Onset of X-linked sideroblastic anemia in the fourth decade

We report the case of a 40-year female who manifested late onset, pyridoxine-refractory X-linked sideroblastic anemia, heterozygous for the first described frameshift ALAS2 mutation, CD506-507 (-C). On presentation she had macrocytic anemia with severe iron overload.

X-linked sideroblastic anemia (XLSA), the most common inherited form of sideroblastic anemia, is associated with several mutations (primarily missense) in the erythroid-specific 5-aminolevulinic acid synthase gene, ALAS2, located on chromosome Xp11.21. In XLSA cases due to missense mutations the anemia usually improves with pyridoxine supplementation.1 In the majority of females heterozygous for ALAS2 mutations, red blood cell (RBC) progenitors with the wild-type ALAS2 allele are sufficient to sustain a normal level of RBC production2-4 and a normal (Hb) hemoglobin concentration, despite dimorphic red cell morphology. Conversely, as in any X-linked disorder, the phenotype of female carriers may be influenced by unbalanced X-chromosome inactivation (Lyonization),5,6 resulting in anemia of different severities.7

A 41-year old Portuguese Caucasian female was referred to our Department for investigation of severe macrocytic anemia with significant iron overload. She had previously been healthy, with no history of drinking or drug abuse. During pregnancies she had been prescribed oral iron; in her last pregnancy (9 years previously) Hb was 101 g/L and mean cell volume (MCV) 96 fl. She reported a recent history of asthenia, mild breathlessness, amenorrhea and skin hyperpigmentation. Her spleen was not palpable and the liver border was just below the costal margin.

Laboratory data: Hb 71 g/L, MCV 107 fl; mean cell hemoglobin (MCH) 36 pg, mean cell hemoglobin concentration (MCHC) 27.6%, red cell distribution width (RDW) 22%, reticulocytes 2%, serum ferritin 3974 μg/L (N 14-150 μg/L), total bilirubin 7.1 μmol/L (N <17), aspartate aminotransferase 54 IU/L (N 5-35 IU/L), alanine aminotransferase 69 IU/L (N 5-35 IU/L), lactate dehydrogenase 175 IU/L (N 211-423 IU/L), and homocysteine 7.9 μmol/L (N 5-10). White cell and platelet counts and morphology were normal. The peripheral blood smear showed anisopoikilocytosis and dimorphism with two distinct red cells populations, one normochromic and the other hypochromic and microcytic. Bone marrow examination revealed dyserythropoesis with megaloblastoid features and 20% of ringed sideroblasts. Liver biopsy showed increased stainable iron; quantification of liver iron by magnetic resonance techniques found more than 350 mmol/g of hepatic tissue. Screening for HFE gene mutations associated with hemochromatosis revealed only H63D heterozygosis. Amplification and sequencing of ALAS2 gene 11 exons with flanking intronic sequence (50 to 100 nt), 250 base pair (bp) pro-

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In summary, we describe the late onset of pyridoxine-refractory X-linked sideroblastic anemia in a female heterozygous for a newly described frameshift mutation in exon 10 of the ALAS2 gene, which occurred de novo in her father's allele. This mutation is predicted to lead to a non-functional truncated polypeptide, explaining the failure to respond to pyridoxine treatment. The patient’s clinical history suggests that during the first three decades of life her erythroid precursors were able to produce enough ALAS-ε to maintain a normal erythropoiesis but that in the fourth decade, an increased skewed Lyonization in favor of the mutated allele caused ineffective erythropoiesis leading to severe anemia. Fortunately, her children did not inherit the mutated allele.

This case emphasizes the need to screen for ALAS2 mutations in female patients with anemia and ringed sideroblasts irrespective of age and the presence of macrocytic red cells, provided platelets and WBC appear normal. Mutation screening is indicated in members of these families, for genetic counselling and early prevention of iron overload.

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References


Figure 2. Hematopoiesis clonal analysis with HUMARA assay performed in DNA from peripheral blood leukocytes, as previously described with minor modifications. The – and + signs indicate sample undigested (–) or digested (+) by the methylation-sensitive restriction endonuclease Hhal. A. The propositus and her sister inherited the same paternal alleles (282 and 292). In Hhal digested DNA the paternally-derived allele (282) is less amplified than the maternal one (292), indicating that the paternally-derived HUMARA allele is less methylated (more active) than the maternal. B. The three offspring inherited the 292 maternal allele. The fact that none of the propositus’ family member have the ALAS2 CD506-507(-C) mutation indicates that it occurred de novo on the same chromosome as the 282 paternally-derived allele.

Figure 1. Hematopoiesis clonal analysis with HUMARA assay performed in DNA from peripheral blood leukocytes, as previously described with minor modifications. The – and + signs indicate sample undigested (–) or digested (+) by the methylation-sensitive restriction endonuclease Hhal. A. The propositus and her sister inherited the same paternal alleles (282 and 292). In Hhal digested DNA the paternally-derived allele (282) is less amplified than the maternal one (292), indicating that the paternally-derived HUMARA allele is less methylated (more active) than the maternal. B. The three offspring inherited the 292 maternal allele. The fact that none of the propositus’ family member have the ALAS2 CD506-507(-C) mutation indicates that it occurred de novo on the same chromosome as the 282 paternally-derived allele.
Imatinib mesylate has been reported to produce positive results in atypical chronic myeloproliferative disorders (CMD) with chromosomal translocations that disrupt the platelet-derived growth factor receptor β gene (PDGFRB). We used imatinib to treat a 49-year-old man with atypical CMD in accelerated phase and the H4 (D10S170)-PDGFRB fusion gene. After 3 months of treatment, we observed grade 4 hematologic toxicity and a lack of response.

Lack of response to imatinib mesylate in a patient with accelerated phase myeloproliferative disorder with rearrangement of the platelet-derived growth factor receptor β-gene

In September 1999, a 49-year-old man presented with asthenia and huge splenomegaly. Blood counts performed 2 years previously had already shown hyperleukocytosis and eosinophilia that were not investigated. The blood count at presentation showed hemoglobin 68 g/L, white blood cell count (WBC) of 7.4×10^9/L with 64% neutrophils, 19% lymphocytes, 6% monocytes, 6% eosinophils, 1% basophils, 2% metamyelocytes, 1% myelocytes, 1% promyelocytes, 1% erythroblasts and a platelet count of 63×10^9/L. Bone marrow aspiration was difficult and showed granulocytic hyperplasia without excess of blast cells and few megakaryocytes. The bone marrow biopsy, stained with hematoxylin-eosin and May-Grünwald Giemsa, showed granulocytic hyperplasia, established myelofibrosis (grade III reticulin) and no evidence of blast cells and few megakaryocytes. The blood counts again recovered. Cytogenetic analysis showed additional abnormalities of t(5;10) in 2 mitoses: add(3)(p21) and monosomy 15.

Imatinib was stopped after 3 months of therapy because of severe hematologic toxicity with the patient requiring platelet and red blood cell transfusions. In the six months following discontinuation of imatinib, hematologic recovery was observed. A second trial of imatinib immediately led to a worsening of thrombocytopenia. Imatinib was definitively stopped and blood counts again recovered. Cytogenetic analysis, repeated after each course of imatinib, showed the persistence of (t(5;10)) in all the mitoses and H4-PDGFRB transcript was still detectable by RT-PCR. Hydroxyurea was subsequently given with persisting good hematologic control until now.

Abnormal activation of PDGFRB was first described as a consequence of the t(5;12)(q33;p13), which fuses the 5′ end of ET6V to the 3′ end of PDGFRB including the entire tyrosine kinase domain, and complete and durable responses to Imatinib were reported in four patients with t(5;12) translocation. Other translocations involving the same region of PDGFRB have been reported: t(5;10)(q33;p21) translocation fusing PDGFRB to H4(D10S170), a gene encoding for a 585-amino acid protein with no significant homology to known genes and with unknown function, has been reported in 3 patients.8,9 H4 is fused to the ret gene as a result of an inv(10)(q22q21) in a subset of papillary thyroid carcinomas. The H4-ret fusion protein is a constitutively active tyrosine kinase.