



Mutation Analysis of X-linked Sideroblastic Anemia in a 12-Month-Old Boy by Massively Parallel Sequencing

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Dear Editor,

Hereditary sideroblastic anemia (SA) is characterized by anemia with ringed sideroblasts in the bone marrow (BM) [1]. Phenotypic expression is highly variable, and the most common hereditary SA is X-linked sideroblastic anemia (XLSA, OMIM #300751) caused by mutation of the erythroid-specific δ -aminolevulinic synthase gene, *ALAS2* [2-4].

We present a case of a 12-month-old boy with severe congenital anemia who was finally diagnosed as having XLSA after simultaneous BM study and molecular genetic analysis by massively parallel sequencing (MPS). The boy was referred from an outpatient pediatric clinic because of severe anemia and progressive pallor. He was a full-term baby, and no one in his family had been diagnosed as having anemia. The pallor was considered pathological, and his weight gain of 7.9 kg was insufficient, leaving him below the third percentile for his age. He was transfused packed red blood cells (RBCs) to correct his Hb level of 59 g/L; however, his pallor persisted, and Hb level recovered only temporarily. He was subsequently referred to the Depart-

ment of Pediatrics at Kangbuk Samsung Hospital. The initial complete blood count, as determined at our institute, was as follows: Hb, 70 g/L; hematocrit, 27.5%; mean corpuscular volume, 63.2 fL; mean corpuscular hemoglobin, 15.9 pg; mean corpuscular hemoglobin concentration, 25.1 g/dL; red cell distribution width, 31.3%; white blood cells, $7.0 \times 10^9/L$; and platelets, $417 \times 10^9/L$. A peripheral blood smear revealed a dimorphic red cell population due to transfusion (Fig. 1A).

His iron profile suggested plentiful body iron stores, as indicated by the following parameter values: elevated serum iron, 49.8 $\mu\text{mol/L}$ (laboratory reference range, 11.6–43.3); normal ferritin, 267.1 pmol/L (36.0–898.8); and total iron-binding capacity, 52.4 $\mu\text{mol/L}$ (42.2–68.0). Hemolytic anemia was excluded by negative direct and indirect Coombs' tests, and he had normal lactate dehydrogenase (324 IU/L [0–250]) and haptoglobin (4.76 $\mu\text{mol/L}$ [3.00–20.00]) levels.

BM aspirate smear revealed an estimated myeloid:erythroid ratio of 1.6:1 and showed small erythroblasts with abnormal condensation of nuclear chromatin and ragged cytoplasm (Fig. 1B,

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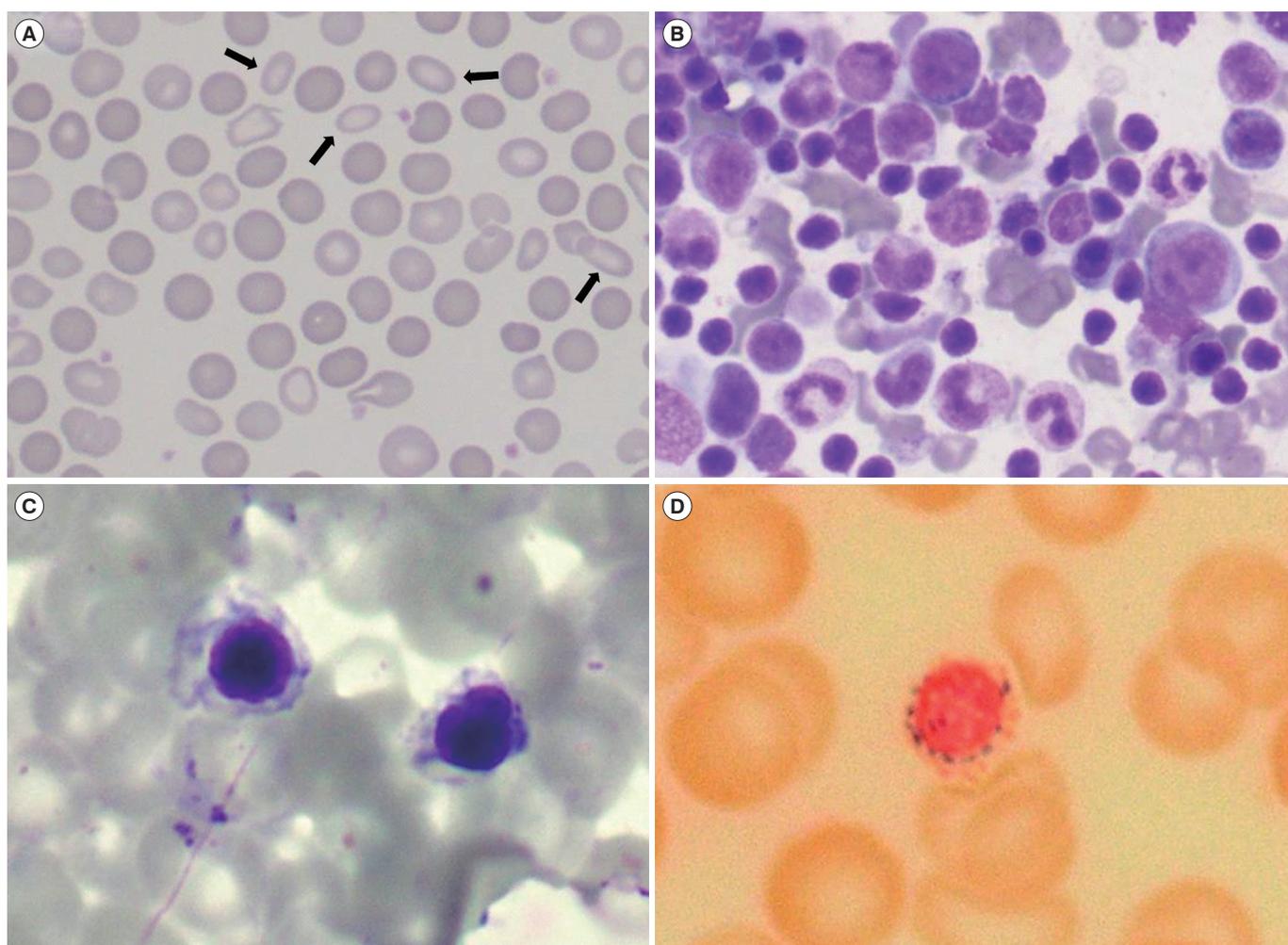


Fig. 1. Peripheral blood smear, bone marrow aspiration, and iron staining results of the patient. (A) Wright-Giemsa-stained peripheral blood smear ($\times 400$) shows microcytic hypochromic anemia with anisopoikilocytosis (black arrows). (B, C) Wright-Giemsa-stained bone marrow aspirate ($\times 400$, $\times 1,000$) shows small erythroblasts with abnormal condensation of nuclear chromatin and ragged cytoplasm. (D) Iron staining ($\times 1,000$) reveals multiple ringed sideroblasts, which comprise over 40% of erythroblasts.

C). Iron staining on the BM aspirate slide showed ringed sideroblasts, which comprised 40% of erythroid precursors (Fig. 1D).

For molecular diagnosis, we conducted MPS after obtaining written informed consent from the patient's parents. Genomic DNA was extracted from peripheral blood. We used the TruSight One sequencing panel (Illumina, San Diego, CA, USA), which includes more than 125,395 probes targeting a 12-Mb region spanning 4,813 genes, including the six known hereditary SA-related genes (*ALAS2*, *SLC25A38*, *GLRX5*, *HSPA9*, *PUS1*, and *ABCB7*) for library preparation and target enrichment. We performed MPS using the NextSeq platform (Illumina) with 150-bp paired-end sequencing, using the pipeline of the Burrows-Wheeler Aligner MEM algorithm, version 0.7.12 [5]; Picard tools, version 1.96 (<https://broadinstitute.github.io/picard/>); and Genome

Analysis Toolkit (GATK), version 3.5 [6] for data analysis. Variant calling and annotation were performed with GATK Haplotype-Caller, Variant Effect Predictor, and dbNSFP. We identified a hemizygous, known mutation, NM_000032.4:c.508C>A (p.Arg170Ser), in exon 5 of *ALAS2*, and validated this variant by Sanger sequencing. According to the guidelines of the American College of Medical Genetics and Genomics [7], this variant is classified as likely pathogenic based on two moderate (PM1 and 2) and four supporting (PP2-5) pathogenic evidences. Finally, we diagnosed the patient as having XLSA with an *ALAS2* mutation, and found his mother to be a heterozygous carrier by using Sanger sequencing (Fig. 2).

The patient was started on pyridoxine therapy (100 mg/day orally) as standard treatment for the goals of maintaining Hb

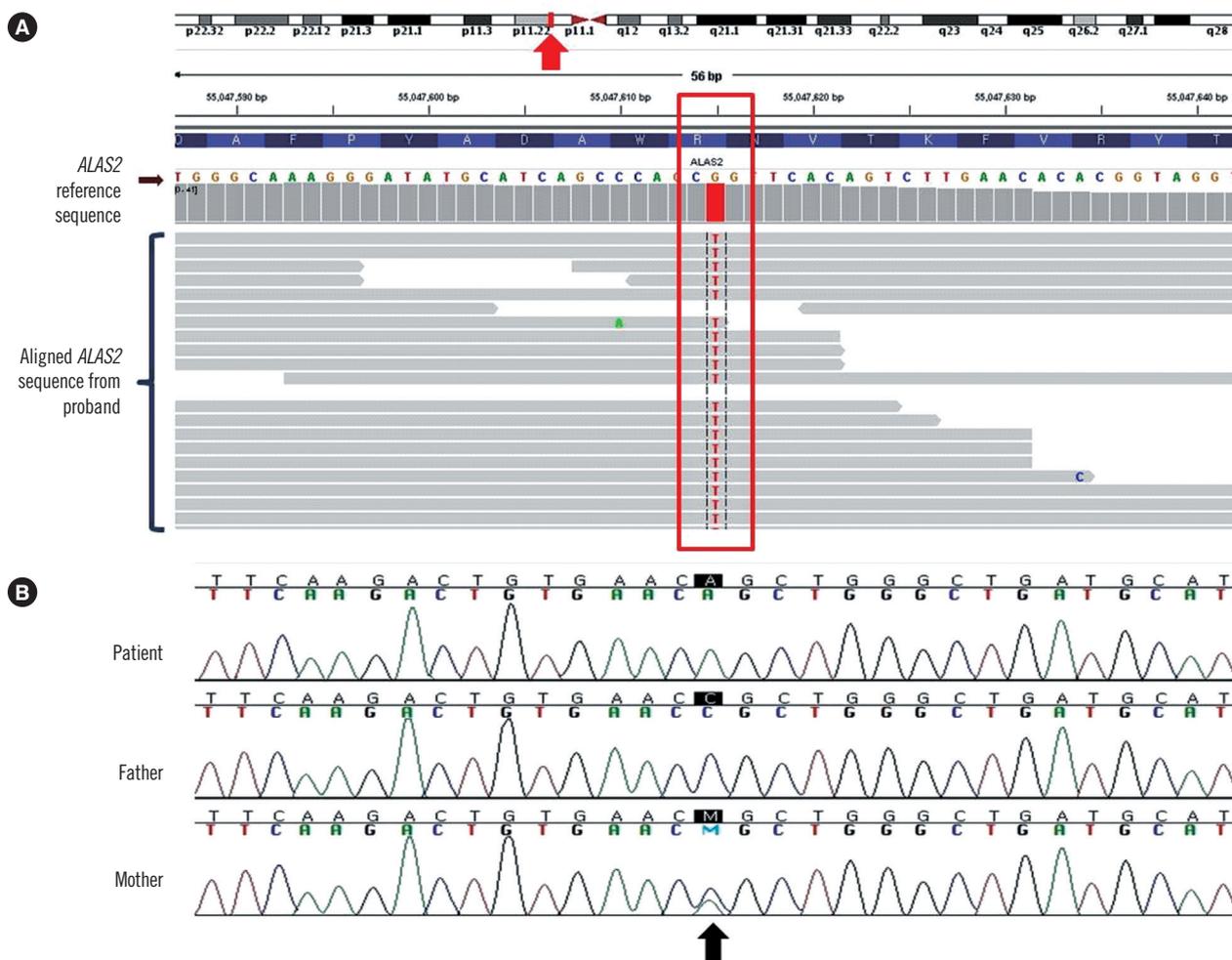


Fig. 2. *ALAS2* variant identified in this family. (A) Massively parallel sequencing (MPS) of candidate genes for sideroblastic anemia, including *ALAS2* (chromosomal location: Xp11.21, red arrow), revealed a hemizygous mutation in this gene (c.508C>A; p.Arg170Ser) in the proband (displayed in reverse read direction in Integrative Genomics Viewer). (B) Sanger sequencing revealed that the mother was heterozygous for the c.508C>A mutation.

above 100 g/L and normal growth for his age with minimal RBC transfusions [8, 9]. Because his Hb level did not show improvement with pyridoxine treatment alone at his one-month follow-up visit, we added a 1-mg oral folate supplement daily. Subsequently, his growth improved, as his weight increased to the 12th percentile at 18 months of age. This study was approved by the Institutional Review Board of Kangbuk Samsung Hospital (KBSMC 2018-02-018).

To date, 64 distinct disease-causing mutations have been described in individuals or families with XLSA [10]. One-by-one Sanger sequencing of candidate genes is generally used to identify pathogenic mutations in hereditary diseases. In our case, MPS enabled the simultaneous analysis of multiple genes, which allowed immediate appropriate treatment. To the best of our knowl-

edge, this is the second case of XLSA caused by missense *ALAS2* p.Arg170Ser mutation, which was reported for the first time by Harigae *et al* in 2010 [6]. Edgar *et al* [11] reported a different *ALAS2* nucleotide substitution, p.Arg170Leu, in a 31-year-old male patient who showed favorable outcome.

Hereditary anemias are often misdiagnosed as iron deficiency anemia, and this can have dangerous clinical consequences, including parenchymal iron overload. In this case, we identified early-onset hereditary SA with severe anemia. Immediate and accurate diagnosis of XLSA led to effective treatment and a favorable clinical course. Therefore, MPS is a useful method for the rapid diagnosis of hereditary anemia in newborns or infants with severe anemia.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

REFERENCES

1. Cazzola M and Invernizzi R. Ring sideroblasts and sideroblastic anemias. *Haematologica* 2011;96:789-92.
2. Barton JC and Lee PL. Disparate phenotypic expression of ALAS2 R452H (nt 1407 G→A) in two brothers, one with severe sideroblastic anemia and iron overload, hepatic cirrhosis, and hepatocellular carcinoma. *Blood Cells Mol Dis* 2006;36:342-6.
3. Cazzola M, May A, Bergamaschi G, Cerani P, Ferrillo S, Bishop DF. Absent phenotypic expression of X-linked sideroblastic anemia in one of 2 brothers with a novel ALAS2 mutation. *Blood* 2002;100:4236-8.
4. Cotter PD, May A, Fitzsimons EJ, Houston T, Woodcock BE, al-Sabah AI, et al. Late-onset X-linked sideroblastic anemia. Missense mutations in the erythroid delta-aminolevulinic synthase (ALAS2) gene in two pyridoxine-responsive patients initially diagnosed with acquired refractory anemia and ringed sideroblasts. *J Clin Invest* 1995;96:2090-6.
5. Li H. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv preprint arXiv:1303.3997 2013.
6. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytzky A, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res* 2010;20:1297-303.
7. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015;17:405-24.
8. Harigae H and Furuyama K. Hereditary sideroblastic anemia: pathophysiology and gene mutations. *Int J Hematol* 2010;92:425-31.
9. Fleming MD. The genetics of inherited sideroblastic anemias. *Semin Hematol* 2002;39:270-81.
10. Cooper DN, Ball EV, Stenson PD, Phillips AD, Evans K, Heywood S, et al. The Human Gene Mutation Database at the Institute of Medical Genetics in Cardiff. <http://www.hgmd.cf.ac.uk> (Updated on December 2016).
11. Edgar AJ, Vidyatilake HM, Wickramasinghe SN. X-linked sideroblastic anaemia due to a mutation in the erythroid 5-aminolevulinic synthase gene leading to an arginine170 to leucine substitution. *Eur J Haematol* 1998;61:55-8.