X-linked sideroblastic anaemia due to ALAS2 mutations in the Netherlands: a disease in disguise


Departments of 1Laboratory Medicine, 3Human Genetics, 4General Internal Medicine, 1Haematology, and 6Paediatric Oncology & Haematology, Radboud University Medical Centre, Nijmegen, the Netherlands, 2Department of Haematology, University Medical Centre Utrecht, Utrecht, the Netherlands, 6corresponding author: tel.: +31 (0)24-3618957; fax: +31 (0)24-3541743, e-mail: dorine.swinkels@radboudumc.nl

ABSTRACT

Background: X-linked sideroblastic anaemia (XLSA; OMIM #300751) is the most common inherited form of sideroblastic anaemia and is associated with several mutations in the erythroid specific 5-aminolevulinate synthase gene (ALAS2). This gene encodes for aminolevulinic acid synthase 2 (ALAS2), the catalytic enzyme involved in the first rate-limiting step of haem biosynthesis. The disorder is characterised by mostly mild hypochromic microcytic anaemia with bone marrow ring sideroblasts. Even untransfused patients with mild or no anaemia are at risk for severe systemic iron overload due to ineffective erythropoiesis. To date, 61 different ALAS2 mutations have been reported in 120 families with XLSA. Descriptions of molecularly confirmed case series from the Netherlands, however, are lacking.

Methods: We reviewed age of presentation, clinical and biochemical features, ALAS-2 defects and treatment characteristics of 15 Dutch patients from 11 unrelated families diagnosed with XLSA.

Results and Conclusions: In one family a novel pathogenic c.1412G>A (p.Cys471Tyr) mutation was found. All other families shared the previously described c.1355G>A (p.Arg452His) mutation. Haplotype analysis in seven probands with the p.Arg452His mutation strongly suggests that six of them were ancestrally related. Nevertheless, their phenotype was very different. Our patients illustrate the phenotypical heterogeneity in the presentation of XLSA patients, the effectiveness of treatment regimens and the various pitfalls associated with the diagnosis, follow-up and treatment of the disease. A timely diagnosis avoids unnecessary investigations and allows adequate treatment that can prevent systemic iron load with subsequent severe life-threatening complications. Therefore, we suggest considering XLSA in both male and female patients with unexplained iron overload and/or (mild) microcytic anaemia, also at older age.

KEYWORDS

ALAS2, iron, sideroblastic anaemia

INTRODUCTION

X-linked sideroblastic anaemia (XLSA; OMIM #300751) is the most common inherited form of sideroblastic anaemia and is associated with several mutations in the erythroid specific 5-aminolevulinate synthase gene (ALAS2), which is the first and rate-limiting step of haem biosynthesis. The disorder is characterised by hypochromic microcytic anaemia with ring sideroblasts in the bone marrow in combination with systemic iron overload due to ineffective erythropoiesis. Phenotypic expression of XLSA is highly variable even in patients with identical mutations, but affected males generally present in the first decades of life with symptoms of anaemia or later with manifestations of parenchymal iron overload. Occasionally patients present later in life. As in most X-linked recessive disorders, the majority of female carriers of XLSA are spared from clinical manifestations. However, sporadically women with ALAS2 mutations may be affected due to inactivation of the normal X-chromosome or age-related skewed X-inactivation in haematopoietic cells. Standard treatment of XLSA consists of high-dose pyridoxine supplementation and iron-reducing strategies such as phlebotomies and iron chelation. The effect of high-dose...
pyridoxine is based on the high prevalence of mutations in the pyridoxine-binding region of the ALAS2 gene. The high dose enhances the half-life of ALAS2; however, this is not true for mutations outside this region. Reduction of iron overload in XLSA improves erythropoiesis and prevents complications of chronic iron overload, especially liver cirrhosis and hepatocellular carcinoma.

In this article we describe 14 male patients and one female patient from 11 unrelated families. All patients are of Dutch origin. These case series are illustrative for the biochemical and clinical presentation of XLSA patients, the effectiveness of treatment regimens and the various pitfalls associated with the (early) diagnosis, follow-up and treatment of the disease.

**PATIENTS AND METHODS**

**Patients**

We reviewed clinical and molecular data of 15 patients (14 male and one female) diagnosed with XLSA in the Netherlands in 2011 and 2012. The diagnosis of sideroblastic anaemia was made at the University Medical Centre Utrecht, Utrecht and the Radboud University Medical Centre, Nijmegen, the Netherlands. We reviewed age at presentation, biochemical and clinical features, treatment regimens and type of ALAS2 mutations.

**Genotyping**

Genotyping was performed by PCR and DNA sequence analysis of the full coding part of the ALAS2 gene. The pathogenicity of a mutation was assessed by review of the literature, association of the mutation with the phenotype in a family, and with bioinformatic tools, which were used to complement the genetic studies in case of a not previously reported mutation. SIFT (=Sorting Intolerant from Tolerant), PolyPhen (Polymorphism Phenotyping) and HOPE (Have (y)Our Protein Explained) provide an *in silico* prediction of the functional consequences of missense mutations.

A search for a founder effect was done in seven of the ten families with the p.Arg452His mutation by genotyping the short tandem repeats (STRs) DXS1044, DXS8032, DXS991 and DXS1950 close to the ALAS2 gene by PCR using fluorescent primers. PCR products were pooled and analysed on an ABI 3730 DNA sequencer.

**RESULTS**

**Overall clinical and biochemical features and treatment strategies of Dutch XLSA patients**

Fifteen XLSA patients from 11 unrelated families were included in the study; all were of Dutch and Caucasian origin (*table 1*). Age at the time of clinical and biochemical diagnosis in our patients ranged from 2-72 years. In the male patients, haemoglobin at diagnosis ranged from 3.9-7.8 mmol/l with the mean corpuscular volume (MCV) between 56-71 fl. Serum ferritin at diagnosis ranged from 99-5040 µg/l.

All patients were treated with high-dose pyridoxine (200 mg daily, except for patient 10 who received 150 mg daily), phlebotomies or chelation. Per phlebotomy, 500 ml blood was withdrawn, except in patient 2B who started on 200 ml per phlebotomy every two weeks for two months. Because of a stable and even increasing Hb, the phlebotomy volume was increased to 400 ml every two weeks until his ferritin became <100 µg/l (*figure 1*).

Also in the other patients, phlebotomies were well tolerated, even in a patient with more severe anaemia (patient 3). In general this treatment regimen resulted in a significant increase of Hb in six out of 15 patients and a decrease of ferritin levels in five out of 15 patients.

The only female proband, patient 1A, died at the age of 79 years due to the complications of diabetes mellitus and heart failure. Patient 2A died at the age of 71 years from a hepatocellular carcinoma (HCC). The other patients are still alive and in good clinical condition. None of them have developed severe complications of systemic iron overload, probably due to timely treatment.

**Molecular features**

Thirteen out of the 15 patients showed hemizygosity for the previously reported pathogenic c.1355G>A (p.Arg452His) mutation in exon 9 of the ALAS2 gene. One female patient was heterozygous for the c.1355G>A (p.Arg452His) mutation (patient 1A). These 13 patients with a p.Arg452His mutation are from ten apparently unrelated families. Haplotype analysis of patients 3 and 6-11 showed that all patients, except for proband 9, carried the same length of the four STRs analysed, suggesting that the p.Arg452His mutation arose from one common ancestor in these probands. The lengths of all four STRs of the patients differed from those found for proband 9. The common haplotype of patients 3, 6-8, 10 and 11 is at least 2.473 kilobase in size.

In two patients (brothers 5A and 5B) a novel mutation was found in exon 9: c.1412G>A (p.Cys471Tyr). For this mutation bioinformatic tools were not consistent in their assessment, i.e. SIFT predicted the mutation as ‘probably damaging’, HOPE reports: ‘the wild-type (cysteine) and mutant amino acids (tyrosine) differed in size. The wild-type residue was buried in the core of the protein’.
Table 1. Haematological, biochemical and molecular data, and treatment characteristics of 15 patients from 11 unrelated families diagnosed with X-linked sideroblastic anaemia

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>Laboratory characteristics</th>
<th>Genotype</th>
<th>Treatment characteristics</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>ID&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Age (yrs)</td>
<td>Hb (mmol/l)</td>
<td>MCV (fl)</td>
<td>Ferritin (µg/l)</td>
</tr>
<tr>
<td>1A</td>
<td>At presentation</td>
<td>72</td>
<td>F</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td>Death</td>
<td>79</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Son</td>
<td>25</td>
<td>M</td>
<td>5.3</td>
</tr>
<tr>
<td></td>
<td>With therapy</td>
<td>46</td>
<td>M</td>
<td>7.2</td>
</tr>
<tr>
<td>2A&lt;sup&gt;++&lt;/sup&gt;</td>
<td>At presentation</td>
<td>66</td>
<td>M</td>
<td>7.8</td>
</tr>
<tr>
<td></td>
<td>With therapy</td>
<td>69</td>
<td>M</td>
<td>7.2</td>
</tr>
<tr>
<td></td>
<td>At presentation</td>
<td>2</td>
<td>M</td>
<td>6.8</td>
</tr>
<tr>
<td></td>
<td>With therapy</td>
<td>16</td>
<td>M</td>
<td>7.4</td>
</tr>
<tr>
<td></td>
<td>With therapy</td>
<td>7.8</td>
<td>71</td>
<td>72</td>
</tr>
<tr>
<td>3</td>
<td>At presentation</td>
<td>35</td>
<td>M</td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td>With therapy</td>
<td>47</td>
<td>M</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>With therapy</td>
<td>62</td>
<td>M</td>
<td>8.3</td>
</tr>
<tr>
<td>4</td>
<td>At presentation</td>
<td>25</td>
<td>M</td>
<td>7.1</td>
</tr>
<tr>
<td></td>
<td>With therapy</td>
<td>34</td>
<td>M</td>
<td>7.0</td>
</tr>
<tr>
<td></td>
<td>With therapy</td>
<td>40</td>
<td>M</td>
<td>7.2</td>
</tr>
<tr>
<td>5A</td>
<td>At presentation</td>
<td>&lt;21</td>
<td>M</td>
<td>3.9</td>
</tr>
<tr>
<td>5B</td>
<td>At presentation</td>
<td>54</td>
<td>M</td>
<td>7.8</td>
</tr>
<tr>
<td></td>
<td>With therapy</td>
<td>23</td>
<td>M</td>
<td>6.2</td>
</tr>
<tr>
<td></td>
<td>With therapy</td>
<td>53</td>
<td>M</td>
<td>8.4</td>
</tr>
</tbody>
</table>

<sup>1</sup>Numerals stand for families (probands are mentioned) for families 1, 2, 5, and 9, also a 2nd affected relative is included; 2hemizygous for men and heterozygous for women; 3At age 28 years; 4patient 2 was originally diagnosed with iron overload at the age of 38 years which later, at age 57, was attributed to hereditary haemochromatosis due to heterozygosity for the p.Cys282Tyr mutation in the HFE gene. Treatment with phlebotomies was started. Because of low Hb levels and ferritin levels within the reference range, phlebotomies were stopped at the age 51; 5Patient previously reported in Cuijpers et al. 18; 6At age 38, no earlier values available; 7At age 32; 8Results of 1 year treatment with pyridoxine (200 mg per day) and phlebotomy every 4-6 weeks. EPO was stopped after diagnosis at age 30 yrs; 9Patient previously reported in thesis of Dr. Ploem 20; 10Low compliance; MDS type RCMD = myelodysplastic syndrome type refractory cytopenia with multilineage dysplasia; 11Probands investigated by haplotype analysis, in subjects indicated by 11* this analysis suggests a common ancestor; ND = not determined or data not available; TS = transferrin saturation; NASH = non-alcoholic steatohepatitis; EPO = erythropoietin.
Table 1. Haematological, biochemical and molecular data, and treatment characteristics of 15 patients from 11 unrelated families diagnosed with X-linked sideroblastic anaemia

<table>
<thead>
<tr>
<th>Patient character</th>
<th>Laboratory characteristic</th>
<th>Genotype</th>
<th>Treatment characteristic</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>ID1</td>
<td>Age (yrs)</td>
<td>Sex (m/f)</td>
<td>Hb (mmol/l)</td>
<td>MCV (fl)</td>
</tr>
<tr>
<td>1A</td>
<td>At presentation</td>
<td>72</td>
<td>F</td>
<td>6.0</td>
</tr>
<tr>
<td>1B</td>
<td>Son</td>
<td>25</td>
<td>M</td>
<td>5.3</td>
</tr>
<tr>
<td>2A</td>
<td>4,5</td>
<td>66</td>
<td>M</td>
<td>7.8</td>
</tr>
<tr>
<td>3</td>
<td>At presentation</td>
<td>35</td>
<td>M</td>
<td>4.3</td>
</tr>
<tr>
<td>4</td>
<td>At presentation</td>
<td>25</td>
<td>M</td>
<td>7.1</td>
</tr>
<tr>
<td>5A</td>
<td>At presentation</td>
<td>&lt;21</td>
<td>M</td>
<td>3.9</td>
</tr>
<tr>
<td>5B</td>
<td>Brother</td>
<td>23</td>
<td>M</td>
<td>6.2</td>
</tr>
<tr>
<td>6</td>
<td>At presentation</td>
<td>32</td>
<td>M</td>
<td>6.8</td>
</tr>
<tr>
<td>7</td>
<td>At presentation</td>
<td>&lt;2.8</td>
<td>M</td>
<td>7.2</td>
</tr>
<tr>
<td>8</td>
<td>At presentation</td>
<td>&lt;2.8</td>
<td>M</td>
<td>6.6</td>
</tr>
<tr>
<td>9A</td>
<td>At presentation</td>
<td>32</td>
<td>M</td>
<td>7.1</td>
</tr>
<tr>
<td>9B</td>
<td>Grandfather</td>
<td>ND</td>
<td>M</td>
<td>ND</td>
</tr>
<tr>
<td>10</td>
<td>At presentation</td>
<td>13</td>
<td>M</td>
<td>6.8</td>
</tr>
<tr>
<td>11</td>
<td>At presentation</td>
<td>14</td>
<td>M</td>
<td>6.8</td>
</tr>
<tr>
<td>12</td>
<td>At presentation</td>
<td>18</td>
<td>M</td>
<td>7.5</td>
</tr>
</tbody>
</table>

*Numbers stand for families (probands are mentioned) for families 1, 2, 5, and 9, also a 2nd affected relative is included: 1hemizygous for men and heterozygous for women; 2at age 28 years; 3patient was originally diagnosed with iron overload at the age of 38 years which later, at age 57, was attributed to hereditary haemochromatosis due to heterozygosity for the p.Cys282Tyr mutation in the HFE gene. Treatment with phlebotomies was started. Because of low Hb levels and ferritin levels within the reference range, phlebotomies were stopped at the age 51; 4patient previously reported in Cuijpers et al.; 5at age 38, no earlier values available; 6at age 32; 7results of 1 year treatment with pyridoxine (200 mg per day) and phlebotomy every 4-6 weeks. EPO was stopped after diagnosis at age 30 yrs; 8patient previously reported in thesis of Dr. Ploem; 9low compliance; 10MDS type RCMD = myelodysplastic syndrome type refractory cytopenia with multilineage dysplasia; 11probands investigated by haplotype analysis, in subjects indicated by * this analysis suggests a common ancestor; ND = not determined or data not available; TS = transferrin saturation; NASH = non-alcoholic steatohepatitis; EPO = erythropoietin.
fact that both brothers share the same mutation and have similar phenotypes suggested the mutation to be pathogenic.

**Case descriptions**

Table 1 shows haematological, biochemical, molecular data and treatment characteristics of the XLSA patients. We will describe some of these patients and relatives in more detail in order to illustrate the biochemical and clinical presentation of XLSA patients, the effectiveness of treatment regimens and the various pitfalls associated with the management of this disease.

Patient 1A illustrates that women may develop a phenotype of XLSA later in life. At the age of 78 years, sideroblastic anaemia was diagnosed after she presented with anaemia (Hb 6.0 mmol/l). Three years earlier, her Hb was 7.7 mmol/l. Post-mortem she was found to have the same ALAS2 defect as her son (patient 1B).

Patient 2A was originally diagnosed with iron overload at the age of 38 years. Treatment with phlebotomies was started. Because of low Hb levels and ferritin levels within reference ranges, phlebotomies were stopped at age 51. After the discovery of the HFE gene in 1996, at age 57, the patient was tested for hereditary haemochromatosis (HH). A heterozygous p.Cys282Tyr mutation in the (haemochromatosis) HFE gene was found. Based on this finding, the patient’s iron overload was attributed to HH. However, HH is an autosomal recessive inherited disorder and complications due to iron overload alone are extremely rare in individuals who are heterozygous for defects in the HFE gene.19

In the same period, a male grandchild (patient 2B) was diagnosed with sideroblastic anaemia. DNA analysis in this child revealed a p.Arg452His mutation in the ALAS2 gene, responsible for XLSA. The same mutation was subsequently found in his grandfather. So, in retrospect, patient 2A suffered from XLSA with secondary systemic iron overload due to ineffective erythropoiesis. At age 70, liver biopsy revealed a hepatocellular carcinoma with substantial iron accumulation in the hepatocytes and some steatosis. The lesion was attributed to iron overload and was not resectable. At age 71, the patient died of this complication. The patient had no history of liver cirrhosis.68

Because of the family history, mutation analysis of the HFE gene was also performed in his grandson, which revealed homozygosity for the p.Cys282Tyr mutation. Because of increasing ferritin levels at age 16, treatment with phlebotomies was started. Within a 30-week period, this resulted in a decrease in ferritin levels from 454 μg/l to 72 μg/l and an increase of Hb from 7.4 mmol/l to 7.8 mmol/l (figure 1).

Patient 3 presented with both severe anaemia (4.3 mmol/l) and very severe and systemic iron overload (ferritin of 5040 μg/l) at age 35. Despite his severe anaemia, phlebotomies were well tolerated and are likely to have contributed to normalisation of his iron stores and Hb in addition to treatment with pyridoxine and iron chelation.

In his teens, patient 5 presented with severe anaemia and ferritin within reference ranges. His younger brother was diagnosed with sideroblastic anaemia at the age of 23 years by family screening. He had no signs and symptoms of anaemia. However, serum ferritin was 1200 μg/l, suggesting severe iron overload. Treatment for sideroblastic anaemia and iron overload was started, consisting of pyridoxine and phlebotomies.

Patient 9 was initially diagnosed with myelodysplastic syndrome (MDS) at the age of 30 years, subtype refractory cytopena with multilineage dysplasia (RCMD). Interestingly, no ring sideroblasts were seen in the bone marrow and the MCV was low, 70 fl. Since his grandfather had previously been described with ‘hereditary primary sidero-achrestic anaemia’ (patient 41 in the study by Bloem20) and since the index patient presented with a hypochromic microcytic anaemia in combination with iron overload, an ALAS2 mutation was suggested and subsequently confirmed.
DISCUSSION

Our Dutch case series is illustrative for the pathophysiology, the biochemical and clinical presentation of XLSA patients, the effectiveness of treatment regimens and the various pitfalls associated with the (early) diagnosis, follow-up and treatment of this disease. In this article we add a novel mutation to the previously described 61 different ALAS2 mutations reported in 120 families with XLSA.22-24 All of our 15 XLSA patients had microcytic anaemia and all had a mutation in the exon 9 domain of the X-chromosome. In 10 out of 11 families (13 out of 15 patients) it concerned the p.Arg452His mutation, making this the most prevalent mutation in Dutch XLSA patients. A search for a founder effect by haplotype analysis in seven of the families with this mutation suggests that this mutation arose from a common ancestor in six of them. Worldwide the p.Arg452His is also the most frequent ALAS2 defect in XLSA. In one patient a novel p.Cys471Tyr mutation was found. Bioinformatic analysis and family genotype-phenotype association study was highly suggestive for a pathogenic defect. Recently, we reported on a 12th Dutch family with XLSA due to a g.55054634G>C mutation in the GATA transcription factor binding site located in a transcriptional enhancer element in intron 1 of the ALAS2 gene.24 Age at diagnosis, degree of anaemia and iron overload widely differed between these patients, illustrating heterogeneity in the clinical and biochemical penetrance of this congenital disease.

One of our patients (patient 3) illustrates that besides anaemia, severe systemic iron overload can occur at early age in transfusion-independent XLSA patients. Preclinical and clinical studies in β thalassaemia major and intermedia and other iron-loading anaemia suggest the ineffective erythropoiesis in these disorders may increase the production of humoral factors, which may include growth differentiation factor 15 (GDF 15), twisted gastrulation (TWSG1) and erythroferrone, leading to decreased production of the iron-regulatory hepatic peptide hormone hepcidin, (reviewed by Kroot et al.25) Heparin acts by inhibiting intestinal iron absorption and macrophage recycling of iron from senescent erythrocytes. Suppression of hepcidin production by these proteins has been suggested to cause inappropriately high intestinal iron absorption and iron release from the reticuloendothelial system (RES), despite iron overload.25-28 We previously reported that patient 2A indeed had elevated serum GDF 15 levels which were associated with an inappropriately low serum hepcidin in relation to his iron stores, as reflected by a low hepcidin/ferritin ratio.28 We did not measure serum GDF 15 and/or serum hepcidin in our other sideroblastic anaemia patients since the results have no therapeutic implications. As far as we know, no studies are available on the above-mentioned humoral factors or hepcidin in sideroblastic anaemia patients due to ALAS2 defects.

In general, systemic iron overload develops in the third or fourth decade, also in patients without overt anaemia.29-31 This emphasises the importance of early diagnosis, since the effects of systemic iron overload are potentially very serious, such as liver cirrhosis and HCC, especially in the presence of concurrent liver toxic conditions (alcohol abuse or non-alcoholic steatohepatitis). Moreover, we suggest that first-degree relatives should be screened for the relevant mutation, because they may develop severe iron overload without any signs and symptoms of anaemia. This phenotype of iron overload with only mild anaemia may lead to a false diagnosis of hereditary haemochromatosis. We suggest that ALAS2 mutations might be the underlying cause of patients (falsely) diagnosed with unexplained forms of HH. In these cases the low MCV should point the clinician to the presence of an iron-loading anaemia such as XLSA. To the best of our knowledge the prevalence of ALAS2 defects among patients with genetically unexplained HH is unknown.

Other genes implicated in iron metabolism and HH may also affect the phenotype of XLSA. Anecdotal data support the suggestion that coinheritance of heterozygosity of the p.Cys282Tyr mutation in the HFE gene is likely increased in XLSA patients with moderate to severe phenotypes.31-33 It is well possible that penetrance of HH due to homozygosity for the p.Cys282Tyr mutation might be modified by ALAS2 mutations and vice versa, as the biochemical presentation of patient 2B suggests, i.e. he developed systemic iron overload already in his teens. The majority of female carriers of XLSA are asymptomatic, as in most X-linked recessive disorders. However, as illustrated by patient 1A, they may be affected due to the predominant inactivation of the normal X-chromosome. Furthermore, physiological age-related skewed X-inactivation in haematopoietic cells may play a role in developing XLSA in female carriers with increasing age. So a combination of congenital and acquired skewing can result in the late onset of XLSA in women.34 Because of the co-existence of normal and affected erythroblasts this anaemia may be normocytic with an increased red cell distribution width (RDW) or even two separate erythrocyte populations.35 Patient 1A also shows that even in elderly patients who present with anaemia, a congenital disorder should be considered. Interestingly, Furuyma et al. describe a male patient with chronic renal failure who developed sideroblastic anaemia at the age of 81 years. This patient was found to have an ALAS2 mutation which only became manifest by an acquired pyridoxine deficiency due to haemodialysis.36
Anecdotal data support the possibility of misdiagnosing XLSA for MDS-RARS (myelodysplastic syndrome-refractory anaemia with ringed sideroblasts) without MDS-specific cytogenetic and genetic abnormalities in elderly people. This may be attributed to the fact that the diagnosis of MDS is solely based on the morphological aspect of the bone marrow, which is often difficult. Our patient 9 was also originally diagnosed with MDS (type RCMD) based on the morphological aspect of bone marrow biopsy, despite low MCH and MCV and a grandfather who was diagnosed with inherited primary sideroachrestic anaemia 50 years ago. Even in retrospect, however, ring sideroblasts, characteristic for sideroblastic anaemia, were not seen in the bone marrow. We have no explanation for this phenotype. To our knowledge, no studies are available on the prevalence of inherited ALAS2 mutation among patients diagnosed with MDS with refractory anaemia (RARS, RA and RCMD). However, in a recent study among 137 cases of sideroblastic anaemia, XLSA patients had MCV levels below the reference range, whereas the MCV of patients with MDS-RARS and MDS-RCMD was within reference range. This indicates that a reduced MCV is important to distinguish XLSA from MDS with refractory anaemia.

As illustrated by our case series, in many patients with XLSA the anaemia is to some extent, responsive to pyridoxine. Pyridoxine is metabolised to pyridoxal 5′phosphate, the cofactor for ALAS2. Pyridoxine responsive XLSA is generally based on missense mutations that reduce the affinity between ALAS2 and pyridoxal 5′phosphate, resulting in a shorter half-life of the enzyme. In these cases treatment with a high dose of the cofactor pyridoxine partly enhances the stability of ALAS2. ALAS2 mutations that alter the posttranslational processing resulting in diminished enzyme activity are mostly pyridoxine unresponsive. Apart from the mutation, the iron status is also important for the pyridoxine responsiveness, because iron overload may compromise mitochondrial function and hence haem-biosynthesis. Therefore, XLSA patients should not be considered refractory to pyridoxine therapy until iron stores have normalised with serum ferritin and transferrin saturation in the normal range. Because of this mechanism it is feasible to phlebotomise in XLSA, even in patients with severe anaemia. Hb typically increases, rather than decreases, after reversal of iron overload by blood removal, as shown by patient 2B and 3. In patients who develop anaemia, frequent withdrawal of a small volume is often feasible (our unpublished observations).

Although 13 out of 15 patients shared the same missense mutation, response to pyridoxine was highly variable. The reason for this remains unclear. Low compliance should be considered, as was the problem in patient 10. If patients are unresponsive to pyridoxine, it is recommended to discontinue it, since increased levels of pyridoxine are associated with peripheral neuropathy. Peripheral neuropathy was not observed in our cases.

In conclusion, our case series describes the biochemical and clinical presentation of XLSA patients and the effectiveness of treatment regime, and it illustrates the various pitfalls associated with diagnosis, follow-up and treatment of the disease. We suspect ALAS2 mutations to be more frequent, but not easy to diagnose. The combination of these data with previously published patient information led us to the following recommendations for the clinical management of patients with XLSA:

1. Diagnosis. Consider XLSA in:
   - Men with unexplained microcytic anaemia, even if the anaemia is mild, since missing the diagnosis might result in severe iron overload and associated morbidity and mortality.
   - Men of all ages presenting with the phenotype of MDS with refractory anaemia (RA), without MDS specific cytogenetic abnormalities, and microcytosis, because patients with MDS-RA have MCV levels within the reference range.
   - Women with unexplained microcytic or normocytic anaemia because of the possibility of late-onset XLSA due to a combination of congenital and acquired unbalanced lyonisation.
   - Patients with unexplained hereditary haemochromatosis and concomitant (mild) microcytic anaemia.

2. Treatment
   - Pyridoxine unresponsiveness in XLSA should not be diagnosed until iron overload has been treated adequately, as iron accumulation is known to reduce pyridoxine activity.
   - Phlebotomies should be considered even in patients with severe anaemia in order to reduce the toxic effects of iron overload and to improve erythropoiesis.

3. Family screening
   - All first-degree family members should be genetically and phenotypically (Hb, MCV, iron, transferrin and ferritin) screened. Even though XLSA is an X-linked disease, women can develop the disease.

ACKNOWLEDGEMENTS

We thank Erwin Wiegerinck for sequencing the ALAS2 gene of the majority of the patients and Siem Klaver for the design and maintenance of the patient database. We report no conflicts of interest.
REFERENCES


