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Haematologica 2020 [Epub ahead of print]

Citation: Immacolata Andolfo, Stefania Martone, Michela Ribersani, Simona Bianchi, Francesco Manna, Rita Genesio, Antonella Gambale, Piero Pignataro, Anna Maria Testi, Achille Iolascon, and Roberta Russo. Apparent recessive inheritance of sideroblastic anemia type 2 due to uniparental isodisomy at the SLC25A38 locus.

Haematologica. 2020; 105:xxx

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Apparent recessive inheritance of sideroblastic anemia type 2 due to uniparental isodisomy at the SLC25A38 locus

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Keywords: uniparental isodisomy, SLC25A38, sideroblastic anemia type II.

Running title: Uniparental isodisomy causes sideroblastic anemia type II.

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The sideroblastic anemias (SAs) are a group of hematological disorders that are characterized by inadequate levels of hemoglobin and ringed sideroblasts in the bone marrow. They can be acquired or congenital. The acquired SAs are part of the myelodysplastic syndromes, with onset in adulthood. The congenital SAs show both early onset in infancy or childhood and in adulthood, and they are the result of germline mutations (inherited or de novo). Several genes have been associated with SAs, and these are involved in mitochondrial pathways, such as for heme synthesis, iron-sulfur cluster biogenesis, and mitochondrial metabolism. Point mutations in the ALAS2, SLC25A38, GLRX5, and HSPA9 genes have been reported for the nonsyndromic SAs. Point mutations in the ABCB7, PUS1, YARS2, LARS2, TRNT1, SLC19A2, and MT-ATP6 genes, and deletion of mitochondrial DNA and the NDUFB11 gene are causal for the syndromic forms of SA.

We report here on a case of an infant with microcytic anemia and paternal isodisomy at the SLC25A38 locus (Figure 1A). We revealed uniparental isodisomy (UPD) in the proband, who carried a homozygous recessive mutation, with only one parent heterozygous for the same variant (excluding a de novo mutation), and in the absence of parental consanguineity. UPD is characterized by the presence of two identical copies of a complete or partial chromosome from one of the parents. This results in homozygosity for an autosomal recessive gene, and thus the clinical expression of a recessive disease. To date, UPD has never been described as a pathogenic mechanism of SAs.

The patient considered here was a newborn male of nonconsanguineous parents from Italy (Figure 1A). At 21 days old, he was admitted to the pediatric Emergency Department due to deep asthenia. He showed mucocutaneous pallor, hypospadia, and micropenis, without any jaundice, fever, or other clinical signs and symptoms. Peripheral blood tests showed severe hypochromic and microcytic anemia, normal white blood cells and platelet counts, and reticulocytopenia (Table 1). A peripheral blood smear showed marked anisopoichilocytosis, with some dacrocyes, microcytes, and spherocytes. His ferritin level was high (747 μg/L), with serum iron and transferrin in the normal range (Table 1); his bilirubin, haptoglobin, and lactate dehydrogenase levels were normal; and his serum erythropoietin (EPO) level was high (114 mUI/mL) (Table 1). Bone marrow smear evaluation was not performed. He received one blood transfusion (10 cm³/kg), and reached a hemoglobin (Hb) of 11.3 g/dL at 23 days old. After the first transfusion, he showed a slow and progressive reduction in Hb (Figure 1B).

The first suspected condition was the sequelae of fetal maternal hemolytic diseases, but the immune-hematological tests were negative. Due to early onset of the condition, the
patient was suspected of having a hereditary anemia. Accordingly, a first level of investigation was performed on the parents. Both parents showed normal red blood cell parameters without abnormal hemoglobins. Thus, a second level of investigation was performed, as genetic testing using a targeted next-generation sequencing (t-NGS) 86-gene custom panel for hereditary anemias. This panel is an updated version of a similar previously published one. The genetic analysis of the proband revealed the presence of the rare nonsense variant c.832C>T, p.Arg278* (rs147431446, minor allele frequency <0.0001 in GnomAD_exome database; NM_017875.4; predicted as pathogenic by ACMG/AMP 2015 guideline) as homozygous (Figure 1A, C). The assumed mechanism of the mutation p.Arg278* is non-expression of the protein due to nonsense mediated decay. Analysis of the inheritance pattern was carried out for both parents. According to the recessive inheritance pattern, the father was a heterozygous carrier of the variant. However, no mutations in the SLC25A38 gene were identified for the mother (Figure 1A, C). We also confirmed the t-NGS data by Sanger sequencing analysis on DNA from the proband and parents. Interestingly, the proband was homozygous for all of the variants located on chromosome 3. In particular, beyond the SLC25A38 nonsense variant, two additional single nucleotide variants (SNV) showed Mendelian violation of inheritance: rs1049296, a coding variant in the TF gene (NM_001063.3:c.1765C>T, p.Pro589Ser); and rs16861582, an intronic variant in the CP gene (NM_000096.3:c.2662-12T>C). These variants resulted in the homozygous state for the proband and the heterozygous state for the father, but none of these were identified in the mother (Figure 1C). Thus, a third level of investigation was performed using single nucleotide polymorphism (SNP) array in the proband and parents, to define either a heterozygous deletion or UPD. No genomic deletions or rearrangements at or around the SLC25A38 locus were identified in the proband or parents at 3p22.1. The SNP array identified large regions of homozygosity that involved the whole paternal chromosome 3, which included the SLC25A38 locus: 3p26.3p25.3(54290_9540289), 3p25.3p12.1(111549397_84682450), 3q11.2q12.3(94565518_101899846), and 3q12.3q29(102540948_197959451) (Figure 1D). Currently, only one case of paternal UPD for whole chromosome 3 was described with no apparent disease phenotype, suggesting the absence of paternal imprinted genes on this chromosome.

Homozygous or compound heterozygous mutations in the SLC25A38 gene are causal for SA type II. A recent retrospective multicenter European study of a cohort of patients with childhood-onset congenital SA showed that in SAs, SLC25A38 is the second most commonly mutated gene after ALAS2. Different recessive loss-of-function mutations (i.e., nonsense,
frameshift, splicing, missense) have been reported for the SLC25A38 along the entire gene (Figure 1E).\(^1,6-12\) These variants impair the transport of glycine in the mitochondria. Indeed, SLC25A38 is a mitochondrial carrier that is expressed in erythroblasts.\(^8\) It is located on the mitochondrial inner membrane and imports glycine into the erythroid mitochondria. The glycine condenses with succinylCoA to form δ-aminolevulinic acid (ALA), a substrate for heme that it is then exported into the cytosol.\(^8,13\) Thus, mutations in SLC25A38 result in alterations to heme synthesis and the hematological phenotype characterized by hypochromic microcytic anemia, and iron depositions around the nucleus in the mitochondria of erythroblasts in the bone marrow.\(^1\) Patients with SLC25A38 mutations showed neonatal microcytic anemia and high ferritin levels (higher than those of patients with mutated ALAS2), as described for the patient here.\(^7\) Of note, patients who carry biallelic pathogenic variants in SLC25A38 gene show iron overload not only in the liver, but also in the myocardium,\(^6\) and the follow-up of this patient will include such evaluation. The same amino acid, arginine 278, was mutated in glycine in a patient previously described (Figure 1E).\(^9\) Comparison of our case and others with nonsense mutations with the patients with biallelic missense mutations reveals no differences. Most of the patients with SLC25A38 mutations present a severe or moderate microcytic anemia, with early onset, transfusion dependence and severe or moderate hepatic iron overload. All patients with a mutated SLC25A38 gene require transfusion at a mean rate of 13.3 transfusions/year (range: 0.3–20/year), with chelation therapy and vitamin B6 supplementation.\(^6\) Accordingly, the patient considered here continued to receive erythrocyte transfusions from 6 months old, with a median interval of 30 days. His median pre-transfusion Hb was 7.3 g/dL, and this transfusion regimen maintained his Hb at ~8.5 g/dL. He also received vitamin supplementation at a standard dose, without high-dose pyridoxine. Pharmacological supplementation with glycine and folate might improve his heme synthesis. In yeast and zebrafish models, exogenous glycine in combination with folate ameliorated the heme levels.\(^14\) This combined supplementation was also administered to three patients with SLC25A38 mutations, although without any improvements.\(^14\) So, to date, the only therapeutic strategy here is chronic blood transfusions and iron chelation. The potentially curative therapy remains hematopoietic stem-cell transplantation.\(^7\)

Due to religious beliefs, families can sometimes refuse permission for blood transfusions, therefore, before the results of the genetic testing, we started the patient on EPO therapy. Initially, this resulted in an apparent lengthening of the transfusion interval (Figure 1B), but later he showed a reduction in Hb. Thus, the treatment with EPO was stopped. To date, there have not been any cases of patients with SAs being treated with EPO described in
the literature. Indeed, EPO should be not effective in this disease, as down-regulation of *SLC25A38* is described in these patients, which is similar to patients with myelodysplastic syndrome with ring sideroblasts. In our patient, we noted a similar picture, with an apparent initial response to EPO, with progressive lengthening of the transfusion interval. Unfortunately, EPO treatment did not improve his Hb.

Currently, the patient is 9 months old, and his psychophysical development is normal. He does not require iron chelation at present. For his sexual phenotype, he has just undergone subcutaneous substitutive testosterone therapy. Testosterone stimulates erythropoiesis, and we believe that this patient will benefit from its administration, for both his anemia and his hypogonadism, although over a short period.

According to the literature, the clinical features in this patient confirm that SAs represent a severe transfusion-dependent disease with no valid options for treatment. From the diagnostic perspective, this case highlights the importance of evaluation of the possible occurrence of UPD for patients with SAs due to *SLC25A38* mutations, and of an assessment of the inheritance pattern of the identified variants. Indeed, incidence of UPD is higher for rare autosomal recessive diseases compared to common autosomal recessive diseases with higher carrier frequencies. Nevertheless, in presence of a normal karyotype, the recurrence risk of a rare autosomal recessive disease caused by UPD of a whole chromosome is negligible since it is a rare, generally sporadic event.
References


Table 1. Clinical characteristics of the proband affected by SA type II.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Units of measure</th>
<th>Patient clinical data (reference range)(^a) according to age at analyses (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Red blood cell count (RBCs)</td>
<td>(\times 10^6/\mu L)</td>
<td>3.5 (4.3–5.9)</td>
</tr>
<tr>
<td>Hemoglobin (Hb)</td>
<td>g/dL</td>
<td>6.8 (13–17)</td>
</tr>
<tr>
<td>Hematocrit (Hct)</td>
<td>%</td>
<td>21.9 (40–52)</td>
</tr>
<tr>
<td>Mean corpuscular volume (MCV)</td>
<td>fL</td>
<td>63.4 (80–96)</td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin (MCH)</td>
<td>pg</td>
<td>19.8 (27–31)</td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin concentration (MCHC)</td>
<td>g/dL</td>
<td>31.2 (32–36)</td>
</tr>
<tr>
<td>Red cell distribution width (RDW)</td>
<td>%</td>
<td>23.5 (11–16)</td>
</tr>
<tr>
<td>Reticulocyte absolute count (ret count)</td>
<td>(\times 10^3/\mu L)</td>
<td>17.0 (31)</td>
</tr>
<tr>
<td>White blood cell count (WBCs)</td>
<td>(\times 10^3/\mu L)</td>
<td>9.5 (4–10.0)</td>
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<tr>
<td>Platelet count (Plt)</td>
<td>(\times 10^3/\mu L)</td>
<td>792</td>
</tr>
<tr>
<td>Mean platelet volume (MPV)</td>
<td>fL</td>
<td>9.4 (7.2–13)</td>
</tr>
<tr>
<td>Total bilirubin</td>
<td>mg/dL</td>
<td>0.5 (0.3–1.2)</td>
</tr>
<tr>
<td>Unconjugated bilirubin</td>
<td>mg/dL</td>
<td>0.25 (&lt;0.6)</td>
</tr>
<tr>
<td>Lactate dehydrogenase (LDH)</td>
<td>U/L</td>
<td>290 (&lt;600)</td>
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<tr>
<td>Haptoglobin</td>
<td>mg/L</td>
<td>n.a.</td>
</tr>
<tr>
<td>Alanine aminotransferase</td>
<td>U/L</td>
<td>26 (10–45)</td>
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<td>Serum iron</td>
<td>(\mu mol/L)</td>
<td>29 (17.9–47.8)</td>
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<tr>
<td>Transferrin saturation</td>
<td>%</td>
<td>9</td>
</tr>
<tr>
<td>Ferritin</td>
<td>(\mu g/L)</td>
<td>747 (21–445)</td>
</tr>
<tr>
<td>Erythropoietin (EPO)</td>
<td>mU/mL</td>
<td>114 (5–25)</td>
</tr>
</tbody>
</table>

\(^a\), reference range from Sapienza University, Rome, Italy
n.a., not available
Acknowledgments
The authors thank the CEINGE Service Facility platforms of Sequencing Core and Oligo Synthesis. This work was supported by an EHA Junior Research Grant to Immacolata Andolfo (3978026), and by Bando Star Linea 1 - Junior Principal Investigator Grants - COINOR, Università degli Studi di Napoli ‘Federico II’ to Roberta Russo. The authors also thank the parents of the patient granting their permission for the case to be communicated to the scientific community.

Authorship contributions
IA, RR designed and conducted the study, and prepared the manuscript; SM contributed to the preparation of the manuscript; FM performed the next-generation sequencing analysis; RG and PP performed the SNP array analysis; AMT, MR and SB took care of the patient and contributed to the preparation of the manuscript; SM and AG performed the genetic clinical counseling of the patient; AI provided critical review of the manuscript.

Conflicts of interest
The authors state that they have no conflicts of interest to disclose.
Figure legend

Figure 1. Description of a patient with sideroblastic anemia type II, and characterization of uniparental isodisomy.

A. Genetic pedigree of the family. Squares, males; circle, female; solid symbols, affected person; arrow, proband. According to uniparental isodisomy, only the father carried the variant c.832C>T, p.Arg278* in the SLC25A38 gene. B. Hemoglobin levels of the patient after blood transfusions and EPO supplementation. C. Alignment track of next-generation sequencing analysis of the proband, mother, and father, showing presence of the c.832C>T variant as homozygous in the proband [mutated allele (T) frequency, 97% (184/190 reads); wt allele (C) frequency, 3% (6/190 reads)], as heterozygous in the father, and absent in the mother. D. Agilent CytoGenomic view of the SNP data. Top panel: number of uncut alleles. Bottom panel: CGH data (log2 ratios). Data from GenetiSure Postnatal Research CGH+SNP 2x400 Array (Agilent), which show large regions of homozygosity on chromosome 3. E. Diagram of the SLC25A38 protein structure and the pathogenic variants, as obtained from the Human Gene Mutation Database Professional [updated in June 2020]. Circles’ colors define the mutation types (for different mutation types at a single position, the color defines the most frequent) (https://www.cbioportal.org/mutation_mapper).
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Supplementary Information: Table of contents
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Methods

Patients, genomic DNA preparation, and NGS analysis
Patient diagnosis was based on medical history, clinical findings, laboratory data, morphological analysis of peripheral blood and aspirated bone marrow. DNA samples were obtained after signed informed consent of the parents and according to the Declaration of Helsinki. The local University Ethical Committees approved the DNA sampling and the collection of patient samples (‘Federico II’ University of Naples). Genomic DNA preparation, genetic testing by targeted next-generation sequencing for hereditary anemias, and validation of the variant were performed as previously described.4

Oligo-SNP array
The oligo-SNP array analysis followed the protocol provided by the manufacturer, with the analysis on the proband and the parents performed using CytoGenomics v5.0.2 (Agilent Technologies, USA).

Statistical analysis
Statistical significances were determined using Student’s t-tests. Two-sided p <0.05 was considered as statistically significant.