Practice guidelines for the diagnosis and management of microcytic anemias due to genetic disorders of iron metabolism or heme synthesis

Running head: rare inherited iron and heme related anemias

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Abstract

During recent years our understanding of the pathogenesis of microcytic inherited anemias has gained from the identification of several genes and proteins involved in systemic and cellular iron metabolism and heme syntheses. Numerous case reports illustrate that the implementation of these novel molecular discoveries in clinical practice has increased our understanding of the presentation, diagnosis and management of these diseases. Integration of these insights in daily clinical practice will reduce delays in time to establish a proper diagnosis, invasive and/or costly diagnostic tests and unnecessary or even detrimental treatments. In order to assist the clinician, we developed an evidence-based multidisciplinary guideline on the management of rare microcytic anemias due to genetic disorders of iron metabolism and heme synthesis. These genetic disorders may present at all ages and therefore this guideline is relevant for pediatricians as well as clinicians treating adults. This article summarizes these clinical practice guideline and includes i) background on pathogenesis, ii) conclusions and recommendations and iii) a diagnostic flow chart to facilitate its use in the clinical setting.

Introduction

Anemia in children, adolescents and adults is commonly encountered in general clinical practice. Multiple causes exist, but with a thorough history, physical examination and limited laboratory evaluations a specific diagnosis can often be established. A standard diagnostic approach is to classify anemia as microcytic (MCV< 80 fl), normocytic or macrocytic (MCV> 98 fl). Nutritional iron deficiency, iron loss by gastro-intestinal disease, iron malabsorption, hemoglobinopathies, including some thalassemia syndromes, and severe anemia of chronic disease are the primary causes of microcytic anemias. In case of normocytic anemia, hemolysis, anemia of chronic disease or bone marrow pathology should
be considered. Macrocytic anemias are often caused by toxic agents such as alcohol, deficiency of folic acid and/or vitamin B12 or less frequently by myelodysplastic syndrome. However, some patients with microcytic anemias remain unexplained by the above-mentioned categorization. In these cases: i) elevation of ferritin and/or transferrin saturation (TSAT) or ii) low TSAT in combination with low-normal ferritin levels (> 20 µg/L) suggest a genetic disorder of iron metabolism or heme synthesis. The family history, an anemia that is refractory or incompletely responsive to iron supplementation and additional features such as neurologic disease and skin photosensitivity may also be indicative of these disorders.

During recent years, defects in genes with roles in systemic and cellular iron metabolism and heme synthesis have been identified to be involved in the pathogenesis of these genetic anemias.\(^1\)\(^-\)\(^4\) We recommend to integrate these clinical and molecular insights in daily practice, to avoid unnecessary delay in diagnosis, invasive or costly diagnostic tests and harmful treatments. Importantly, in some genetic anemias, such as the sideroblastic anemias, iron overload is of greater consequence than the anemia itself.\(^3\)\(^,\)\(^5\) Unrecognized tissue iron loading might lead to severe morbidity and even mortality, underscoring the need for accurate and timely diagnosis of these disorders.

In this article we present an evidence-based multidisciplinary guideline for the diagnosis and treatment of 12 disorders of microcytic anemia due to defects in 13 different genes leading to genetic disorders of iron metabolism and heme synthesis. We included the disorder associated with a defect in \(SLC40A1\) (or \(ferroportin-1\)) in this guideline, since in animal models mutations in this gene cause a microcytic anemia,\(^6\) and narrative reviews classified the related human anemia as microcytic,\(^1\) despite the fact that our literature review showed that microcytic anemia is rarely reported in these patients. We also included (normocytic) patients with XLDPP, since this disorder is a variant of erythropoietic protoporphyria, a disease with patients presenting with microcytic anemia. We describe the methodology used, provide a
short and illustrated introduction in iron homeostasis (Figure 1) and briefly discuss the
pathogenesis, epidemiology, clinical presentation, diagnosis, and treatment. We present i)
case tables comprising characteristics of the individual patients described in the literature
(Supplement 1), except for patients with loss-of-function defects in ALAS2 and defects in
SLC40A1, UROS and FECH because of their relatively high prevalence, ii) evidence-based
conclusions and related references (Supplement 2), iii) recommendations including advice on
family screening (Table 1), and iv) results of literature analysis on prevalence of anemia in
patients with defects in SLC40A1 (Supplement 3). To facilitate the clinician, a table
summarizing the characteristics of the disorders (Table 2) and a flow chart (Figure 2) to aid
the diagnosis of the relevant diseases are included.

Methodology
This guideline has been developed to assist clinicians and patients in the clinical decision
making process for rare anemias due to genetic disorders of iron metabolism and heme
synthesis by describing a number of generally accepted approaches for the diagnosis and
treatment of these disorders.

For the development of this evidence-based guideline, the working group adopted the
methodology described in the Medical specialist guidelines 2.0 of the Netherlands
Association of Medical Specialists. This methodology is based upon the international
AGREE II criteria for assessing the quality of guidelines (http://www.agreetrust.org). In
short, the development of the guideline was started by formulating a number of starting
questions (Supplement 4). Each question guided a systematic literature review in both
Medline and Embase up to December 2010, using MESH headings and free text words related
to the name of the predefined genes and disease of interest (see “definitions” below), and
disease specific symptoms. In addition, we retrieved more recent original and review articles
by using the same key words for searching PUBMED, and checked the references of the
obtained papers. Searches were limited to those written in English, German, French and Dutch. Searches were not limited in time.

The majority of the articles retrieved were case reports or small case series. The searches also showed a number of reviews, however, none of them used a systematic approach. Therefore, we modified the usual therapeutic/intervention based system for grading the evidence of the conclusions in supplement 2 in the following levels: 1. Proven or very likely based on results by numerous investigators, in various populations and settings, 2. Probable based on moderate number of reports 3. Indicative based on small number of reports 4. Expert opinion of members of the working group.

**Working group**

The working group consisted of the authors of this article.

**Definitions**

This guideline covers microcytic anemias due to genetic defects in iron metabolism or heme synthesis (Table 2). For the selection of these diseases, we used recent reviews, and added disorders that were described in more recent years, i.e. sideroblastic anemia due to defects in STEAP3 and X-linked dominant protoporphyria (XLDPP).

We excluded microcytic anemia caused by hemoglobinopathies and genetic diseases not predominantly characterized by a primary defect in iron metabolism or heme synthesis.
Introduction in iron metabolism and heme synthesis

Iron plays an essential role in many biochemical processes, in particular in the production of heme for the incorporation in hemoglobin and myoglobin, and iron-sulphur clusters, which serve as enzyme cofactors. In case of iron deficiency, cells lose their capacity for electron transport and energy metabolism. Clinically, iron deficiency causes anemia and may result in neurodevelopmental deficits. On the other hand, iron excess leads to complications such as endocrine disorders, liver cirrhosis and cardiac dysfunction.

Therefore, tight regulation of body iron homeostasis on systemic and cellular level is paramount. These processes comprise several proteins, most of which have been discovered in the last 20 years. Defects in these proteins lead to disorders of iron metabolism and heme synthesis that are characterized by iron overload, iron deficiency, or iron maldistribution. Cells involved in iron homeostasis are duodenal enterocytes, hepatocytes, macrophages and erythroid precursors. To illustrate the description on pathophysiology for the different disorders, in Figure 1 we schematically present the function of the above-mentioned cells in iron homeostasis, and the proteins involved.

1. Disorders due to low iron availability for erythropoiesis

1A. Iron refractory iron deficiency anemia (IRIDA) due to defects in TMPRSS6

Pathogenesis and epidemiology

TMPRSS6 (OMIM 609862) encodes matriptase-2 (a type II plasma membrane serine protease), that senses iron deficiency and blocks hepcidin transcription by cleaving hemojuvelin (HJV). Consequently, pathogenic TMPRSS6 defects result in uninhibited hepcidin production, causing iron refractory iron deficiency anemia (IRIDA). At the population level Genome Wide Association Studies (GWAS) show that TMPRSS6 is polymorphic with a relatively large amount of high frequency polymorphisms of which some
(particularly p.Ala736Val) are associated with significant decrease of the concentrations of iron, hemoglobin (Hb) and Mean Cellular Volume (MCV) of the red blood cell.\textsuperscript{17,18} The prevalence of pathogenic mutations leading to IRIDA is unknown, but under-diagnosis seems likely. IRIDA patients due to a suspected homozygous or compound heterozygous \textit{TMPRSS6} defect are described in 61 cases in 39 families. Since functional studies are not always performed, it is unclear whether all these mutations are pathogenic. A few cases with microcytic anemia, low TSAT, and low-normal ferritin are reported to be heterozygous for \textit{TMPRSS6} defects (Supplement 1).

\textit{Clinical presentation and diagnosis}

Most IRIDA patients present in childhood with a microcytic anemia, which tends to become less severe with increasing age,\textsuperscript{19} in combination with a remarkably low TSAT and – if untreated- a low to normal ferritin. Most patients fail to respond to oral iron (see below), but since this feature is also observed in iron deficient anemic patients with autoimmune atrophic gastritis, \textit{Helicobacter pylori} infection and celiac disease, these (non-genetic) disorders should be considered in the diagnostic work up.\textsuperscript{20}

In IRIDA, serum hepcidin is inappropriately high given the low body iron status. Consequently, the hepcidin/TSAT ratio is high.\textsuperscript{21} In the absence of inflammation, an increased hepcidin/TSAT ratio is specific for IRIDA, whereas a low hepcidin/ferritin ratio is characteristic for many genetic iron loading disorders.

In affected children, growth, development and intellectual performances are normal.\textsuperscript{22} Although the pedigree structure of most IRIDA patients shows an autosomal recessive inheritance, anecdotal reports suggest that heterozygous pathogenic \textit{TMPRSS6} mutations might cause a mild IRIDA phenotype (Supplement 1). It is still unclear whether this can be explained by environmental factors, a combination with modulating polymorphisms or a low
expressing allele, or whether the current Sanger sequencing strategy misses certain defects in the exons, introns of the gene or its regulatory regions, or whether defects in other genes are involved. Therefore, we conclude that IRIDA due to a *TMPRSS6* defect can only be diagnosed with certainty when the patient is homozygous or compound heterozygous for a pathogenic mutation.

*Treatment*

Case reports indicate that the pathogenicity of the *TMPRSS6* defect determines the response to oral iron. Severe *TMPRSS6* defects usually lead to oral iron resistance. Only few cases (partially) respond to oral iron (Supplement 1). Ascorbic acid (3mg/d) supplementation along with oral ferrous sulfate has been reported to improve Hb and iron status in an infant resistant to oral iron supplementation only. Case series show that repeated administration of intravenous iron (iron sucrose or iron gluconate) increase Hb and ferritin and to a lesser extent MCV and TSAT, although complete normalization of Hb is seldomly achieved. Attempting to correct the Hb level into the reference range may place the patient at risk for iron overload. We found no evidence for a threshold of circulating ferritin levels above which the iron in the reticulo-endothelial (RE) macrophages becomes toxic on the long term. However, following guidelines for iron treatment in patients with chronic kidney disease, we recommend to monitor serum ferritin levels and not exceed a concentration of 500 µg/L to avoid this risk, especially in children and adolescents. The role of erythropoietin (EPO) treatment in IRIDA is controversial. Trials with novel hepcidin lowering compounds in these patients have not been performed.
1B. Ferroportin disease due defects in SLC40A1

Pathogenesis and epidemiology

SLC40A1 (or ferroportin-1, IREG1, MTP1, SLC11A3) (OMIM 606069) encodes the protein ferroportin,\(^2\) the only known human cellular iron exporter (Figure 1).\(^1\)\(^,\)\(^2\)\(^,\)\(^9\) In 2000, ferroportin was identified in the zebrafish mutant weisscherbst, as the defect gene responsible for the hypochromic anaemia in these animals that was ascribed to inadequate circulatory iron levels.\(^6\) In man only heterozygous mutations in SLC40A1 are reported and microcytosis is not observed. The resulting hereditary hemochromatosis (HH) type 4, or ferroportin disease, is an autosomal dominant condition primarily characterized by iron overload with a heterogeneous phenotype.\(^30\)\(^,\)\(^31\) In almost all case series and the available narrative reviews, mutations causing ferroportin disease are classified into two groups: loss-of-function (LOF, HH type 4A or classical HH) and gain-of-function (GOF, HH type 4B or atypical HH) mutations.\(^32\)\(^,\)\(^33\) Functional studies and clinical data show that a LOF mutation typically leads to iron retention in the duodenal cell and macrophages due to reduced ferroportin activity and preserved inhibitory capacity of hepcidin. The phenotype is characterized by an elevated serum ferritin level in association with a low to normal TSAT and predominant iron deposition in macrophages with low tolerance to phlebotomy in some patients.\(^32\) GOF mutations lead to increased iron absorption due to increased ferroportin activity, which is resistant to the inhibitory effect of hepcidin. As a consequence, the iron overload phenotype of these patients is indistinguishable from other forms of hereditary hemochromatosis,\(^30\)\(^,\)\(^31\) and therefore this subtype of ferroportin disease does not fit into the category of “disorders due to low iron available for erythropoiesis”.

For certain genotypes, however, the functional studies and the biochemical and histological phenotypes vary between studies and patients.\(^34\) Therefore, the distinction between LOF and GOF mutations is not always straightforward. In order to define the type of mutation, we
developed for the purpose of this guideline a classification system based on both phenotypic and functional characteristics (Supplement 3). Based on this classification we identified 36 different LOF mutations and 15 GOF mutations. A total of 207 patients with a LOF mutation and 73 patients with a GOF were found worldwide.

*Clinical presentation and diagnosis*

Symptoms are related to iron overload and are non-specific. Since the current guideline is on anemias, in our evaluation of the literature on ferroportin disease we investigated the occurrence of anemia (defined by the WHO)\(^35\) as presenting symptom.

In 76 (27.7%) and 24 (8.5%) out of 280 patients, both Hb and MCV were numerically noted. Eight (10%) of the patients fulfilled the WHO criteria of anemia without additional causes of microcytic anemia (Table 1 in Supplement 3). Five males had a Hb between 12.0 and 12.9 g/dL and three females had Hb between 11.1 and 11.5 g/dL without microcytosis. Seven of the 8 anemic patients were annotated a LOF mutation.

We conclude that of the total of 76 patients diagnosed with iron overload due to both LOF and GOF mutations in *SLC40A1* and documented Hb levels only \(\approx 10\%\) was anemic. This anemia is described as mild and normocytic.

*Treatment*

Iron overload in patients with both LOF and GOF ferroportin disease is treated with phlebotomy. Both the application of phlebotomy and its effects were described for 94 patients. In 14 of the 94 patients (14.8%) a transient mild or profound anemia during phlebotomy treatment was reported. Our literature evaluation showed that the risk for the development of anemia during phlebotomy was associated with older age and a LOF mutation phenotype, e.g.
a relatively low TSAT (Table 2 in Supplement 3). Data from case reports suggest that patients who develop anemia upon phlebotomies benefit from extension of the phlebotomy interval.

1C. Aceruloplasminemia (ACP) due to defects in \( CP \)

Pathogenesis and epidemiology

\( CP \) (OMIM 604290) encodes ceruloplasmin (CP), which is secreted into plasma and carries 95% of circulating plasma copper. CP catalyzes cellular efflux of iron by oxidation of \( \text{Fe}^{2+} \) to \( \text{Fe}^{3+} \) for binding to circulating transferrin (Figure 1).\(^{36}\) In vitro studies and mice studies show that CP is also required for the stability of the iron exporter ferroportin, especially in glial cells.\(^{37}\) Mice studies demonstrate that the absence of CP leads to wide spread iron overload in parenchymal and reticuloendothelial organs, including the nervous system.\(^{36}\)

The incidence of ACP in Japan is estimated to be approximately 1 per 2,000,000 in non-consanguineous marriages. There are no reliable data on the incidence and prevalence in Western European countries.\(^{38}\) In total 35 pathogenic \( CP \) mutations have been described in 50 families. ACP is a rare autosomal recessive disease. However, 7 patients with a clinical phenotype of ACP were heterozygous for a \( CP \) defect (Supplement 1).

Clinical presentation and diagnosis

Iron accumulation in ACP affects the liver, pancreas and central nervous system.\(^{39}\) ACP patients develop the classical triad of i) diabetes mellitus, ii) retinal degeneration and iii) neurodegenerative disease with extrapyramidal and cerebellar symptoms in combination with mental dysfunction. A mild normo- to microcytic anemia with low serum iron and elevated serum ferritin is a constant feature in ACP.\(^{40}\) However, in none of the described patients, anemia was the presenting symptom. Disease onset typically occurs in the 4-5th decade of life with neurodegenerative symptoms. Most patients die in the 6th decade due to neurological
complications. Despite the hepatic iron overload, ACP has not been described to be associated with liver disease.

Absent or very low serum ceruloplasmin in combination with low serum copper and iron, high serum ferritin and increased hepatic iron concentration is indicative of ACP. The diagnosis is supported by characteristic findings on MRI that are compatible with iron accumulation in liver, pancreas and brain. Homozygous or compound heterozygous CP defects confirm the diagnosis of ACP.

Treatment

Case series describe normalization of serum ferritin and decrease of hepatic iron overload after treatment with iron chelation. In 6 cases neurological improvement, and in 2 other cases reduction of insulin demand was described on treatment with iron chelators. Oral administration of zinc sulphate in a symptomatic heterozygous ACP patient has been reported but effect on neurological symptoms remains unclear. Three homozygous ACP patients were treated with fresh frozen plasma; their outcome was not reported. The anemia in ACP is mild and does not need any intervention.

2. Disorders due to defects in iron acquisition by the erythroid precursors

2A. Hypotransferrinaemia due to defects in TF

Pathogenesis and epidemiology

Genetic hypotransferrinaemia is a rare autosomal recessive disease due to a defect in TF (OMIM 19000). Transferrin deficiency leads to low concentrations of transferrin bound iron with iron deficient erythropoiesis and high concentrations of non-transferrin bound iron with subsequent iron overload in nonhematopoietic tissues. Functional studies in a limited number of patients indicate that the intestinal iron absorption is increased with augmented plasma iron...
clearance and reduced red cell iron utilization.\textsuperscript{49,50} The increased intestinal iron absorption results from the strongly reduced hepatic hepcidin production ascribed to iron deficient erythropoiesis and low transferrin concentration.\textsuperscript{51,52}

Since 1961 at least 13 cases in 11 families with of hypotransferrinemia are reported worldwide.\textsuperscript{49,50,53-56} Underlying molecular defects in TF are recognized in 6 patients (Supplement 1).

\textit{Clinical presentation and diagnosis}

Patients present in early life with microcytic and hypochromic anemia. The anemia is characterized by low serum iron and high ferritin levels. The transferrin level varies between below limits of detection to 20 \% of normal and is fully saturated. Serum hepcidin levels are low. The iron content in the bone marrow is reduced with a decreased myeloid to erythroid ratio in most cases.\textsuperscript{49,50,53-56}

Affected children may show a failure to thrive with occasionally retardation in the mental development. There are signs of widespread iron overload with hepatomegaly and strikingly early endocrinopathy, skin iron deposition, and sometimes fatal, cardiomyopathy. Some patients have osteoporosis.\textsuperscript{55,56}

The diagnosis is confirmed by molecular analysis of TF. Heterozygous relatives have decreased transferrin concentrations, without anemia or systemic iron loading.

\textit{Treatment}

Treatment consists of infusions of apotransferrin either directly or as plasma. Data on the dosage and frequency of these infusions are limited. Monthly infusions of plasma has been reported to be sufficient to normalize Hb and serum ferritin levels. To date, only 3 patients have been treated with apotransferrin on a compassionate use-basis.\textsuperscript{50,55} The calculated
elimination half-life reported varied between 4.8 and 10 days. Apotransferrin is not on the market for clinical purposes, but has a orphan drug status (EU/3/12/1027; 2012). A clinical trial in patients with hypotransferrinemia is ongoing (ClinicalTrials.gov Identifier: NCT01797055). Reluctance with repeated erythrocyte transfusion or iron substitution is advocated to avoid further iron overload.

2B. Anemia with systemic iron loading due to defects in SLC11A2 (DMT1)

Pathogenesis and epidemiology

SLC11A2 (OMIM 600523) encodes Divalent Metal Transporter-1 (DMT1), a cellular membrane bound iron transporter (Figure 1). Molecular studies indicate that SLC11A2 defects cause defective enterocyte and erythroid iron uptake. Systemic iron loading suggests the presence of an additional pathway for intestinal iron absorption, e.g. as heme. Another explanation might be the low hepcidin levels, resulting in increased dietary iron absorption in case SLC11A2 is not completely eliminated. Anemia with systemic iron loading due to defects in SLC11A2 is a rare autosomal recessive disorder: 7 patients from 6 families have been described due to homozygous (n=2) or compound heterozygous defects (n=5).

Clinical presentation, diagnosis

Four out of 7 described patients presented at birth with microcytic anemia and increased TSAT. Serum ferritin levels varied from low to moderately increased, with some association with erythrocyte transfusions or iv iron supplementation. Liver iron loading was demonstrated by MRI or biopsy in 5 out of 7 patients at ages varying between 5-27 yrs despite normal or only mildly increased ferritin concentrations in 3 of them.
Treatment

The 7 described patients with severe anemia were treated with erythrocyte transfusions. Three patients received oral iron, which increased Hb and led to transfusion independency in one patient.

Three patients received EPO, resulting in an increase of Hb, but - based on the clinical course of 1 patient - not in prevention of liver iron loading. Erythrocyte transfusions and probably also oral or iv iron cause additional liver iron loading. Chelation was not effective in reducing liver iron and resulted in decrease of Hb (unpublished data, Tchernia and Beaumont). 1,62

2C. Sideroblastic anemia due to defects in \textit{STEAP3}

Pathogenesis and epidemiology

\textit{STEAP3} (OMIM 609671) encodes a ferroreductase, responsible for the reduction of \textit{Fe}^{3+} to \textit{Fe}^{2+} in endosomes of erythroblasts (Figure 1). Mice studies show that absence or reduced activity of ferroreductase results in severe microcytic anemia, which can be corrected by introduction of a functional \textit{STEAP3}. The first and so far only human \textit{STEAP3} mutation was recently described in three siblings born to healthy, non-consanguineous parents.

Clinical presentation and diagnosis

The 3 siblings displayed a transfusion-dependent severe hypochromic anemia with a normal to slightly decreased MCV. Serum iron and ferritin were normal to increased, while TSAT was markedly increased. A bone marrow smear in the index patient showed ring sideroblasts. Liver biopsy after multiple erythrocyte transfusions showed iron loading. All patients suffered from gonadal dysfunction, as described for \textit{STEAP3} deficient mice. A heterozygous nonsense \textit{STEAP3} mutation was inherited from the father, while no defect was found in the mother. The authors explain the normal phenotype of the father by the \textit{STEAP3} expression of...
the ‘healthy’ allele in lymphocytes, which was significantly higher than in his affected children.

*Treatment*

Treatment consisted of a combination of erythrocyte transfusions and chelation while EPO increased the transfusion interval.

3. Disorders due to defects in the heme and/or iron-sulphur cluster synthesis

3A. Sideroblastic anemia due to defects in *SLC25A38*

*Pathogenesis and epidemiology*

*SLC25A38* (OMIM 610819) encodes a protein on the inner membrane of the mitochondria of hematopoietic cells, especially erythroblasts, and is essential for the heme synthesis (Figure 1). Its specific function is not known, but it has been hypothesized that *SLC25A38* facilitates 5-aminolevulinic acid (ALA) production by importing glycine into mitochondria or by exchanging glycine for ALA across the mitochondrial inner membrane. Defects in *SLC25A38* result in severe congenital sideroblastic anemia with microcytic hypochromic erythrocytes. Until now 26 patients have been reported with 20 different mutations. The inheritance pattern is autosomal recessive; heterozygous relatives are not anemic.

*Clinical presentation and diagnosis*

Patients present with severe, often transfusion dependent, microcytic hypochromic anemia in childhood, which is clinically similar to thalassemia major. Bone marrow smears show ring sideroblasts. Serum ferritin levels and TSAT are increased, even before treatment with erythrocyte transfusions.
Treatment

Symptomatic treatment consists of erythrocyte transfusions and iron chelation. Hematopoietic stem cell transplantation (HSCT) is the only curative treatment and was performed in 8 out of 29 patients and resulted in disease free survival in 4 patients (follow up < 5 years)(Supplement 1).

3 B. X-linked sideroblastic anemia with ataxia due to defects in ABCB7

Pathogenesis and epidemiology

ABCB7 (OMIM 300135) on the X-chromosome encodes a protein in the inner membrane of the mitochondria, which is the putative mitochondrial exporter of Fe-S complexes (Figure 1).3,68 Defects in ABCB7 result in disrupted iron metabolism and heme synthesis causing a mild, slightly microcytic, sideroblastic anemia but also cerebellar ataxia.69 Sideroblastic anemia with ataxia due to defects in ABCB7 is a rare, X-linked disease.69 Seventeen patients with 4 different pathogenic ABCB7 defects have been described in case reports, of which 5 were female, which might be explained by skewed X-inactivation. None of the women showed neurological defects. There is no apparent genotype-phenotype correlation.

Clinical presentation and diagnosis

The presenting symptom in all male patients was cerebellar ataxia that developed in childhood. Cerebral MRI showed cerebellar hypoplasia in 4 patients. Mild, slightly microcytic, sideroblastic anemia was found, usually in the second decade. In 10 patients free erythrocyte protoporphyrin IX was increased. None of the patients showed systemic iron loading.
Treatment

Treatment of the mild anemia is not reported.

3C. X- linked sideroblastic anemia due to defects in ALAS2

Pathophysiology and epidemiology

ALAS2 (OMIM 301300) is located on chromosome X and encodes for ALAS2, an erythroid specific isoform of the catalytic enzyme involved in heme synthesis in the mitochondria (Figure 1).70 Almost all ALAS2 defects are missense mutations, most commonly in domains important for catalysis or pyridoxal phosphate (vitamin B6) co-factor binding.71 Recently also defects in the binding site of the transcription factor GATA1 in the first intron of ALAS2 have been described.72 ALAS2 defects result in decreased protoporphyrin synthesis and subsequent reduced iron incorporation and heme synthesis, causing microcytic anemia and erythroid mitochondrial iron loading. Mitochondrial iron loading exacerbates the anemia through decreased pyridoxine sensitivity.73 Heme deficiency is associated with ineffective erythropoiesis, followed by increased intestinal iron uptake and tissue iron accumulation. X-linked sideroblastic anaemia (XLSA) is the most common genetic form of sideroblastic anaemia.3 Since Cooley described the first patients with XSLA in 1945,74 61 different pathogenic ALAS2 defects have been described in 120 non-related families.75,76 As in most X-linked disorders, most female carriers of XLSA are asymptomatic. However, women with ALAS2 mutations may be affected due to skewed X–inactivation.77 Furthermore, physiologic age related skewed X –inactivation in hematopoietic stem cells may play a role in developing XLSA in female carriers with increasing age.78 Estimates on the prevalence of ALAS2 defects are not available. Since the phenotype might be mild, under-diagnosis is likely.
Clinical presentation and diagnosis

XLSA is characterized by mild hypochromic, microcytic, sideroblastic anemia in combination with systemic iron overload. Elevated Red cell Distribution Width (RDW) has been described in female carriers of the mutation and is ascribed to the presence of 2 erythrocyte populations. Phenotypic expression of XLSA is highly variable even in patients with identical mutations. Case reports indicate that affected males generally present in the first 2 decades of life with symptoms of anemia or later with either manifestations of anemia or those of parenchymal iron overload. Manifestation at elderly age due to an acquired pyridoxine deficiency is described.

Treatment

The available evidence indicates that initial doses of oral vitamin B6 (pyridoxine) 50-200 mg/day are effective in improving anemia and iron overload in all responsive XLSA patients. Occasionally high doses may be considered. Once a response is obtained evidence suggests the life-long maintenance dose may be lowered to 10-100 mg/day, since too high doses may give neurotoxicity. Since iron overload may compromise mitochondrial function and hence heme biosynthesis, XLSA patients should not be considered pyridoxine refractory until iron stores are normalized. Most patients can be treated with phlebotomies for iron overload, since the anemia is mild. Hb typically increases, rather than decreases, after reversal of iron overload by phlebotomies.
3D. Sideroblastic anemia due to defects in \textit{GLRX5}

\textit{Pathogenesis and epidemiology}

\textit{GLRX5} (OMIM 6095588) encodes for the mitochondrial disulfide glutaredoxin (GLRX) 5, that is highly expressed in early erythroid cells and is essential for the biosynthesis of Fe-S clusters (Figure 1).

\textit{In vitro} data show that in GLRX5 deficient erythroblasts, Fe-S cluster production is decreased, and more active Iron Responsive Protein 1 (IRP1) is present in the cytosol. This reflects a low-iron state of the cell and causes repression of target genes, including \textit{ALAS2}, resulting in reduced heme synthesis, in mitochondrial iron accumulation, and increased turnover of ferrochelatase. Sideroblastic anemia due to defects in \textit{GLRX5} is a rare autosomal recessive disease: only 1 male patient has been described.

\textit{Clinical presentation and diagnosis}

The patient born from consanguineous parents, presented at age 44 years with type 2 diabetes mellitus and at 60 years with icterus and hepatosplenomegaly. There was a progressive microcytic anemia with increased TSAT, serum ferritin and iron accumulation in both bone marrow macrophages and erythroblast mitochondria.

\textit{Treatment}

Treatment with iron chelation and erythrocyte transfusion resulted in an increase of Hb and a decrease in serum ferritin. Pyridoxine supplementation was not effective.
3E. Erythropoietic ProtoPorphyria due to defects in *FECH* (EPP) and gain-of-function mutations in *ALAS2* (XLDPP)

**Pathophysiology, epidemiology**

Erythropoietic ProtoPorphyria comprises 2 variants, EPP and (X-linked dominant protoporphyria) XLDPP, and belongs to the cutaneous porphyrias characterized by accumulation of free protoporphyrin IX. Autosomal recessive EPP (OMIM 177000) is caused by defects in *FECH* (OMIM 612386), encoding for ferrochelatase in the mitochondrion that is responsible for iron insertion into protoporphyrin IX to form heme (Figure 1). The prevalence differs worldwide and mode of inheritance is complex.

XLDPP results from gain-of-function mutations in *ALAS2* (OMIM 301300) leading to protoporphyrin IX overproduction. XLDPP is found in 29 families worldwide and in \( \approx 2\% \) (UK) to \( \approx 10\% \) (USA) of patients with the EPP phenotype.

The diminished ferrochelatase activity in the EPP variant results in reduced heme synthesis, with occasionally ringed sideroblasts, but without systemic iron overload. This suggests that the iron absorption and supply in these patients match the requirement for reduced erythropoiesis.

**Clinical presentation and diagnosis**

The variants EPP and XLDPP are clinically indistinguishable. The predominant clinical presentation is a painful photosensitivity, erythema, stinging and burning, beginning in childhood on sunlight exposed skin. In severe patients the liver is affected. Twenty to 60% of the patients show a microcytic anemia, with a mean decrease in hemoglobin in adult male patients of 1.2 g/dL and reduced iron stores.
Fluorescent erythrocytes can be seen in a fresh, unstained blood smear. Free protoporphyrin is found in plasma and erythrocytes. Zn protoporphyrin is typically increased in XLDPP, due to the relative iron deficiency for the amount of protoporphyrin IX.91

Treatment
Since anemia is mild, its treatment is not warranted.91 The therapy of EPP is focused on minimizing the harmful effects of exposure to sunlight and on managing the hepatotoxic effects of protoporphyrin.

3F. Congenital Erythropoietic Porphyria (CEP), due to defects in UROS or GATA1

Pathogenesis and epidemiology
UROS (OMIM 606938) encodes uroporphyrinogen III synthase, the 4th enzyme of the heme biosynthetic pathway. CEP (OMIM 263700) is a rare autosomal genetic disease caused by defects in UROS, leading to erythroid accumulation of the non-physiological uroporphyrin I and coproporphyrin I.91 A reduced red cell survival due to excess porphyrin in erythrocytes contributes to hemolytic anemia found in most patients with CEP.92 To date, approximately 45 UROS mutations have been described in > 200 individuals.93 An X-linked variant of CEP (OMIM 314050) caused by a mutation in GATA1 (OMIM 305371) on the X-chromosome has been reported for 1 patient.94 GATA binding factor 1 regulates expression of UROS in erythroblasts.

Clinical presentation and diagnosis
CEP typically presents with passage of red urine shortly after birth. Lifelong bullous cutaneous photosensitivity to visible light starts in early infancy, leading to scarring with photomutilation.91 Other manifestations include hypertrichosis, erythrodontia, osteoporosis
and corneal ulceration with scarring. Age of onset and severity of CEP are both highly variable. In a retrospective study of 29 CEP patients, 66% suffered from chronic hemolytic anemia with variable severity. Iron parameters are not described in these patients.

The patient with CEP caused by the GATA1 defect had a severe hypochromic microcytic, hemolytic anemia mimicking the phenotype of thalassemia intermedia, in combination with thrombocytopenia. Biochemically, CEP patients have uro- and coprophorphyrin accumulation in erythrocytes, plasma, urine, faeces and increased UROS activity in erythrocytes.

**Treatment**

Allogenic HSCT is the only curative option for CEP. Chronic erythrocyte transfusion in severe cases to suppress erythropoiesis is described. Protection of eyes and skin from sunlight is essential and minor skin trauma should be avoided.

**Conclusions**

We present a clinical guideline on orphan diseases of which many aspects are still unknown. Consequently, the level of evidence for the management of these disorders is relatively low. We therefore recommend centres of excellence with expertise with these diseases to join forces in order to identify new mechanisms, biomarkers and treatments and to optimize the management of these patients. Until more evidence is available, this guideline can be used to assist clinicians and patients in their understanding, diagnosis and management of microcytic genetic anemias of iron metabolism or heme synthesis.
Acknowledgements

We thank M. Wessels, literature specialist, for her help in the systematic literature review of the various databases. Financial support was provided by a grant from the Quality Foundation Funds Medical Specialists (SKMS) of the Netherlands association of Medical specialists (OMS).

Authorship

Contribution: AED, RAPR, PPTB, ND, LTV, NVAMK, RT, DWS were members of the working group, made literature and case-tables, drafted, discussed and finalized the guidelines and edited the manuscript; TvB: advisor and support in the use of the methodology for guideline development; DWS, initiated and coordinated the project and acquired funding. All authors approved the final version of the manuscript.

Conflict-of-interest disclosure: D.W.S. is medical director of the www.hepcidinanalysis.com initiative that serves the scientific and medical community with hepcidin measurements at a fee-for service basis. She is an employee of the Radboud University Medical Centre that offers genetic testing for the genes described in the current guideline (at http://www.umcn.nl/Informatievoorverwijzers/Genoomdiagnostiek/en/Pages/default.aspx). The remaining authors declare no competing financial interests.
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### Table 1. Recommendations

#### Part 1: Anemia due to low iron bioavailability for erythropoiesis

<table>
<thead>
<tr>
<th>1A. Iron refractory iron deficiency anemia (IRIDA) due to defects TMPRSS6</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical presentation and diagnostics</strong></td>
</tr>
<tr>
<td>• In patients with unexplained microcytic anemia with low transferrin saturation (TSAT) and normal or reduced serum ferritin concentration, not or partially responding to oral iron and (partially) responsive to intravenous iron supplementation, Iron Refractory Iron Deficiency Anemia (IRIDA) due to a TMPRSS6 defect should be considered. Determination of serum hepcidin is recommended in case the diagnosis IRIDA is suspected.</td>
</tr>
<tr>
<td>• Increased serum hepcidin in relation to TSAT (hepcidin/TSAT ratio &gt; p.97.5 of local reference value) is suggestive of IRIDA. TMPRSS6 mutation analysis is recommended.</td>
</tr>
<tr>
<td>• In case of a homozygous or compound heterozygous TMPRSS6 defect, IRIDA due to a dysfunctional matriptase-2 protein should be diagnosed.</td>
</tr>
<tr>
<td>• No recommendation can be made on the clinical significance of heterozygous TMPRSS6 defects with or without concomitant polymorphisms, because of lack of evidence.</td>
</tr>
<tr>
<td><strong>Treatment</strong></td>
</tr>
<tr>
<td>• In a patient with iron deficiency anemia due to pathogenic TMPRSS6 defects initial treatment with oral iron or oral iron combined with ascorbic acid should be considered.</td>
</tr>
<tr>
<td>• Patients, for whom this initial treatment does not result in acceptable Hb levels, should be treated with intravenous iron supplementation.</td>
</tr>
<tr>
<td>• In IRIDA patients, the choice of the chemical form of iv iron should be based on its registration for the specific age group OR a proven good safety profile in adults during several years of post marketing surveillance.</td>
</tr>
<tr>
<td>• The total iv iron cumulative doses should be calculated based on formulas of the deficit on body iron allowing for the correction of the Hb deficit and rebuilding the iron stores. Doses should be repeated every 3-7 days until the total dose is administered. Single doses should not exceed the maximum single dose.</td>
</tr>
<tr>
<td>• Serum ferritin levels should be monitored and preferably not exceed 500 µg/L to avoid toxicity of iron overload, especially in children and adolescents.</td>
</tr>
<tr>
<td>• No recommendation can be made on the efficacy of the combination of iv supplementation and erythropoietin (EPO) treatment in IRIDA patients, because of low evidence. This combination therapy might prevent toxic iron loading in some patients.</td>
</tr>
<tr>
<td><strong>Family screening</strong></td>
</tr>
<tr>
<td>• The proband should be informed about the mostly autosomal recessive inheritance pattern of IRIDA. We recommend to screen relatives of the proband for the IRIDA phenotype: siblings and spouse in case of consanguinity and reproductive age. If the proband is diagnosed at young age, and his/her parents are of reproductive age, phenotyping of the parents is recommended. In case of a clinical IRIDA phenotype in the above mentioned relatives, mutation analysis is recommended.</td>
</tr>
<tr>
<td>• Children of the proband should only be phenotyped and, in case of an IRIDA phenotype, genotyped in case of consanguinity of the proband and his/her spouse or in case of proven carriership of both proband and his/her spouse.</td>
</tr>
<tr>
<td>• Because of the complex genotype-phenotype correlation in IRIDA, we recommend referral to a clinical geneticist in case of an IRIDA phenotype and a pathogenic heterozygous TMPRSS6 defect.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>1B. Ferroportin disease due to defects in SLC40A1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical presentation and diagnostics</strong></td>
</tr>
<tr>
<td>• Ferroportin disease due to loss-of-function (LOF) mutations should NOT be considered as a cause of microcytic anemia.</td>
</tr>
<tr>
<td>• When anemia occurs in a patient with primary iron overload during treatment with repeated phlebotomies the presence of LOF ferroportin disease may be considered.</td>
</tr>
<tr>
<td><strong>Treatment</strong></td>
</tr>
<tr>
<td>• Patients with iron overload due to LOF and gain-of-function (GOF) ferroportin disease should be treated with repeated phlebotomies.</td>
</tr>
<tr>
<td>• For patients that develop anemia during phlebotomies despite elevated ferritin levels extension of the phlebotomy interval is recommended</td>
</tr>
<tr>
<td>• In patients who develop anemia during phlebotomies additional treatment with EPO may be considered.</td>
</tr>
<tr>
<td><strong>Family screening</strong></td>
</tr>
<tr>
<td>• The proband should be informed about the mostly autosomal recessive inheritance pattern of IRIDA. We recommend to screen relatives of the proband for the IRIDA phenotype: siblings and spouse in case of consanguinity and reproductive age. If the proband is diagnosed at young age, and his/her parents are of reproductive age, phenotyping of the parents is recommended. In case of a clinical IRIDA phenotype in the above mentioned relatives, mutation analysis is recommended.</td>
</tr>
<tr>
<td>• Children of the proband should only be phenotyped and, in case of an IRIDA phenotype, genotyped in case of consanguinity of the proband and his/her spouse or in case of proven carriership of both proband and his/her spouse.</td>
</tr>
<tr>
<td>• Because of the complex genotype-phenotype correlation in IRIDA, we recommend referral to a clinical geneticist in case of an IRIDA phenotype and a pathogenic heterozygous TMPRSS6 defect.</td>
</tr>
</tbody>
</table>
The proband should be informed about the autosomal dominant inheritance pattern of ferroportin disease. We recommend to screen the first degree relatives (parents, siblings and children) and additional family members (via cascade screening) for the SLC40A1 mutation identified in the proband. Mutation carriers should be screened for the ferroportin disease phenotype.

1C. Aceruloplasminemia (ACP) due to defects in CP

Clinical presentation and diagnostics
- In patients with the combination of insulin dependent diabetes, neurodegenerative disease, retinal degeneration and mild anemia with systemic iron loading, CP defects should be considered.
- In patient with absent or very low ceruloplasmin in combination with low serum copper and iron, high serum ferritin, and characteristic findings on MRI that are compatible with iron accumulation in liver, pancreas and brain, ACP should be considered.
- In case of homozygous or compound heterozygous CP defects, ACP should be diagnosed.

Treatment
- In patients with ACP anemia is mild and therefore treatment is not recommended.
- Iron chelation therapy should be considered for the treatment of ACP.

Family screening
- The proband should be informed about the autosomal recessive inheritance pattern of ACP. Siblings of the proband may be also affected. Since CP defects are very rare, the chance that children of the proband are affected is negligible.
- We recommend to screen for pathogenic CP mutations: siblings and spouse in case of consanguinity and reproductive age. In case a proband is diagnosed with ACP after the fourth decade, his or her parents are not likely to be of reproductive age, and genotyping is not recommended.
- Children of the proband should only be checked for CP mutations in case of consanguinity of the proband and his/her spouse or in case of proven carriership of both proband and his/her spouse.
- Individuals heterozygous or compound heterozygous for CP mutation should be screened for the ACP disease phenotype.

Part 2: Defects in iron acquisition by the erythroid precursors

2A. Hypotransferrinaemia due to defects in TF

Clinical presentation and diagnosis
- In patients with unexplained hypochromic microcytic anemia, low iron binding capacity/serum transferrin concentrations and increased ferritin concentrations, hypotransferrinemia should be considered. Mutation analysis of the TF gene is recommended.
- In case of a homozygous or compound heterozygous TF defect, hypotransferrinemia due to a TF defect should be diagnosed.

Treatment
- Transferrin supplementation by either plasma transfusion or apotransferrin infusion is recommended in patients with hypotransferrinemia due to a TF defect.
- Iron status should be monitored in patients with hypotransferrinemia due to a TF defect in order to detect toxic iron loading early.
- In case of systemic iron loading, phlebotomies are recommended. If phlebotomies are not tolerated due to a decreasing Hb, chelation therapy is recommended.

Family screening
- Recommendations are identical to those described in 1C.

2B. Anemia with systemic iron loading due to defects in SLC11A2 (DMT1)

Clinical presentation and diagnosis
- In patients presenting in childhood with unexplained microcytic anemia with increased TSAT, (among others) SLC11A2 defects should be considered. Genotyping of SLC11A2 is recommended.
- In case of a homozygous or compound heterozygous SLC11A2 defect, diagnosis of microcytic anemia due to a SLC11A2 defect is confirmed.

Treatment
- Patients with microcytic anemia due to pathogenic SLC11A2 defects should be treated with oral iron supplementation and/or EPO and/or erythrocyte transfusions, according to the needs of the individual patient.
- In case of treatment with oral iron supplementation and/or erythrocyte transfusions, iron status should be monitored in order to detect toxic iron loading in an early stage.
• Since a normal serum ferritin concentration does not exclude liver iron loading in patients with SLC11A2 defects, MRI of the liver should be considered.

**Family screening**
- Recommendations are identical to those described in 1C.

### 2 C. Sideroblastic anemia due to defects in STEAP3

**Clinical presentation and diagnosis**
- In patients with unexplained hypochromic sideroblastic anemia with low or normal MCV defects in the STEAP3 gene should be considered.
- In case of the combination of hypochromic anemia and gonadal dysfunction, STEAP3 defects should be considered.

**Treatment**
- Patients with hypochromic anemia due to STEAP3 defects can be treated with erythrocyte transfusions in combination with EPO. Systemic iron loading should be treated with iron chelation.

**Family screening**
- Since sideroblastic anemia due to a STEAP3 defect has been described in only 1 family, the inheritance pattern is uncertain and the proband should be referred to a clinical geneticist.

### Part 3: Defects in the heme and/or iron sulphur cluster synthesis.

#### 3A. Sideroblastic anemia due to defects in SLC25A38

**Clinical presentation and diagnosis**
- In children with severe unexplained microcytic sideroblastic anemia defects in SLC25A38 should be considered.

**Treatment**
- Hematopoietic stem cell transplantation (HSCT) is recommended since this is the only curative option.
- Symptomatic treatment consists of erythrocyte transfusions and chelation therapy.

**Family screening**
- Recommendations are identical to those described in 1C.

#### 3B. X-linked sideroblastic anemia with ataxia due to defects in ABCB7

**Clinical presentation and diagnosis**
- In male patients presenting with the combination of a mild microcytic anemia and ataxia, a defect in ABCB7 should be considered.
- Increased protoporphyrin IX concentrations in red blood cells are suggestive of this disorder.

**Treatment**
- Treatment of (mild) anemia is not indicated.

**Family screening**
- The proband should be informed about the X-linked inheritance pattern of anemia and ataxia due to ABCB7 defects. Brothers of the proband may also be affected. Sons of the proband are not affected. Daughters of the proband are obligate carriers of the relevant ABCB7 defect and have no or a mildly anemic clinical phenotype.
- We recommend to offer screening for the ABCB7 mutation: the mother and sisters (for carriership) and the brothers. The spouse should only be checked in case of consanguinity and reproductive age. Daughters of the proband should be offered to be checked for carriership.

#### 3C. X-linked sideroblastic anemia due to defects in ALAS2

**Clinical presentation and diagnosis**
- XLSA due to an ALAS2 defect should be considered in patients of both gender and of all ages with pyridoxine responsive or unresponsive (mild) microcytic sideroblastic anemia with or without iron loading and in patients with unexplained iron loading.
- In patients suspected for XLSA, iron parameters (ferritin, TSAT) should be checked to detect iron loading, as well as liver enzymes, and signs of liver fibrosis or hepatocellular carcinoma.
- In case of elderly patients presenting with MDS-RARS or MDS-RCMD without specific cytogenetic abnormalities, the presence of ALAS2 defects should be considered, especially if the anemia is microcytic.

**Treatment**
- Management of patients with XLSA should involve treatment of anemia, and prevention and treatment of iron overload.
- Initial treatment with pharmacological doses pyridoxine (50-200 mg/day) is recommended. Occasionally high doses (up to 300 mg/day) in heavy, active or elderly may be considered.
- In case of pyridoxine responsiveness, lifelong supplementation of pyridoxine 10-100 mg daily is recommended.
- Once a response is obtained evidence suggests the life-long maintenance dose may be lowered to 10-100 mg/day, since too high doses may give neurotoxicity.
- Iron loading should be treated, preferably by phlebotomies.

**Family screening**
- The proband should be informed about the X-linked inheritance pattern of anemia and/or iron overload due to ALAS2 defects. Brothers of the proband may be also affected. Sons of the proband are not affected.
- Daughters of the proband are obligate carrier of the relevant ALAS2 defect.
- We recommend screening for the ALAS2 mutation: the brothers and also the mother, sisters and daughters (for carriage and for the reason that women may develop a XLSA phenotype later in life). The spouse should only be checked in case of consanguinity and reproductive age.
- Female carriers and male hemizygous individuals should be screened for the XLSA phenotype.

### 3D. Sideroblastic anemia due to defects in GLRX5

**Clinical presentation and diagnosis**
- In patients presenting with microcytic sideroblastic anemia and iron loading (among others) defects in GLRX5 should be considered.
- In case of microcytic sideroblastic anemia without ALAS2, or SLC25A38 defects a lymphoblastic culture should be considered. Decreased activity of mitochondrial acitonase and succinate dehydrogenase (complex I-IV) as a manifestation of a defective Fe-S cluster synthesis, is suggestive for a GLRX5 defect.

**Treatment**
- In patients with sideroblastic anemia and iron loading due to GLRX5 defects, monitoring of the iron status and possible complications of iron overload is recommended. Iron loading should be treated with chelation therapy. Severe anemia with blood transfusions.

**Family screening**
- Recommendations are identical to those described in 1C.

### 3E. Erythropoietic ProtoPorphyria due to defects in FECH and gain-of-function mutations in ALAS2 (XLDPP)

**Clinical presentation and diagnosis**
- In patients with an unexplained mild normo- to microcytic anemia, a low to normal serum ferritin and cutaneous protoporphyria, a painful photosensitivity in childhood, EPP has to be considered, both in men and women.
- The diagnosis EPP should be confirmed by fluorescent erythrocytes in an unstained smear, and/or enhanced protoporphyrin in plasma and/or erythrocytes.
- To prove autosomal recessive EPP FECH activity should be measured or FECH mutations determined.
- In case EPP is not explained by the FECH genotype, the ALAS2 gene should be investigated for the presence of gain-of-function mutations.

**Treatment**
- In case anemia in EPP is present, it is mild and treatment is not recommended.

**Family screening**
- Because of complex genetics, influenced by genetic background, we recommend referral to a clinical geneticist in case of EPP. Family screening for a proband with XLDPP due to and ALAS2 gain-of-function mutation should initially include phenotyping and genotyping of all first degree family members (including women): mother in case of a male proband, both parents in case of a female proband, siblings and children.

### 3F. Congenital Erythropoietic Porphyria (CEP) due to defects in UROS or GATA1

**Clinical presentation and diagnosis**
- In patients with an unexplained hemolytic anemia in combination with a painful cutaneous photosensitivity autosomal recessive CEP has to be considered, both in men and women.
- In cases with combined cutaneous photosensitivity and severe microcytic, hypochromic hemolytic anemia X-linked CEP should be considered.
- Since disease severity and onset of first symptoms is highly variable, CEP should be considered both in children and adults in case of the above-mentioned phenotype.
- Diagnosis should be based on increased urinary levels of uroporphyrin I and coproporphyrin I and
confirmed by decreased UROsynthase activity in the erythrocytes or the presence of pathogenetic homozygous or compound heterozygous mutations in \textit{UROS} or in \textit{GATA1}.

\textbf{Treatment}

\begin{itemize}
  \item In patients with CEP, allogenic HSCT should be considered as the only curative treatment
  \item Chronic erythrocyte transfusions is recommended as a symptomatic treatment, and iron chelation is recommended according to guidelines for chronic transfusions.
\end{itemize}

\textbf{Family screening*}

\begin{itemize}
  \item Recommendations for CEP due to UROS defects are identical to those described in 1C.
  \item Recommendations are identical to XLDPP due to gain-of-function \textit{ALAS2} mutations, see 3E.
\end{itemize}

*, Recommendations on family screening are based on Borry et al\textsuperscript{99}, Godard et al\textsuperscript{100}
### Table 2. Overview of characteristics of rare microcytic anemias due to genetic disorders of iron metabolism or heme synthesis.

<table>
<thead>
<tr>
<th>Pathophyisis</th>
<th>Low iron availability for erythropoiesis</th>
<th>Defect in iron acquisition of erythroid progenitor cells</th>
<th>Defect in heme synthesis or iron-sulfur cluster biogenesis</th>
<th>Erythropoietin protoporphyria</th>
<th>Congenital Erythropoietic Porphyria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disorder</td>
<td>IRIDA</td>
<td>Ferroportin disease&lt;sup&gt;†&lt;/sup&gt;</td>
<td>Aceruloplasmiaemia</td>
<td>Hypotransferrinemia</td>
<td>Microcytic anemia with iron loading</td>
</tr>
<tr>
<td>Gene</td>
<td>TMPRSS6</td>
<td>SLC40A1</td>
<td>CP</td>
<td>TF</td>
<td>DMT1</td>
</tr>
<tr>
<td>Protein</td>
<td>matriptase-2</td>
<td>ferroportin-1</td>
<td>ceruloplasmin</td>
<td>transferrin</td>
<td>DMT1</td>
</tr>
<tr>
<td>Patients desc.</td>
<td>20-100</td>
<td>&gt;200</td>
<td>5-20</td>
<td>5-20</td>
<td>&lt;5 (&lt;3)</td>
</tr>
<tr>
<td>Inheritance</td>
<td>AR&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>AD</td>
<td>AR/AD</td>
<td>AR</td>
<td>AR/AD&lt;sup&gt;‡&lt;/sup&gt;</td>
</tr>
<tr>
<td>Age at presentation</td>
<td>child</td>
<td>adult</td>
<td>40-50 yrs</td>
<td>variable</td>
<td>child</td>
</tr>
<tr>
<td>Symptoms -neurological -skin</td>
<td>no</td>
<td>no</td>
<td>yes</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>Anemia</td>
<td>variable</td>
<td>mild in 10%&lt;sup&gt;++&lt;/sup&gt;</td>
<td>mild</td>
<td>variable</td>
<td>variable</td>
</tr>
<tr>
<td>MCV</td>
<td>micro</td>
<td>normo</td>
<td>micro/ normo</td>
<td>micro</td>
<td>micro</td>
</tr>
<tr>
<td>Ring sideroblasts</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>Iron loading Ferritin</td>
<td>no normal-low</td>
<td>yes high</td>
<td>yes high</td>
<td>yes high</td>
<td>variable&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td>Transferrin saturation</td>
<td>&lt;10%</td>
<td>normal/ high&lt;sup&gt;†&lt;/sup&gt;</td>
<td>normal/ low</td>
<td>100%</td>
<td>high</td>
</tr>
<tr>
<td>Hepcidin</td>
<td>high&lt;sup&gt;†&lt;/sup&gt;</td>
<td>variable</td>
<td>no data</td>
<td>low</td>
<td>normal/low</td>
</tr>
<tr>
<td>Treatment</td>
<td>oral iron iv iron/EPO&lt;sup&gt;†&lt;/sup&gt;</td>
<td>phlebotomy</td>
<td>**</td>
<td>chelation</td>
<td>eryth apoTF plasma chelation phlebotomy</td>
</tr>
</tbody>
</table>

EPO, erythropoietin; erytx, erythrocyte transfusion; TF, transferrin; HSCT, hematopoietic stem cell transplantation; AD, Autosomal Dominant; AR, Autosomal Recessive;<sup>†</sup> both loss-of-function and gain-of-function have been described, data in column reflect those of the combined group unless stated otherwise;<sup>‡</sup> loss-of-function;<sup>§</sup> gain-of-function;<sup>†</sup> also autosomal dominant inheritance pattern described;<sup>††</sup> heterozygous pathogenic mutation in combination with decreased expression of normal allele;<sup>§</sup> in some families only women affected since the defect is lethal in man;<sup>#</sup> gonadal dysfunction;<sup>**</sup> neurologic symptoms manifest in childhood, anemia may develop later in life (young adolescent);<sup>+++</sup> in case of loss-of-function mutations, anemia is more likely to occur and TSAT is lower;<sup>‡</sup> anemia resolves by pyridoxine treatment in most XLSA patients;<sup>b</sup> MCH decreased;<sup>c</sup> liver iron loading has been described demonstrated by MRI and liver biopsy, even if serum ferritin is normal;<sup>d</sup> iron loading may be secondary to erythrocyte transfusion;<sup>e</sup> hepcidin increased in relation to iron parameters, hepcidin/TSAT ratio > upper limit of reference range in absence of inflammation;<sup>f</sup> measured after treatment with transfusions;<sup>g</sup> only 1 human study available on EPO.
Legends

Figure 1. Cells and proteins involved in iron homeostasis and heme synthesis

a) The enterocyte: iron enters the body through the diet. Most iron absorption takes place in the duodenum and proximal jejunum. The absorption or iron takes place in different phases. In the luminal phase iron is solubilized and converted from trivalent iron into bivalent iron by duodenal cytochrome B (DcytB). During the mucosal phase iron is bound to the brush border and transported into the mucosal cell by the iron transporter dimetaltransporter (DMT1). In the cellular phase iron is either stored in cellular ferritin or transported directly to the opposite side of the mucosal side. In the last phase of iron absorption Fe\textsuperscript{2+} is released into the portal circulation by the basolateral cellular exporter ferroportin. Enterocytic iron export requires hephaestin, a multicopper oxidase homologous to ceruloplasmin, which oxidases Fe\textsuperscript{2+} to Fe\textsuperscript{3+} for loading onto transferrin. This cellular efflux of iron is inhibited by the peptide hormone hepcidin by binding to ferroportin and subsequent degradation of the ferroportin-hepcidin complex.

b) The hepatocyte: serves as the main storage for the iron surplus (most body iron is present in erythrocytes and macrophages). Furthermore this cell, as the main producer of hepcidin, largely controls the systemic iron regulation. The signal transduction pathway runs from the membrane to the nucleus, where bone morphogenetic protein (BMP) receptor, the membrane protein hemoujuelin (HJV), the HFE protein and transferrin receptor (TfR) -1 and -2, and matriptase-2 play an essential role. Through intracellular pathways, a signal is given to hepcidin transcription. The membrane associated protease matriptase-2 (encoded by TMPRSS6), detects iron deficiency and blocks hepcidin transcription by cleaving HJV.

c) The macrophage: belongs to the group of reticulo-endothelial cells and breaks down senescent red blood cells. During this process iron is released from heme proteins. This iron can either be stored in the macrophage as hemosiderin or ferritin, or may be delivered to the erythroid progenitor as ingredient for new erythrocytes. The iron exporter ferroportin is
responsible for the efflux of \( \text{Fe}^{2+} \) into the circulation. In both hepatocytes and macrophages this transport requires the multicopper oxidase ceruloplasmin (CP), which oxidases \( \text{Fe}^{2+} \) to \( \text{Fe}^{3+} \) for loading unto transferrin.

d) The erythroid progenitor: transferrin saturated with 2 iron molecules is endocytosed via the transferrin receptor 1 (TfR1). After endocytosis the iron is released from transferrin, converted from \( \text{Fe}^{3+} \) to \( \text{Fe}^{2+} \) by the ferroreductase STEAP3, and transported to the cytosol by DMT1, where it is available mainly for the heme synthesis. Erythropoiesis has been reported to communicate with the hepatocyte by the proteins TWSG1, GDF15 and erythroferrone (Erfe) that inhibit signaling to hepcidin.\(^{11,27,101}\)

e) The mitochondria of the erythroid progenitor: in the mitochondria the heme synthesis and iron-sulfur cluster (Fe-S clusters) synthesis takes place. In the first rate-limiting step of heme synthesis, 5-aminolevulinic acid (ALA) is synthesized from glycine and succinyl-CoA by the enzyme delta-aminolevulinic acid synthase (ALAS2) in the mitochondrial matrix. The protein SLC25A38 is located in the mitochondrial membrane and is probably responsible for the import of glycine into the mitochondria and might also export ALA to the cytosol. In the heme synthesis pathway, the uroporphyrinogen III synthase (UROS) in the cytosol is the fourth enzyme. It is responsible for the conversion of hydroxymethylbilane (MHB) to uroporphyrinogen III, a physiologic precursor of heme. In the last step, ferrochelatase (FECH) located in the mitochondrial intermembrane space is responsible for the last step, i.e. the incorporation of \( \text{Fe}^{2+} \) in protoporphyrin IX (PPIX) to form heme. GATA binding factor I (GATA 1) is critical for normal erythropoiesis, globin gene expression and megakaryocyte development and among others regulates expression of UROS and ALAS2 in erythroblasts. The enzyme glutaredoxin-5 (GLRX5) plays a role in the synthesis of the Fe-S clusters, which are transported to the cytoplasm, probably via the transporter ABCB7.

Figure adapted from van Rooijen et al.\(^{2}\) Professional illustration created by Debra T. Dartez.
Figure 2. Diagnostic flow chart for microcytic anemias caused by inherited defects in iron metabolism or heme synthesis.

Genes are given in italics and refer to the disorders of this review (Table 2). After clinical and laboratory assessment clinicians may proceed the diagnostic work-up with either bone marrow smears or gene analysis. Iron parameters should be interpreted in the context of the age of the patient and the given treatment: older patients are more likely to have developed iron overload (increased TSAT and ferritin) due to increased and ineffective erythropoiesis and iron supplementation and transfusions. Note that for some diseases the decision tree is based on only few patients (Table 2).

Neur. Dis, neurologic disease; DM, diabetes mellitus; FPP, free protoporphyrin; ZnPP, zinc protoporphyrin; TSAT, transferrin saturation; BMRS, bone marrow ring sideroblasts

*, patients have normocytic anemia, gain-of-function mutation; +, only 1 family described; ±, patients have normocytic anemia and the majority has loss-of-function mutations; ^, in rare cases these patients present with elevated ferritin levels.
Figure 1.
Figure 2.
Practice guidelines for the diagnosis and management of microcytic anemias due to genetic disorders of iron metabolism or heme synthesis

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