

Rapid Communication

Diagnostic Conundrum: Anemic New born and Identification of Novel Red Cell Gene Mutation Supporting Sideroblastic Anemia

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Abstract

Term newborn usually have a higher Hemoglobin (Hb) compared to older children and adults and majority of their Hb consists of HbF. However, babies born with congenital sideroblastic anemias can be anemic at birth in absence of hemolysis or bleeding complications. Diagnosis can be challenging and Red Cell Gene Panel (RCGP) can be helpful in such circumstances. We report such a newborn here who had low HbF% at birth and he was diagnosed with Autosomal Recessive pyridoxine refractory congenital Sideroblastic Anemia (ARSA) and we identify a novel red cell gene mutation (the SLC25A38 c. 706c>T).

Keywords: Hemoglobin F; Anemia; Red cell gene mutation; Sideroblastic anemia

Introduction

Term newborn usually have a higher Hemoglobin (Hb) compared to older children and adults. This increased Hb is a normal compensatory mechanism in these infants for the relative tissue-level hypoxia that is prevalent in the intrauterine environment, and it is exacerbated by the high affinity of fetal hemoglobin for oxygen [1,2]. In utero, the oxygen saturation in arterial blood is low and erythropoietin levels are high, hence there is a rapid red blood cell production in fetuses. Soon after birth, the oxygen saturation goes as high as up to 95%, which in turn down regulates the erythropoietin mediated red cell production and haemoglobin levels fall [3]. At birth the Hb levels are 149 g/L-237 g/L in term and 191 g/L-221 g/L in preterm babies [4].

Congenital Sideroblastic Anemias are rare disorder caused by mutations in genes that are involved in heme synthesis, iron-sulfur cluster biogenesis, or mitochondrial metabolism [5]. For heme synthesis, the gene defect is on the x-chromosome, forming mutations in the Aminolevulinic Synthase (ALAS2), Adenosine Triphosphate-Binding Cassette B7 (ABCB7) or Glutaredoxin 5 (GRLX5) enzymes. Other causes include mutations in the mitochondrial transporter (SLC25A38), thiamine transporter SLC19A2, RNA modifying enzyme Pseudouridine Synthase (PUS1), mitochondrial Tyrosyl-Trna Synthase (YARS2) and mitochondrial DNA deletions [6].

A male baby born at term underwent blood tests as he was clinically listless. His blood test showed he was anemic but his White Blood Count (WBC) and Platelet counts was normal. His MCV as well as MCH were also exceptionally low. Hemoglobin Electrophoresis (HbE) using high performance liquid chromatography did not show any abnormal Haemoglobin (Hb) but Hishemoglobin F (HbF) level was much lower than expected at 22.5% for a new born infant (Table 1 & Figure 1). There was no history of intra-uterine bleeding complications or any intra-uterine transfusion which could have explained his low HbF%. His vital parameters remain

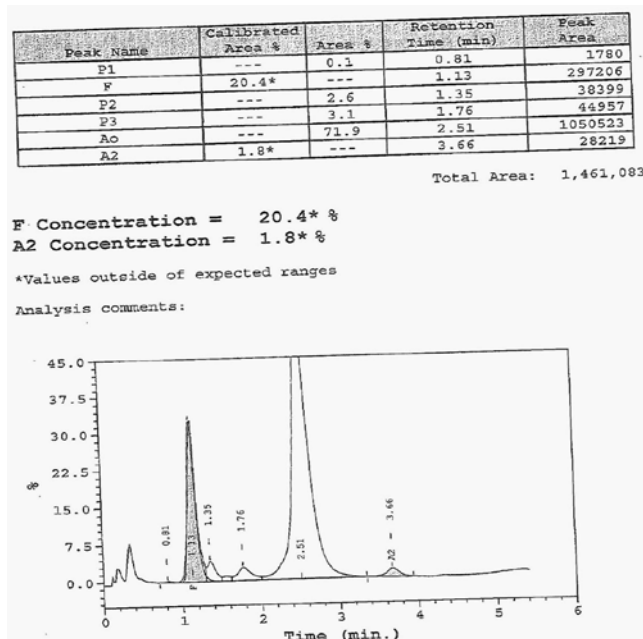


Figure 1: Baby's Hb Electrophoresis.

stable. Neonatologist started him on antibiotic suspecting infection. However, he remained clinically stable but listless and his blood parameters did not improve. Both his parents were physically well, and they had normal blood parameters (Table 2).

Her parents were not in consanguineous marriage and they are of Pakistani origin. As his Hb did not improve over the course of 3 weeks and no obvious causes were identified, his blood sample was sent for Red Cell Gene Panel (RCGP) studies (Table 3). Test for pathogenic variants in genes known to be associated with hemolytic anemia (e.g. red cell membranopathy, enzymopathy, hemoglobinopathy), Congenital Dyserythropoietic Anemia (CDA), Diamond Black fan

Table 1: Baby' blood results.

Day 0	Day 1	Day 10
WBC- 19.1x 10 ⁹ /L (10.0 - 26.0) Neutrophils -13x 10 ⁹ /L (4.0 - 14.0) HB-87 g/L (140 - 220) MCV -68.5 fL (100.0-120.0) MCH-18.4 pg (31.0-37.0) PLT- 166x10 ⁹ /L (150-410) C-Reactive Protein<1 mg/L (0 - 5) Total Bilirubin- 14 umol/L (<21) Direct Bilirubin-9 umol/L (0.0 - 5.0) LDH -581 iu/L (225 - 600) Baby Blood Group- A Rh D NEGATIVE DAT Screen-NEGATIVE Blood Culture-No growth after 5 days Hb electrophoresis-HB A+F Haemoglobin A2- 1.7 % (2.4 - 3.5) Haemoglobin F- 22.5 % Reticulocytes Absolute174.1x10 ⁹ /L (30.0 - 100.0)	WBC- 25.2x10 ⁹ /L (10.0 - 26.0) HB-95 g/L (140 - 220) MCV-66.3fL (100.0 - 120.0) MCH-18.7 pg (31.0 - 37.0) PLT- 201x10 ⁹ /L (150 - 410) Reticulocytes Absolute194.7x 10 ⁹ /L (30.0 - 100.0) Ferritin-892ug/L (13 - 150) Transferrin Saturation 67.3 % (20 - 55) Vitamin B12- 376 ng/L (197 - 771) Serum Folate>20.0 ug/L (3.9 - 26.8)	WBC-9.6 x 10 ⁹ /L (5.0-19.0) Neutrophils- 3.9 x10 ⁹ /L (4.0-14.0) HB-95 g/L (101 - 183) MCV-63.3 fL (90.0 - 120.0) MCH-18.0pg (24.0 - 30.0) PLT – 256x10 ⁹ /L (150 - 410) C-Reactive Protein-3mg/L (0 - 5) Total Bilirubin-24 umol/L (<21) Direct Bilirubin- 8umol/L (0.0 - 5.0)

Table 2: Parents blood results.

Mother	Father
Blood group-O Rh D negative NO ATYPICAL RED CELL ANTIBODIES DETECTED WBC 8.8x 10 ⁹ /L (3.9 - 11.1) HB 123 g/L (120 - 150) MCV 95.2 fL (83.0 - 101.0) MCH 32.8 pg (27.0 - 32.0) PLT 152x 10 ⁹ /L (150 - 410)	WBC-5.9x 10 ⁹ /L (3.7 - 9.5) HB -154g/L (133 - 167) MCV -83.9 fL (82.0 - 98.0) MCH-27.4 pg (27.3 - 32.6) PLT -221x10 ⁹ /L

anemia (DBA) and Sideroblastic anemia using Agilent Sure-Select enrichment Technology and Illumina DNA sequencing. Reported variants were confirmed by Sanger sequencing. Pathogenic variants in the SLC25A38 genes are associated with Autosomal Recessive Pyridoxine Refractory Congenital Sideroblastic Anemia (ARSA) [7].

However, the SLC25A38 c. 706c>T variant has not been previously reported in English literature and we are not sure where the clinical phenotype associated with this new mutation will be different from SLC25A38 associated other ARSA. It is also not clear why the Hb F is low in this infant. The RCGP result did not indicate any abnormality in red cell membranopathy subpanel. However, there is obviously something affecting the haemoglobin switching and HbA production with adult production up-regulated early. However, it is not clear why HbA production is upregulated and we think there is likely other factors involved which we do not fully understand at this time. Parents testing including RCGP would have been helpful however it was not possible due to their refusal.

Congenital sideroblastic anemias showed characteristic pathological deposition of iron in mitochondria of erythroid precursors leading to ineffective erythropoiesis with iron overload,

and in the peripheral blood there are hypochromic red cells [8]. They can be subdivided into syndromic and non-syndromic forms and are genetically heterogeneous and others due to mitochondrial DNA deletion. Non-syndromic forms are either X-linked or autosomal recessive. Their morphological hallmark is the presence of ring sideroblasts in the bone marrow. These represent iron-laden mitochondria which form a ring around the nucleus of erythrocyte precursors [9]. This feature points to a common pathophysiology due to genetic mutations that disrupt heme-iron biosynthesis in the mitochondria [10].

Unlike X-linked sideroblastic anemias ARSA is not responsive to treatment with pyridoxine and they are phenotypically severe. Supportive care is the mainstay of treatment which includes hematological monitoring; the surveillance of iron levels and almost always requires chronic blood transfusions. Iron chelation is required for transfusion hemosiderosis. In some cases, Allogeneic stem cell transplantation is considered as a successful treatment option and it is the only cure for this condition at present [11].

Author Contribution

MSI conceived the article, MSI has written the manuscript reviewed the literature. DH has analysed the sample, MSI and DH have interpreted the results. Both authors have agreed on the final manuscript.

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Table 3: Red cell gene panel results.

Apparent homozygous SLC25A38 c. 706c>T; p. (Gln236*)-likely pathogenic variant detected.
Interpretation: The SLC25A38 c. 706c>T variant has not been previously reported in literature and is predicted to introduce a premature termination codon in the mRNA. This variant is therefore likely to be pathogenic. This result is consistent with a diagnosis of congenital sideroblastic anemia in this patient.
Following genes were analysed:
Membranopathy subpanel: ABCG5, ABCG8, ADD1, ADD2, AK, ANK1, APOB, EPB41, EPB42, DMTN, KCNN4, MTTP, PIEZ01, RhAG, SLC2A1, SLC4A1, STPA1, STPB, STOM, TMOD1, TPM3, XK,
Enzymopathy subpanel: ALDOA, BPGM, CYB5A, CYB5R1, CYB5R2, CYB5R3, CYB5R4, CYB5RL, ENO1G6PD, GAPDH, GCLC, GPI, GPX1, GSR, GSS, HK1, HK2, NT5C3A, PFKM, PGAM1, PGD, PGK1, PGM1, PKLR, TPI1
Hemoglobinopathy Subpanel: AHSP, ATRX, HBA1, HBA2, HBB, HBD, HBE1, HBG1, HBG2, HBM, HBQ1, HBZ
Congenital dyserythropoietic anemia subpanel: CDAN1, C15ORF41, COX4I2, GATA1, GATA2, KIF23, KLF1, LPIN2, SEC23B, TAL1
Diamond-Blackfan anemia subpanel: GATA1, RPL11, RPL15, RPL19, RPL26, RPL27, RPL35A, RPL5, RPL9, RPS10, RPS19, RPS24, RPS26, RPS29, RPS7, CECR1
Sideroblastic anemia subpanel: ABCB6, ABCB7, ALAS1, ALAS2, GLRX5, PUS1, SF3B1, SLC19A2, SLC25A38, YARS2

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