Clinical Notes

Late-onset familial Diamond–Blackfan anemia with neutropenia caused by *RPL35A* variant

Running title: Late-onset DBA caused by *RPL35A* variant

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Diamond–Blackfan anemia (DBA) is a rare inherited bone marrow failure syndrome (IBMFS) with distinctive characteristics of erythroblastopenia, congenital anomalies, and cancer predispositions.[1] Because its symptoms are heterogeneous, diagnosis of DBA with atypical findings is challenging. DBA patients often carry pathogenic variants in ribosomal protein genes, and molecular genetic analyses can enable clinicians to reach a diagnosis in atypical patients.[2] Here, we report a case of familial DBA with *RPL35A* in-frame variant and atypical clinical manifestations.

A 7-year-old boy visited a clinic for persistent airway symptoms and repeated otitis media. Blood tests revealed anemia and neutropenia, and he was referred to our hospital. His blood test data were: white blood cell count, 2.15×10^9/L (19.0% segmented cells, 1.0% stab cells, 79.0% lymphocytes, 1.0% eosinophils, 0% blasts); red blood cell count, 2.08×10^{12}/L; hemoglobin, 68 g/L; hematocrit, 0.192; mean corpuscular volume, 92.3 fL; reticulocyte count, 15×10^9/L; platelet count, 352×10^9/L; lactate dehydrogenase, 248 U/L; ferritin, 89.8 ng/mL; haptoglobin, 45 mg/dL; IgG, 1161 mg/dL; Hb-F, 2.4%; erythropoietin, 4480 mIU/mL. A bone marrow aspirate showed slightly hypocellular marrow without blasts or dysplastic cells (nucleated cell count, 66×10^9/L). Erythroblasts were detected and myeloid-to-erythroid ratio was 1.44 (Fig. 1). Cytogenetic analysis showed no karyotype abnormalities, and chromosome-

breakage test was negative. He had normal stature (125.4 cm; +0.29 SD) and no obvious congenital anomalies.

Regarding familial history, his 39-year-old father had received treatment with prednisolone for pure red cell aplasia or DBA since the age of 5 years. His father also had no congenital anomalies. The last bone marrow aspirate for his father conducted at 27 years of age showed almost-normal marrow cellularity without abnormal findings. Thus, we initiated the boy on prednisolone 1 mg/kg/day and his hemoglobin level recovered to approximately 100 g/L. His neutrophil count, which had been approximately 0.30×10^9/L to 0.50×10^9/L, increased to approximately 1.0×10^9/L after the initiation of prednisolone. Subsequently, we tapered prednisolone to 1 mg alternate days and his hemoglobin level and neutrophil count were maintained. Furthermore, the frequency of his infectious episodes decreased after the initiation of prednisolone.

Targeted next-generation sequencing (NGS) for DBA was performed after receiving informed consent from his parents. An in-frame variant of *RPL35A* (NM_000996.4:c.79CTT[1] (p.Leu28del); variant allele frequency, 0.51) was detected. His father was also analyzed and found to carry the same variant (variant allele frequency, 0.29). This variant was considered to be likely pathogenic for their hematological symptoms.

DBA is a genetically and clinically heterogeneous IBMFS, although most cases are diagnosed within the first year of life.[1] In our patient, erythroblasts in bone marrow, neutropenia, late onset age, and absence of congenital anomalies made it difficult to diagnose DBA. Although some DBA cases with neutropenia have been reported, the frequency and etiology of neutropenia in DBA remain unknown.[3,4] When limited to cases with *RPL35A* variants, neutropenia was documented in 33 of 45 cases in a retrospective study.[4] We consider neutropenia to be a symptom of DBA in our patient and partially related to his susceptibility to infection. In this sense, neutropenia may be a characteristic symptom in DBA with *RPL35A* variants. In addition, our patient is unique in having a considerably late onset age.

Pathogenic variants are estimated to be identified in 60%–70% of DBA patients by genetic analysis, and *RPL35A* variants affect approximately 3% of patients.[1] ~~Although the functional significance of the variant detected in our cases has not been fully analyzed, we consider the variant to be likely pathogenic.~~ The phenotypes and family history of our patient were suggestive of DBA, and some *RPL35A* variants are known to be pathogenic for DBA. ~~Furthermore,~~ T~~t~~he p.Leu28del in-frame variant, which was detected in our cases, was estimated to be the most common *RPL35A*

variant.[4] Some familial cases with *RPL35A* variants were described in a Japanese cohort, but accurate data on their frequency and clinical characteristics are limited.[5]

In conclusion, we have described a case of late-onset familial DBA presenting with neutropenia. We conducted targeted NGS and achieved a diagnosis. Although DBA is a genetically and clinically heterogeneous syndrome, patients with *RPL35A* variants involving a non-large deletion may present with atypical symptoms. Further research on genotype-phenotype associations for this rare disease is expected to improve the selection of patients for genetic analysis and accurate diagnosis.

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Disclosure

The authors declare no conflicts of interest.

Ethical statement

Informed consent was obtained from the patient and his parents for the publication of this case report.

Author contributions

K.T. wrote the manuscript. M.M. and A.S. carried out medical care of the patient. M.W. designed and performed the genetic analysis. K.W. and A.S. revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

References

1 Costa L da, Leblanc T, Mohandas N. Diamond-Blackfan anemia. *Blood*. 2020;**136**:1262–73.

2 Muramatsu H, Okuno Y, Yoshida K, Shiraishi Y, Doisaki S, Narita A, et al. Clinical utility of next-generation sequencing for inherited bone marrow failure syndromes. *Genet. Med.* 2017;**19**:796–802.

3 Schofield KP, Evans DIK. Diamond-Blackfan syndrome and neutropenia. *J Clin Pathol*. 1991;**44**:742–4.

4 Gianferante MD, Wlodarski MW, Atsidaftos E, da Costa L, Delaporta P, Farrar JE, et al. Genotype-phenotype association and variant characterization in Diamond-Blackfan anemia caused by pathogenic variants in RPL35A. *Haematologica*. 2021;**106**:1303–10.

5 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**:854–64.

Figure Legend

Fig. 1 Staining of the bone marrow aspirate smear. (a) The bone marrow aspirate showed slightly hypocellular marrow. The nucleated cell count was 66×10^9/L. (b) Trilineage cells without dysplastic change were observed. (c) Myeloid cells at each stage of maturation were observed in the bone marrow. However, mature myeloid cells were less frequent than normal. The myeloid-to-erythroid ratio was 1.44. (d) Erythroid precursor cells were detected in the bone marrow smear (total erythrocytic series, 24.4%).