Brief Report

Late-onset X-linked sideroblastic anemia following hemodialysis

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Abstract

X-linked sideroblastic anemia (XLSA) is due to deficient activity of erythroid-specific 5-aminolevulinate synthase (ALAS2). We report here a patient who developed sideroblastic anemia at the age of 81 while undergoing hemodialysis. The diagnosis of sideroblastic anemia was established by the presence of ringed sideroblasts in the bone marrow, and treatment with oral pyridoxine completely eliminated the ringed sideroblasts. We identified a novel point mutation in the 5th exon of this patient’s ALAS2 gene, which resulted in an amino acid change at residue 159 from aspartic acid to asparagine (D159N). *In vitro* analyses of recombinant D159N ALAS2 revealed that this mutation accounted for the pyridoxine-responsiveness of his disease. The very late onset in this case of XLSA emphasizes that nutritional deficiencies caused either by dietary irregularities in the elderly or, as in this case, by maintenance hemodialysis therapy, may uncover occult inherited enzymatic deficiencies in the heme biosynthetic pathway.

Key Word: X-linked sideroblastic anemia, hemodialysis, erythroid-specific 5-aminolevulinate synthase, ALAS2, Heme
Introduction

X-linked sideroblastic anemia (XLSA) is an X-linked recessive hypochromic, microcytic anemia with ringed sideroblasts in the bone marrow, resulting from the deficient activity of erythroid-specific 5-aminolevulinate synthase (ALAS2, or ALAS-E; EC 2.3.1.37). XLSA typically presents by the 4th decade of life, however, there are rare cases that may manifest later in life. Late-onset XLSA merits special mention not only because it is extremely rare (only three cases have been reported), but also because there has been no direct evidence that the mutations of ALAS2 resulted in the disease.\textsuperscript{1-3} In this study, we report a patient with late-onset XLSA who developed the disease following hemodialysis. The diagnosis was confirmed by molecular analysis of the ALAS2 gene.
Case Report

An 81-year-old male patient was admitted to Kyoto Min-I-Ren Central Hospital in September 2000, with the diagnosis of chronic renal failure and sideroblastic anemia. Prior to admission, he had been treated with hemodialysis for two and one-half years. Erythropoietin was administered (3,000 U subcutaneously three times weekly) yet he remained anemic (RBC, 285 x 10^10/L; Hb, 8.1 g/dL; Ht, 24.5%; MCV, 85.9 fl; on June 27, 2000), although blood transfusions were not required. On July 25, 2000, anemia worsened (RBC, 232 x 10^10/L; Hb, 6.3 g/dL; Ht, 19.1%; MCV, 82.4 fl). There were no signs of intestinal bleeding and he received eight units of packed red blood cells. On admission to the hospital in September 2000, laboratory findings revealed a severe normocytic anemia (RBC, 195 x 10^10/L; Hb, 5.6 g/dL; Ht, 17.0%; MCV, 87.3 fl; MCH, 29.0 pg; MCHC, 33.2%; and WBC, 4,600 x 10^6/L) with an increased red cell distribution width (RDW) of 19.8% (Normal, 11.6-15.0). The peripheral blood smear revealed both normocytic and microcytic erythrocyte populations. The MCV dropped to 82.4 fl before additional packed red cell transfusions were administered. A bone marrow examination revealed a hypocellular marrow with a normal myeloid/erythroid ratio. Prussian blue staining of bone marrow cells revealed the presence of numerous ringed sideroblasts (49% of the erythroblasts). The serum ferritin concentration was elevated at 797 µg/l. A myelodysplastic syndrome was suspected, namely refractory anemia with ringed sideroblasts (RARS) and additional packed red cell transfusions were administered. A serum pyridoxal level was below 2.0 ng/mL (normal, 6.0 - 40.0) and oral pyridoxine (200 mg/day) was started. After two weeks of pyridoxine therapy, the anemia stabilized and no further transfusions were required. The bone marrow examination was repeated and ringed sideroblasts were no longer evident. Hemoglobin levels were maintained at or above 7.0 g/dL.
with continued pyridoxine treatment but the patient died of aspiration pneumonia in November 2000.
Materials and Methods

Identification of an ALAS2 mutation

An autopsy sample of the patient’s liver, which had been stored in a 3.7% formaldehyde solution, was used as a source of DNA. After informed consent was obtained from the patient’s family, genomic DNA was extracted from the liver sample. Using the genomic DNA as a template, each exon of the ALAS2 gene was amplified using polymerase chain reaction (PCR), then PCR products were directly sequenced. The primers which were used for PCR and direct sequencing have been previously described. The mutation of the ALAS2 gene in the patient was confirmed by three independent PCR reactions followed by direct sequencing.

Enzymatic characterization of the mutant ALAS2 protein

Using a QuickChange Site-Directed Mutagenesis kit (Stratagene, La Jolla, CA), the point mutation of ALAS2 gene of the patient was introduced into the wild type (WT) ALAS2 cDNA, which had been subcloned into a pGEX-3X expression vector for expression of the mature ALAS2 enzyme as a GST-fusion protein. After confirming the sequence of mutant ALAS2 cDNA, BL21(DE3)pLysS E. coli strain was transformed by the expression vector. Induction of the recombinant protein expression, purification and determination of enzymatic activity were carried out as described previously.
Results and Discussion

All eleven exons and the 250 bp promoter region of the ALAS2 gene were sequenced, and a single nucleotide transition (from guanine to adenine at the 527th nucleotide) was identified in exon 5 (Figure 1). This point mutation resulted in an amino acid change from aspartic acid to asparagine at residue 159 (D159N). The proband had five brothers, two sisters and three daughters but none had a history of anemia. Consent for genetic analysis of living family members could not be obtained. The D159N mutation does not appear to be a polymorphism, however, as it was not found in 44 control ALAS2 alleles from the Japanese population (data not shown). Recently, a D159Y ALAS2 mutation was reported in another family with pyridoxine-responsive XLSA.5 In this family, there were two males with sideroblastic anemia that was expressed in childhood. Early expression of the D159Y mutation may be due to the fact that tyrosine, unlike asparagine, is positively charged and would be predicted to have a greater effect on the catalytic activity of ALAS2. These findings suggest that the aspartic acid normally found at residue 159 is important for enzymatic activity and that mutations at this residue are causative for XLSA.

ALAS requires pyridoxal 5'-phosphate (PLP), the active form of pyridoxine, as a cofactor. Pyridoxine deficiency may cause sideroblastic anemia6 and pyridoxine deficiency is prevalent in patients undergoing chronic hemodialysis.7 In spite of these points only one patient has been reported to develop sideroblastic anemia following chronic hemodialysis.8 In our patient, treatment with pyridoxine eliminated the ringed sideroblasts from the marrow and resulted in independence from transfusions. Most patients with XLSA are responsive to pyridoxine (24 of the 29 known ALAS2 mutations).9,10 To confirm that the D159N mutation was responsible for the sideroblastic anemia in our patient, we examined the effect of PLP on
the activity of recombinant D159N mutant enzyme *in vitro*. The specific activity of the D159N mutant was 31.4% of the wild type enzyme in the absence of PLP. Activity of the mutant enzyme increased to 60.3% of wild type in the presence of PLP (Table 1).

Our study indicates that clinically silent mutations of ALAS2 might become evident later in life if cofactor deficiency develops due to dietary peculiarities or, as in this case, if an intervention such as hemodialysis occurs.
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References


Table 1:

Enzymatic activity of the recombinant ALAS2 protein

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<th>GST-WT</th>
<th>GST-D159N</th>
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<tr>
<td>without PLP</td>
<td>24,666 ± 421.6</td>
<td>7,755 ± 392.1</td>
</tr>
<tr>
<td>with PLP (0.2 mM)</td>
<td>37,743 ± 1,022.2</td>
<td>22,767 ± 421.6</td>
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nmol ALA/h/mg protein, (mean ± S.D., n = 3)
Legend to Figure

Figure 1. Nucleotide sequence analysis of the ALAS2 gene.

Adenine substituted for Guanine at position 527 in this patient. This transition resulted in an amino acid change from aspartic acid to asparagine at the 159th amino acid residue.
Figure 1
Late-onset X-linked sideroblastic anemia following hemodialysis

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