protein level could partly rescue defects in DBA associated RP haploinsufficiency (46,47).



### **Mechanisms of erythroid failure due to ribosomal protein deficiency**

DBA typically presents with erythrocyte aplasia in the first one year of life and both quantitative and qualitative defects of erythroid progenitors contribute to the abnormal erythropoiesis. Majority of the genes responsible for the development of DBA are ribosomal proteins suggesting that insufficiency in ribosomal function may be the underlying cause of red cell aplasia in DBA cases. Ribosomal haploinsufficiency leads to disrupted ribosome biosynthesis and a consequent cytoplasmic buildup of numerous free RPs which results in the stabilization and activation of p53 (38,48-50). In ribosomal stress conditions, several cytoplasmic free RPs such as RPL11, RPI5, RPL23, RPS7, and RPS27 will bind to Murine double minute (MDM2) and hinders its interaction with p53, leading to the stabilization of p53 (51-53). This pathway has been shown to be upstream of apoptosis and cell-cycle arrest, eventually which leads to DBA erythroid hypoplasia (54,55).

Homozygous inactivation of p53 in RPS19 mutant mice totally corrected the DBA erythroid hypoplasia, further corroborating the MDM2/p53-mediated DBA pathogenesis caused by RPS19 mutations and cytoplasmic free RPL26 could influence the translation of p53 mRNA (30,56). However, it is believable that permanent overexpression of RPS19, by gene transfer may result in a persistent activation of MDM2 and/or inhibition of p53 (55).

Moreover, when global translation was blocked by inhibition of the translation initiation factor Eukaryotic Transcription Initiation factor 4- Erythroid (eIF4E), the investigators observed a down regulation of full-length GATA1 while the other proteins persisted unaffected. These observations suggest that ribosome biosynthesis faults could impact translation of specific proteins, as GATA1. Thus, in addition to the

cytoplasmic free RPs, full length GATA1 level reduction may participate in the stabilization of p53 and, consequently, the DBA erythroid cell phenotype (47).

Although the mechanism by which mutations in the RP genes caused explicit defects in erythroid cell maturation is not copiously understood, several lines of evidences indicate that p53 activation induced by ribosomal malfunction may be fundamental for the pathogenesis of DBA (56). Disruption of 40S biosynthesis provoke the release of RPL11 and other RPs into the nucleoplasm results in the binding with MDM2. This phenomenon can compromise MDM2 activity; thereafter the consequent accumulation of p53. The exhibition of phenotypes such as growth retardation, macrocytic anemia with reticulocytopenia and increased apoptosis in bone marrow progenitors by RPS19 mutant mice suggested the presence of a direct link between DBA phenotypes and accumulation of p53 (51).

The pathophysiology for erythroid defect in DBA might therefore be because of the failure of a particular protein to achieve a threshold level at a critical stage; for example, by disturbing the stoichiometry of multi-protein erythroid-specific complexes or by a more selective influence on the translation of a vital protein. A global reduction in translation could also be significant for the pathophysiology of DBA (57).

In conclusion, defective erythropoiesis in DBA is mostly the consequence of either ribosome biogenesis defects due to mutations in ribosomal protein (RP) genes or as consequence of mutation in the GATA1 gene, leading to a reduction of full-length GATA1 and synthesis of a short isoform, GATA1s. In normal conditions, p53 is degraded because of its interaction with MDM2. In DBA, the ribosome biogenesis defect induces a release of several free RPs in the cytoplasm, which can inhibit p53 degradation. p53 degradation is also inhibited by the reduction

in full-length GATA1 synthesis (15). Treatment includes gene therapy strategies such as gene addition (to increase copy numbers of the wild-type form of the mutated proteins), gene silencing (leading to the degradation of the mutated mRNA) and genome editing (such as zinc-finger nucleases, transcription-activator like effector nucleases, or clustered, regulator).

## **GENETICS AND INHERITANCE OF DIAMOND-BLACKFAN ANEMIA**

### ***Inheritance***

According to the report of recent studies, approximately 40 – 45% of DBA cases are hereditary which are inherited with autosomal dominant inheritance which mean that a single copy of altered gene in each cell is adequate to cause the disorder (58) whereas the remaining 55 – 60% of the DBA patients are sporadic, i.e., resulted from new aberrations in the gene which occur in people who have no history of this disorder in their family (12).

Even though autosomal dominant inheritance is the frequently observed pattern of inheritance, autosomal recessive inheritance, which is defined as the presence of DBA siblings from unaffected consanguineous parents, with a lesser frequency has been reported. DBA classically presents at 2 - 3 months of age; only 25% of affected offsprings are anemic at birth and hydrops is occasional (59,60).

### ***Genetics***

As shown in the pathophysiology of DBA, it mainly arises from an abnormal ribosomal protein gene with the exception of the rare form resulting from mutation of transcriptional factor GATA1 (6,13,22,24,25,28,61-63). The genes encoding ribosomal proteins belonging to both the large and small ribosomal subunits are found to be mutated in DBA (7,11). This disorder is described by genetic heterogeneity,

disturbing different ribosomal gene loci. More than a decade of heterozygous mutations resulting in haploinsufficiency have been identified for the genes that encode ribosomal proteins (40) (Table 1).

Mutations in ribosomal genes account for 60 – 70% of DBA patients. Among these ribosomal gene mutations, about 20% of the cases involve large deletions that require analysis of copy number variation for detection. On the other hand, overall it is estimated that around 35% of the DBA cases remain yet genetically indeterminate. These ribosomal gene aberrations may be inherited in an autosomal dominant pattern or may arise spontaneously-linked mutations in the transcription factor GATA1 (11).

## **GENOTYPE AND PHENOTYPE CORRELATION**

In general, the phenotypic spectrum of DBA embraces a wider domain of severity, even within the same families, it ranges from the classical syndrome to individuals with a solitary increase in eADA. No correlation has yet been found between the identity of the DBA gene and hematological severity, including response to steroids. However, craniofacial abnormalities are more linked to mutations affecting either RPL5 or RPL11 (22,29).

Clinical figures from European and American DBA cases disclosed that the incidence of malformations in DBA patients having RPS19 mutations is 31% and this is not significantly different from that of the entire DBA population (29,64,65). RPS19 mutations are found in some first-degree families presenting only with isolated high eADA and/or macrocytosis. However, large deletions at the 19q13 locus and unbalanced translocation t (X; 19) are always linked to mental retardation, which points to a contiguous gene syndrome. Conversely, the patient with balanced translocation t(X;19)

**Table 1** Mutations of ribosomal protein genes in Diamond Blackfan Anemia

Sr. No.	Study	Year	Gene	Chromosome	Subunit	Frequency*
1	Mirabello et al	2017	RPL18 RPL35	19q13.33 9q33.3	60S 60S	2 2
2	Gazda et al	2012	RPS26	17p13	40S	6.5
3	Farrar et al	2011	RPS17	15q	40S	5†
4	Doherty et al	2010	RPS7 RPS10	2p25 6p	40S 40S	1 2-5
5	Gazda et al	2008	RPL5 RPL11	p22.1 p36.1-p35	60S 60S	7 6.5
6	Farrar et al	2008	RPL35A	3q29-qter	60S	2
7	Cmejla et al	2007	RPS17	15q	40S	5†
8	Gazda et al	2006	RPS24	10q22-q23	40S	2
9	Draptchinskaia et al	1999	RPS19	19q13.2	40S	25
10	-	-	Others	-	GATA1, RPS28	-

\* Frequency may depend on the cohort size.

† Including gene deletions.

which interrupts RPS19, without loss of other genes, has normal mental development (66). Comparatively, mutations in RPL5 and RPL11 possibly will be accompanied by a more severe phenotype than mutations in RPS19, particularly with respect to skeletal deformations (22). Current studies advise that the patients with an RPL5 and RPL11 mutation have an increased probability to present with craniofacial, thumb and heart anomalies (9,22,61).

Physical anomalies are observed in up to 50% of the cases with a wide range of severity. These are classically craniofacial, including hypertelorism, flat nasal bridge, and high arched or cleft palate. Thumb abnormalities have also been seen in 20%

of the DBA cases, including the usual triphalangal thumb (67). In RPS5 alterations, craniofacial, congenital heart, and thumb defects are more severe than those seen with pathogenic variants in RPL11 and RPS19 (22,68). Curiously, patients with RPL5 mutations incline to have cleft lip and/or palate or cleft soft palate, solitarily or in combination with other physical abnormalities.

Consistent with these reports, patients with RPL5 mutations also had physical malformations and cleft palate, and in contrary, patients without an RPL5 mutation presented with cleft palate (69). Cleft lip and/or cleft palate was also reported in almost 50% of the affected babies with RPL5 pathogenic variants (22). Small

gestational age was reported in individuals with an RPL5 and RPS19 pathogenic variant as well, which is higher in individuals with RPS5 pathogenic variant (68). Pathogenic variants in RPL11 are largely associated with thumb abnormalities (22); individuals with RPL11 pathogenic variants have been also identified with cleft lip or palate (CL/P) (68).

Individuals with mutated variants of RPS10, RPS19, RPS26 have not yet shown any genotype-phenotype correlations (24). Till now, no genotype-phenotype correlations have been also identified in persons with RPS29 pathogenic variants. DBA with mandibulofacial dystostosis were identified in mutants of RPS28 and TSR2 whereas persons with the variants of RPL27, RPL31 and RPL27 have no identified genotype-phenotype correlations (70).

## CLINICAL PRESENTATIONS AND DIAGNOSIS

### *Clinical presentations*

#### *Hematologic features*

DBA babies typically present with an erythrocyte aplasia in the first twelve months of life, with the median age of exhibition of two months but occasionally its presentation may be delayed up to adulthood. By definition, all DBA patients are anemic (71), and it is present at birth in only 15% of patients and fetal hydrops has occasionally been reported, suggesting that erythropoiesis is spared *in utero* perhaps this is due to transiently being rescued by maternal or placental factors with a post-natal switch from effective to ineffective erythropoiesis (17,60,72).

Red blood cells are usually macrocytic; reticulocyte counts are reduced or zero but the other hematological lineages are not involved as a rule with the exception of slightly an abnormal lower leukocyte and increased platelet

counts reported at diagnosis. Bone marrow aspirates indicate isolated erythroblastopenia, (usually <5% of nucleated cells on bone marrow smears) in more than 90 percent of the patients. Another unusual bone marrow pattern is erythroid hyperplasia with maturation detention; apparently normal numbers and maturation of erythroblasts have been exceptionally described (17,73).

Bone marrow analysis also demonstrated normal cellularity and morphology except for the erythroid line in all patients (74). Erythroid entire aplasia and hypoplasia have been found in DBA cases presented with erythroid maturation arrest with an elevated number of juvenile precursors and indicated dyserythropoietic morphology (71,74,75).

The colony assessment for BFU-E confirmed totally absent/reduced growth in 83% of patients. Addition of stem cell factor (SCF) induced a noticeable increment of erythroid colonies in all the tested subjects. The activity of eADA which is a crucial enzyme in the purine salvage pathway, is usually high in DBA patients (71,74,76). A moderately increased risk of developing hematological malignancies also exists and initial clinical manifestations such as pallor, shortness of breath while suckling, failure to thrive and systolic murmur are observed during infancy (16). The risk of developing solid tumors, myelodysplastic syndrome, or leukemia is elevated in DBA patients (77).

#### *Physical abnormalities*

More than a third of the disordered persons present with a variety of associated congenital physical anomalies. Especially, thumb and upper limb malformations as well as craniofacial anomalies including short stature are common. A cute snub nose and wide spaced eyes, and other craniofacial anomalies are also seen. Other defects frequently observed include urogenital



anomalies, atrial or ventricular septal defects, and prenatal or postnatal growth retardation. A distinct facial appearance and triphalangeal thumbs have been characteristically explained in DBA (27,70,78,79).

According to some studies, the incidence and severity of physical abnormalities have not been gender-related, whereas other researchers revealed that a greater severity of abnormalities was observed among males, compared to females (66).

### Diagnostic criteria

Diagnosing DBA is usually tough due to its partial phenotypes and the wide inconsistency of clinical expressions (16,71). Having the variability, the International Clinical Consensus Conference stated diagnostic and supporting criteria for the diagnosis of DBA (71) (Table 2). The diagnosis of DBA is made when the requirements of major criteria outlined in Table 2, are fulfilled and the Parvovirus infection and Fanconi's anemia are ruled out. However, some essentials are not

**Table 2** Diagnostic criteria for DBA\*

Diagnostic criteria
<ul style="list-style-type: none"> <li>• Normochromic, often macrocytic anemia developing in the first year of life</li> </ul>
<ul style="list-style-type: none"> <li>• Profound reticulocytopenia</li> </ul>
<ul style="list-style-type: none"> <li>• Normocellular bone marrow with selective deficiency of erythroid precursors</li> </ul>
<ul style="list-style-type: none"> <li>• Normal or slightly reduced leukocyte count</li> </ul>
<ul style="list-style-type: none"> <li>• Normal or slightly increased platelet count</li> </ul>
Major supporting criteria
<ul style="list-style-type: none"> <li>• Gene mutation described in “classical” DBA</li> </ul>
<ul style="list-style-type: none"> <li>• Positive family history</li> </ul>
Minor supporting criteria
<ul style="list-style-type: none"> <li>• Elevated erythrocyte adenosine deaminase activity</li> </ul>
<ul style="list-style-type: none"> <li>• Congenital anomalies described in “classical” DBA</li> </ul>
<ul style="list-style-type: none"> <li>• Elevated HbF</li> </ul>
<ul style="list-style-type: none"> <li>• No evidence of another inherited bone marrow failure syndrome</li> </ul>

\* Accepted by the DBA working group of the European Society for Paediatric Haematology and Immunology, ESPHI (17).

included in these criteria such as the presence of typical malformations, the response to steroids and the chronic course of the anemia can also help to diagnose DBA (73).

Onset after the age of two years or the absence of isolated bone marrow erythroblastopenia induce a big caution in the diagnosis of DBA. The only identification of pathogenic mutants in one of the DBA genes conclusively establishes a diagnosis of DBA (61,80). Furthermore, molecular diagnosis enables the detection of carriers, and the exclusion of hematopoietic stem cell transplantation from sibling donors living with the mutations. However, determining the effects of missense mutations may be difficult, whereas nonsense and frameshift mutations will probably be pathogenic in the majority of cases (73). The bone marrow is classically normocellular, with a scarcity of erythroid precursors. The erythroid burst-forming units (BFU-E) and erythroid colony-forming units (CFU-E) *in vitro* are severely reduced, with relative sparing of the granulocyte-monocyte colony-forming units (CFU-GM) (81). The erythrocyte adenosine deaminase activity (eADA) levels are often elevated (74).

DBA may be misdiagnosed with transient erythroblastopenia of childhood (TEC). Therefore, to avoid misdiagnosing of DBA, TEC must be ruled out. TEC is a common disorder of children age greater than one year. It is an acquired, short-lived failure of red cell production usually of a month or so in duration. TEC, most likely a post infectious, transient autoimmune IgG-mediated disorder, typically occurs in children probably as the result of infections acquired through contact (82). As in DBA, children with TEC often present with profound anemia as a result of pure red cell aplasia. On the other hand, DBA is a dominantly inherited disorder observed in children younger than one year. It is characterized by presence of congenital anomalies; elevated MCV, fetal Hgb and erythrocyte adenosine deaminase activity (75,76,83,84).

## **TREATMENT AND PROGNOSIS OF DBA**

Heterogeneity of DBA is also shown in response to treatment and in the follow-up of DBA cases. In 2008, a group of veteran clinicians established a consensus for the diagnosis and treatment of DBA. This document represents a gold standard for therapeutic decisions. There are no obvious phenotypic or genotypic differences between remission and non-remission patients (71,83).

Although corticosteroid therapy is endeavored with varying regimens for almost all DBA cases, treating the patients with RBC transfusion may be used primarily in the first year of life to reduce corticosteroid associated side effects and adverse effects in neonates which may include noteworthy growth disturbances. Even so there is currently no way to select the patients who will respond or not to the treatment, around 80% of the patients initially respond to steroids but those DBA patients who initially responded to this therapy suffered from many side effects. Some DBA cases remained responsive to steroids, while efficacy vanished in others taking the treatment (18,71,83). The mode of action of corticosteroids in DBA is particularly obscure. Apoptosis at the progenitor level seems to be the cause of the anemia and corticosteroids seem to have a non-specific anti-apoptotic effect in erythroid progenitors particularly at the colony forming unit-erythroid (CFU-E) proerythroblast interface (84).

Patients who are not responsive to steroids or are unable to tolerate the treatment may require chronic RBC transfusion therapy. This therapy is given to most patients every 35 weeks with a goal of sustaining the Hb level greater than 8 g/dL. Based on the child's growth and function, Hb values may need to be higher for some patients. Since the transfusion therapy of RBC is chronic, iron overload becomes challenging, so careful monitoring of serum ferritin levels and other parameters indicative of iron overload is

mandatory (71). Pediatric hematologists with expertise in the treatment of DBA will frequently commence iron chelation with intravenous or oral agents after 15 units of red cell transfusions. Intravenous chelation is frequently needed for the establishment of nonmetallic ports that are magnetic resonance imaging compatible (84,85).

Performing allogeneic Hematopoietic Stem Cell Transplantation (HSCT) is another alternative to cure hematological aspects of DBA. This therapy is performed when DBA patients become unresponsive to repeated transfusions or to prevent iron overload and organ damage due to dependency of frequent transfusions. However, the adverse events due to HSCT may exceed from those adverse events due to iron overloading. In the future, gene therapy is presumed promising for RPS19 deficient DBA patients. Other treatments, have been used in DBA over the last three decades. However, these drugs appear to be largely ineffective and there is currently no evidence that any of these has a major role in the management of DBA (71). The prognosis is generally good. However, complications of treatment and a higher incidence of cancer may reduce life expectancy. Disease severity depends on the quality and response to treatment. For patients undergoing regular transfusions, quality of life is clearly altered (85).

## CONCLUSIONS

DBA is a clinically heterogeneous disorder accompanied by hypoplastic anemia and also manifested by congenital malformations. Even if Ribosomal protein haploinsufficiency have been coined as a primary causative agent of DBA, non-ribosomal proteins such as GATA1 have been identified to have a vital role in the pathogenesis of DBA. The molecular mechanisms which are fundamental for the pathogenesis of DBA are still not entirely understood. The

discovery of RPS19 as the foremost DBA gene led to an exciting scientific research in DBA and other ribosomal disorders. Even though there is convincing evidence for the involvement of p53 in the pathogenesis of DBA, no single proposed mechanism so far accounted for all facets of DBA and yet none is mutually exclusive. Even though a number of genes are responsible for DBA, mutations in RPS19 are the major causes of impairment of ribosomal protein biosynthesis which lead to nucleolar disorganization and activation of the p53 family. Many treatment options have been tried over the past several years with inconsistent success. Despite many treatments having been tried for the management of DBA, chronic red blood cell transfusion, corticosteroids and hematopoietic stem cell transplantation remained the cornerstones of therapy.



## Abbreviations

**CL/P:** Cleft of Lip or Palate

**DBA:** Diamond Blackfan Anemia

**eADA:** elevated erythrocyte Adenosine Deaminase Activity

**HDM:** House Dust Mites

**MDM:** Murine Double Model

**RP:** Ribosomal Protein

**RPL:** Large Ribosomal Protein

**RPS:** Small Ribosomal Protein

**TEC:** Transient Erythroblastopenia of Childhood



## Availability of data and materials

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

### Competing interest

The authors declare that they have no competing interests.

### Authors' contributions

MS & BE performed literature searching and drafted the manuscript. MM involved in drafting of the manuscript along with MS & BE. All authors read and approved the final manuscript.



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