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LETTER TO THE EDITOR

A novel heterozygous *ALAS2* mutation in a female with macrocytic sideroblastic anemia resembling myelodysplastic syndrome with ring sideroblasts: a case report and literature review

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Dear Editor,

Sideroblastic anemias are a group of disorders with the common feature of bone marrow ring sideroblasts (RS), reflecting excess mitochondrial iron deposition [1]. In adults, these syndromes are commonly associated with myelodysplastic syndrome (MDS), and a significant portion of cases result from a mutation in the RNA splicing machinery component splicing factor 3b, subunit 1 (*SF3B1*) [2]. Congenital sideroblastic anemia is rare and represented by X-linked sideroblastic anemia (XLSA), which is attributed to mutations in the X-linked gene erythroid-specific 5-aminolevulinate synthase (*ALAS2*) [1, 3]. *ALAS2* encodes the enzyme catalyzing the first and rate-limiting steps in the heme biosynthesis pathway in erythroid cells. XLSA is predominantly observed in hemizygous males and manifests as microcytic anemia with systemic iron overload, whereas most heterozygous female carriers are asymptomatic and may exhibit only minor red cell abnormalities; however, some cases develop into microcytic sideroblastic anemia due to unbalanced lyonization, skewed X chromosome inactivation, or age-related clonal hematopoiesis [4–6]. Here, we review recently recognized adult female cases of macrocytic sideroblastic anemia caused by heterozygous *ALAS2* mutation.

A 61-year-old woman had macrocytic anemia since adulthood and was receiving iron chelation therapy. Her son is healthy, but her mother had anemia of unclear etiology. At her first visit to our hospital, the white blood cell count was 2400/μL; red blood cell count, 1.96×10^6/μL; hemoglobin, 7.3 g/dL; hematocrit, 22.1%; mean corpuscular volume, 112.8 fL; and platelet count, 8.6×10^4/μL. Serum biochemical analysis demonstrated increased ferritin (913.2 ng/mL). Bone marrow analysis revealed RS (60% of all erythroblasts) (Fig. 1a), with normal karyotype and mild megaloblastoid changes in the erythroid lineage (Fig. 1b), implying MDS with RS (MDS-RS). After obtaining written informed consent, we conducted Sanger sequencing to identify the genetic alteration associated with RS formation. Surprisingly, genomic DNA sequencing of whole blood revealed a novel 488 G > A heterozygous *ALAS2* mutation (Fig. 1c). The mutation was germline because it involved the buccal cells (Fig. 1d), and it resulted in an amino acid substitution of arginine for histidine at residue 163. No mutation was identified in *SF3B1*. In vitro enzymatic analysis with bacterially expressed recombinant ALAS2 protein [7] confirmed that the mutation severely abrogated its enzymatic activity (Fig. 1e), supporting the diagnosis of congenital sideroblastic anemia rather than MDS-RS. Similar to previous reports [8, 9], we observed complete skewing toward the expression of wild-type *ALAS2* mRNA in reticulocytes (Fig. 1f, g). Thus, the severe loss-of-function mutation of *ALAS2* might perturb terminal erythroid maturation, leading to sideroblastic anemia in heterozygous females and embryonic lethality in hemizygous males, which is supported by the observation that anemia was detected exclusively in females (Fig. 1g). Regarding the mechanisms of macrocytic anemia occurrence, the compensatory stimulation of erythropoiesis within wild-type *ALAS2*-expressing erythroblasts might play a role. Nevertheless, further accumulation of cases and the establishment of animal models are required.

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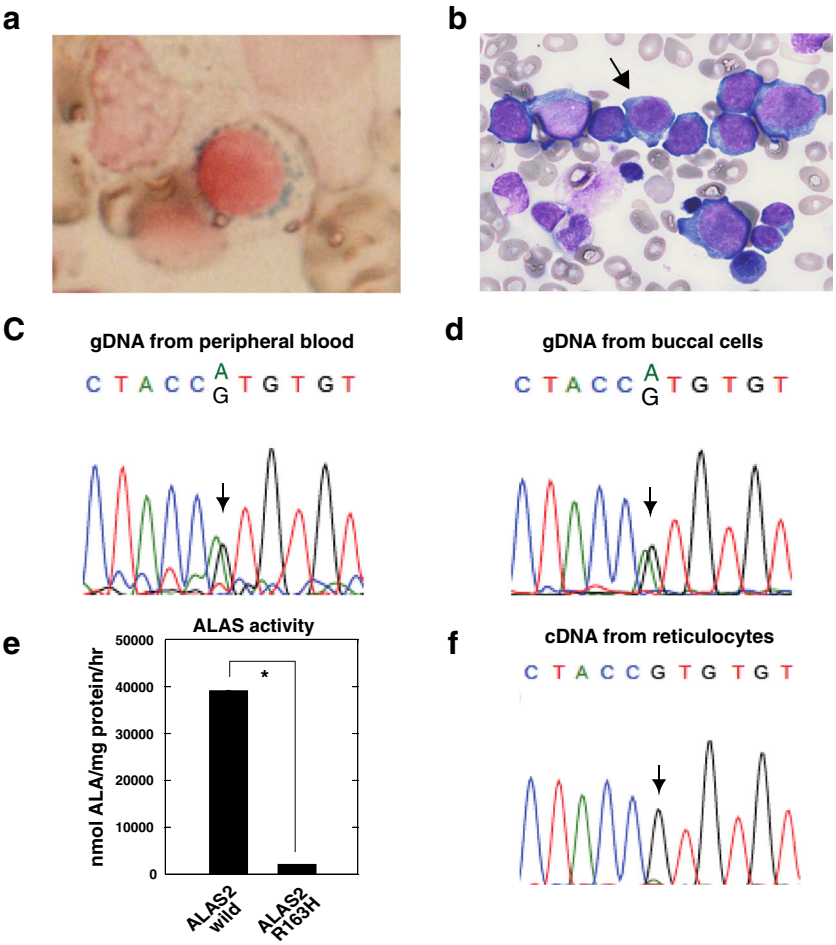

Fig. 1 Macrocytic sideroblastic anemia in a female with a novel heterozygous *ALAS2* mutation. **a** Representative picture of ring sideroblast (Prussian blue stain). **b** May-Grunwald-Giemsa stain of bone marrow cells. Mild megaloblastic change was indicated by arrow. **c**, **d** Heterozygous *ALAS2* mutation was confirmed using genomic DNA from whole blood (**c**) and buccal cells (**d**). The mutation site is indicated by an arrow. **c**, **d** ALAS2 enzymatic activity with recombinant protein. **e** In vitro ALAS2 enzymatic activity with recombinant protein. For the analysis, 1 μg of the protein was used in the presence of 200 μM of pyridoxal 5′-phosphate as a cofactor of ALAS2 [7]. The data are expressed as mean ± standard deviation ($n = 3$). *$p < 0.05$ based on Student's t test. **f** Sanger sequencing for ALAS2 with cDNA derived from reticulocytes. The mutation site is indicated by an arrow. **g** Summary of the reported cases of macrocytic sideroblastic anemia among females with a novel heterozygous *ALAS2* mutation, including the present case

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Case	1	2	3
Proband's gender	Female	Female	Female
Age at diag.	61	42	32
Family History of Anemia	Yes (Mother)	Yes (Mother; Macrocytic)	Yes (Mother & Sister; Macrocytic)
ALAS2 mutation	R163H	R227C	Y365C
Hb at diag. (g/dL)	7.3	6.3	Unknown (Hct 24%)
MCV at diag. (fL)	112.8	112	114-122
Response to Vit. B6	No	No	N/D
Skewed X inactivation	N/D	N/D	No
ALAS2 mRNA expression in reticulocytes	Wild-type	Wild-type	Wild-type
Reference	Present case	(8)	(9)

Because MDS cases typically exhibit macrocytic anemia [10], the possibility of heterozygous *ALAS2* mutations should be considered in the diagnosis of MDS-RS, especially among females without *SF3B1* mutation, and with a family history of anemia and/or minor dysplastic morphological changes. Our report provides a definite conceptual framework for better understanding of the pathophysiology of sideroblastic anemia.

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Compliance with ethical standards After obtaining written informed consent, we conducted Sanger sequencing to identify the genetic alteration associated with RS formation

Conflict of interest The authors declare that they have no conflict of interest.

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