# Monolobated Megakaryocytes in Diamond Blackfan Anemia with RPL5 Mutation at Disease Presentation Mimicking Myelodysplastic Syndrome

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Monolobated Megakaryo<br/>cytes in Diamond Blackfan Anemia with  $RPL5\,$  Mutation at Disease Presentation M<br/>imicking Myelodysplastic Syndrome

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Diamond-Blackfan anemia (DBA) is congenital erythroid aplasia associated with defects in ribosomal biogenesis, classically presenting with macrocytic anemia in infancy and congenital anomalies<sup>1</sup>. Ribosomal protein S19 (*RPS19*) is the most commonly mutated gene, while Ribosomal protein L5 (*RPL5*) mutation accounts for the minority (7%) of cases and is associated with more severe phenotype than *RPS19* mutation<sup>2,3</sup>. Approximately 80% of patients with DBA respond to corticosteroid therapy, whereas 20% require chronic red blood cell (RBC) transfusion<sup>4</sup>. However, they are also at risk of developing myeloid malignancies later in life, including myelodysplastic syndrome (MDS)<sup>4,5,6</sup>. There is an overlap in presentation between DBA and MDS, therefore careful bone marrow assessment with ancillary testing are crucial to detect disease transformation. Megakaryocytic dysplasia at disease presentation in DBA is a highly atypical finding that has not been well described in the literature. We report two cases of DBA with *RPL5* mutation (DBA type 6) showing peculiar megakaryocytic morphology with monolobated nuclei at baseline, mimicking MDS.

Two unrelated infants presented with reticulocytopenic anemia, mild dysmorphic features and cardiac anomalies at birth. Patient 1 was a term male infant with hemoglobin (Hb) of 81 g/L, reticulocyte count of 10 x 109/L, and mean corpuscular volume (MCV) of 99.2 fL. Patient 2 was a female infant born at 36 weeks gestation with Hb of 67 g/L, reticulocyte count of 56 x 109/L, and MCV of 110 fL. Both otherwise had normal blood cell counts and smears. Bone marrow biopsies were first performed at three months of age for patient 1 and one year of age for patient 2. Bone marrow in both patients showed normal to mildly increased cellularity with paucity of erythroid precursors and no dysplasia in erythroid or granulocytic lineages. Blasts were not increased (<1%). Unexpectedly, however, both marrows showed increased number of monolobated megakaryocytes, observed in approximately half of the lineage (Figs. 1A-C). Exome sequencing revealed heterozygous mutation in *RPL5* gene c.[67C>T];[=], p.[Arg23\*];[=] for patient 1 and c.67C>T, p.[Arg23Ter] for patient 2, leading to the diagnosis of DBA type 6. Cytogenetic analysis were normal and next generation sequencing of the bone marrow samples were negative for mutations in myeloid-malignancy related genes.

Both patients were managed with red blood cell transfusions (RBC) as needed and steroids. Patient 1 had follow-up bone marrow biopsies at age five (Fig. 1B) and eight years, which revealed similar findings as previous with persistence of monolobated megakaryocytes and no clonal evolution. At nine years of age, he had been weaned off steroids and only required one RBC transfusion in the past 2 years. Patient 2 was started on steroids at one year of age and was able to discontinue transfusion therapy.

Both DBA and MDS can present with macrocytosis and reticulocytopenic anemia. However, bone marrow in DBA is classically characterized by the absence or <5% of erythroid progenitors and absence of dysplasia at baseline. Megakaryocytic monolobation is considered a dysplastic feature in MDS, particularly the 5q deletion subtype<sup>5</sup>. This observation raised a concern of transformation to MDS. However, megakaryocytic atypia was present as early as three months of age for Patient 1, there was no dysplasia in other cell lineages, genetic testing was negative for MDS-related mutations, and both patients were eventually weaned off steroids and became independent of transfusions years later, following the expected clinical course of DBA. Although megakaryocytes in infants may show less nuclear segmentation or ploidy compared to older children, the number of non-lobated megakaryocytes in these cases was out of proportion to normal age-related changes, and persisted beyond five years of age in Patient 1. To our knowledge, baseline megakaryocytic dysplasia has never been published in DBA literature, apart from one poster abstract that also described an increased number of non-lobulated megakaryocytes in patients with *RPL5* and *RPL11* mutations<sup>7</sup>.

These findings shed light into common pathogenesis between the two disorders. Deletion of 5q in MDS results in haploinsufficiency of RPS14, a ribosomal gene closely related to those implicated in DBA. RPS14 is involved in the MDM2/p53 axis, which is essential in erythroid and megakaryocytic differentiation<sup>8,9</sup>. The inability to assemble ribosomes leads to free ribosomal proteins which bind Murine Double Minute-2 (MDM2) and in turn suppress ubiquitination of p53. This allows for accumulation of p53, resulting in erythroid hypoplasia and dysmegakaryopoiesis<sup>8,9</sup>. Interestingly, both RPL5 and RPL11 proteins have also been shown to be implicated in p53 activation through interaction with the MDM2 protein<sup>10,11,12</sup>. Through this shared MDM2/p53 pathway, it is plausible that RPL5 and RPL11 haploinsufficiency leads to dysmegakaryopoiesis similar to that in MDS with 5q deletion.

Childhood MDS is often considered a higher risk disease, with 5-year overall survival of 63% and usually requires stem cell transplant<sup>5,6</sup>. It is imperative to recognize that the "dysplastic" megakaryocytes may represent a baseline finding in DBA with RPL5 mutations instead of progression to MDS, since the long-term prognosis and management for the two conditions are different. Further studies are needed to investigate whether DBA with RPL5 mutations represent a distinct subcategory that shares similar pathophysiology with MDS with 5q deletion and explore common therapeutic strategies for both conditions.

#### Consent

Informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available upon request by the Editor-in-Chief of this journal.

#### **Competing interests**

The authors declare that they have no competing interests.

#### Authors' contributions

AS and CS put together the patient data, took pathology images, and reviewed literature on the topic. AS, NA, AL and AA were all involved in the patient's care and all authors contributed to writing the manuscript. All authors read and approved the final manuscript.

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## References

- 1. Engidaye G, Melku M, Enawgaw B. Diamond Blackfan Anemia: Genetics, Pathogenesis, Diagnosis and Treatment. EJIFCC. 2019 Mar 1;30(1):67-81.
- Quarello P, Garelli E, Carando A, Brusco A, Calabrese R, Dufour C, Longoni D, Misuraca A, Vinti L, Aspesi A, Biondini L, Loreni F, Dianzani I, Ramenghi U. Diamond-Blackfan anemia: genotype-phenotype correlations in Italian patients with RPL5 and RPL11 mutations. Haematologica. 2010 Feb;95(2):206-13.
- Gazda HT, Sheen MR, Vlachos A, 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 Dec;83(6):769-780.
- 4. Vlachos A, Muir E. How I treat Diamond-Blackfan anemia. Blood. 2010 Nov 11;116(19):3715-23.
- 5. Niemeyer CM, Kratz CP. Paediatric myelodysplastic syndromes and juvenile myelomonocytic leukaemia: molecular classification and treatment options. Br J Haematol. 2008 Mar;140(6):610-24.
- 6. Strahm B, Nöllke P, Zecca M, Korthof ET, Bierings M, Furlan I, Sedlacek P, Chybicka A, Schmugge M, Bordon V, Peters C, O'Marcaigh A, de Heredia CD, Bergstraesser E, Moerloose BD, van den Heuvel-Eibrink MM, Starý J, Trebo M, Wojcik D, Niemeyer CM, Locatelli F; EWOG-MDS study group. Hematopoietic stem cell transplantation for advanced myelodysplastic syndrome in children: results of the EWOG-MDS 98 study. Leukemia. 2011 Mar;25(3):455-62.
- Leak S, Harrington Y, de la Fuente J. RPL5 and RPL11 Diamond Blackfan Anaemia Patients Have An Increased Number Of Hypolabated and Non-Lobulated Megakaryocytes In The Bone Marrow. Blood. 2013; 122 (21): 1229.
- 8. Kang J, Brajanovski N, Chan KT, Xuan J, Pearson RB, Sanij E. Ribosomal proteins and human diseases: molecular mechanisms and targeted therapy. Signal Transduct Target Ther. 2021 Aug 30;6(1):323.
- 9. Schneider RK, Schenone M, Ferreira MV, Kramann R, Joyce CE, Hartigan C, Beier F, Brümmendorf TH, Germing U, Platzbecker U, Büsche G, Knüchel R, Chen MC, Waters CS, Chen E, Chu LP, Novina CD, Lindsley RC, Carr SA, Ebert BL. Rps14 haploinsufficiency causes a block in erythroid differentiation mediated by S100A8 and S100A9. Nat Med. 2016 Mar;22(3):288-97.
- Dai MS, Lu H. Inhibition of MDM2-mediated p53 ubiquitination and degradation by ribosomal protein L5. J Biol Chem. 2004 Oct 22;279(43):44475-82.
- Dai MS, Zeng SX, Jin Y, Sun XX, David L, Lu H. Ribosomal protein L23 activates p53 by inhibiting MDM2 function in response to ribosomal perturbation but not to translation inhibition. Mol Cell Biol. 2004 Sep;24(17):7654-68.
- 12. Bhat KP, Itahana K, Jin A, Zhang Y. Essential role of ribosomal protein L11 in mediating growth inhibition-induced p53 activation. EMBO J. 2004 Jun 16;23(12):2402-12.

#### Figure Legend:

FIGURE 1 Bone marrow aspirate slides showing paucity of erythroid precursors and hypo/monolobated megakaryocytes (pointed by black arrows) in A) Patient 1 at three months of age (50x objective, Wright stain), B) Patient 1 at five years of age (50x objective, Wright stain), and C) Patient 2 at one year of age (50x objective, Wright stain).

