Iron-Refractory Iron Deficiency Anemia (IRIDA)

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KEYWORDS

• Iron-refractory iron deficiency anemia • Inherited iron deficiency • Hepcidin
• *TMPRSS6* • Matriptase-2

KEY POINTS

- Iron-refractory iron deficiency anemia (IRIDA) is an inherited disorder of systemic iron balance in which both absorption and utilization of iron are impaired.
- Patients with IRIDA show iron deficiency anemia that is refractory to oral iron therapy but partially responsive to parenteral iron.
- IRIDA is caused by mutations in the gene *TMPRSS6*.
- *TMPRSS6* encodes matriptase-2, a transmembrane serine protease expressed by the liver that regulates the production of the iron regulatory hormone hepcidin.
- Studies conducted in tissue culture systems and mouse models have enhanced our understanding of the underlying pathogenesis.

INTRODUCTION

Iron is an essential metal for many biologic processes in mammals. Its primary role is to bind oxygen in the heme moiety of hemoglobin. Iron also plays a central role in the enzymatic transfer of electrons performed by cytochromes, peroxidases, ribonucleotide reductases, and catalases. This reactivity of iron also has the potential to cause damage to biologic systems if iron is "free" and not bound and transported by a finely regulated and complex system of proteins that maintain iron homeostasis.

Under normal physiologic conditions in the adult male, only 1 to 2 mg of the 20 to 25 mg of iron required daily to maintain erythropoiesis enters the body through carefully regulated intestinal absorption.[1] Most of the daily iron need is derived from the recycling of erythroid iron through phagocytosis of senescent red cells by

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reticuloendothelial macrophages and degradation of hemoglobin. As humans have no physiologically regulated mechanism for excreting iron from the body, control of iron balance occurs almost entirely at the level of intestinal absorption.

Virtually all plasma iron exists bound to the circulating glycoprotein transferrin (TF), which allows the iron to remain soluble, renders iron nonreactive, and facilitates its cellular import through the transferrin cycle.[2] Iron-loaded plasma transferrin binds to transferrin receptors (TFR) on the cell surface. The TF/TFR receptor complex is endocytosed, and acidification of the endosome results in the release of iron from TF. The iron is transported out of the endosome into the cytoplasm, and the empty TF and TFR return to the cell surface and are released into the plasma to repeat this cycle.

Most nonerythroid intracellular iron is stored in hepatocytes and macrophages in the form of ferritin, a multimeric iron storage protein whose structure facilitates iron bioavailability in response to cellular need. Intracellular iron, either absorbed by the duodenal enterocyte or liberated by macrophages from heme recycling, is either stored as ferritin or exported into the plasma by ferroportin.[3] Ferroportin, which is the sole known mammalian cellular iron exporter, is highly expressed on the basolateral membrane of enterocytes and on the cell membrane of reticuloendothelial macrophages.

Iron homeostasis requires carefully coordinated regulation of intestinal iron absorption, cellular iron import/export, and iron storage. Hepcidin, a small circulating peptide released by the liver, is the master regulator of systemic iron balance. Hepcidin limits both iron absorption from the intestine and iron release from macrophage stores by binding to ferroportin and triggering ferroportin's internalization and degradation. Hepcidin expression is modulated in response to several physiologic and pathophysiological stimuli, which include systemic iron loading, erythropoietic activity, and inflammation.[4]

As is the case with many physiologic processes, spontaneous mutations leading to disease in animals and humans have revealed much about the normal regulation of iron transport and storage in humans. In particular, the identification of *TMPRSS6* as the gene mutated in cases of iron-refractory iron deficiency anemia (IRIDA), has increased our knowledge of the molecular mechanisms that regulate hepcidin expression.

CLINICAL PRESENTATION

In 1981, Buchanan and Sheehan[5] described 3 siblings with iron deficiency anemia despite adequate dietary iron intake and no evidence of gastrointestinal blood loss. All 3 failed to respond to oral ferrous sulfate therapy. In 2 of the siblings, a formal oral iron "challenge" (see **Box 2**) to assess for impaired intestinal iron absorption failed to show evidence of a rise in serum iron 2 hours after the oral administration of 2 mg/kg elemental iron as ferrous sulfate. Following intramuscular injection of iron dextran, the 3 siblings also showed only a partial hematological response assessed by hemoglobin and red cell indices. In addition, although intramuscular iron administration raised the serum ferritin level, suggesting restoration of iron stores, the patients nevertheless remained hypoferremic. The investigators postulated that the phenotype was explained in part by an inherited, iron-specific absorptive defect, which was further compounded by a defect of iron utilization reflected in the partial response to parenteral iron therapy.

Pearson and Lukens[6] subsequently described 2 affected siblings. In addition to recognizing the intestinal iron uptake defect reflected in the failed response to oral iron challenge, these investigators also documented a discordance in the rate of

decline of transferrin saturation (rapid) and serum ferritin (slower) after iron dextran administration. Given that iron dextran must be phagocytosed and "recycled" by macrophages before the iron can be made available for erythropoiesis, the investigators postulated that a macrophage iron retention phenotype/macrophage iron recycling defect contributed to the phenotype.

Further cases of familial iron deficiency anemia with similar clinical presentations were subsequently reported, which provided additional insight into the mode of genetic transmission as well as the underlying pathophysiological defect.[7–11] Brown and colleagues[8] reported 2 affected female siblings of Northern European ancestry whose parents exhibited normal hematological parameters, thus suggesting a recessive mode of transmission for the disorder. Galanello and colleagues[11] provided additional evidence for autosomal recessive transmission in a large kindred that originated in a small village in southern Sardinia; the structure of this pedigree, which contained 5 affected individuals, suggested that the disorder might be caused by homozygosity for a mutation that arose in a common ancestor. Hartman and Barker[9] reported an affected African American sibling pair in whom bone marrow biopsies performed after parenteral iron therapy failed to demonstrate normal sideroblasts (erythroid normoblasts containing stainable nonhemoglobin iron in the cytoplasm), despite the presence of stainable iron in bone marrow macrophages. This observation thus provided evidence in support of the iron utilization defect that had been postulated by Buchanan and Sheehan.[5]

Review of these case reports identified several unifying features that suggested that these cases represented the same underlying disorder, a condition that has been termed iron-refractory iron deficiency anemia (IRIDA).[12] These key features of IRIDA include (**Box 1**) (1) congenital hypochromic, microcytic anemia (hemoglobin 6–9 g/dL); (2) very low mean corpuscular volume (45–65 fL); (3) very low transferrin saturation (<5%); (4) abnormal oral iron absorption (as indicated by a lack of hematological improvement following treatment with oral iron or failure of an oral iron challenge); (5) abnormal iron utilization (as indicated by a sluggish, incomplete, and transient response to parenteral iron); and (6) an inheritance pattern compatible with autosomal recessive transmission. In these cases, acquired causes of iron deficiency (eg, gastrointestinal blood loss) and inherited causes of microcytosis (eg, thalassemias, lead toxicity) were excluded by extensive laboratory testing. Furthermore, no case showed clinical evidence of a chronic inflammatory disorder or generalized

Box 1
Key clinical features and typical laboratory data from untreated IRIDA probands at diagnosis in childhood

- Lifelong/congenital, usually presents in childhood
- Severe microcytosis (mean corpuscular volume 45–65 fL)
- Moderate/severe anemia (hemoglobin 6–9 g/dL)
- Severe hypoferremia with very low transferrin saturation (<5%)
- No or minimal response to oral iron supplementation
- Abnormal oral iron absorption/failure of an oral iron challenge
- Incomplete and transient response to parenteral iron
- Autosomal recessive transmission
- Anemia often ameliorates into adulthood, although hypoferremia persists

intestinal malabsorptive defect. Hemoglobinopathies and sideroblastic anemias were excluded as potential causes of the microcytosis by hemoglobin electrophoresis and bone marrow examinations, respectively.

In the published IRIDA cases, subjects were generally healthy and growing normally, and the anemia was typically detected during routine screening usually conducted before the age of 2 years. Thus, from the clinical histories, it was not known if patients with IRIDA were already iron-deficient at birth. Of note, the proband reported by Brown and colleagues,[8] who was diagnosed with microcytic anemia at age 9 months, showed plentiful reticuloendothelial iron stores on an initial bone marrow examination performed after a 3-month failed course of oral iron therapy but showed an absence of stainable iron on repeat bone marrow examination performed at age 4 years after a course of intramuscular iron. These findings, along with reports of normal birth weights for IRIDA patients,[10] raise the possibility that in utero iron transfer may be normal in these patients, with the depletion of iron stores occurring only after birth.[13]

For unclear reasons, the clinical signs and symptoms of IRIDA appear distinct from severe acquired iron deficiency anemia. Although IRIDA subjects have laboratory evidence of severe iron deficiency, clinical signs observed in acquired iron deficiency have been noted inconsistently in the reported IRIDA cases. Moderate to severe pallor was described in the kindred reported by Melis and colleagues.[14] The 18-month-old proband reported by Andrews[10] was described as pale with dry skin. Studying the kindred originally reported by Brown and colleagues,[8] Pearson and Lukens[6] noted that the affected siblings developed angular cheilitis (crusted, painful lesions at corners of their mouths) that receded after intravenous iron therapy. Only rarely are signs and symptoms associated with iron deficiency, such as koilonychias or hair loss, described in IRIDA.[15] Although IRIDA has been considered a rare clinical entity based on the small number of cases reported in the literature, it is possible that in the absence of routine laboratory screening for anemia, many cases never come to clinical attention because of the normal growth and development of the affected individuals. Remarkably, despite congenital, severe iron deficiency, long-term follow-up of the affected subjects has shown normal growth and normal intellectual development,[6,14] with no evidence of the cognitive concerns on which iron deficiency screening in infancy have been founded.[16]

Given the small number of reported cases, experience with the natural history and long-term management of IRIDA is, at present, limited. Pearson and Lukens[6] proposed a treatment regimen that involved the parenteral administration of iron dextran every 2 to 4 years, or when serum ferritin levels fell below 50 to 75 ng/mL or the mouth ulcerations observed in their patients recurred. Hartman and colleagues[17] described the course of 5 patients with IRIDA who had been followed for 15 years. They noted that repeated iron infusions that elevated the serum ferritin to levels greater than 200 ng/mL resulted in considerable improvement in both the anemia and microcytosis. Although the serum iron and transferrin saturation occasionally reached the normal range, the patients generally developed recurrent hypoferremia. With the cessation of iron infusions, microcytosis returned, but not to the severe degree present in infancy. In the affected family members studied by Galanello and colleagues (who on last report range from 18 to 48 years of age), the anemia was more severe during childhood, requiring intermittent intravenous iron administration. However, hemoglobin levels of 10.0 to 13.9 g/dL were maintained in the adult affected subjects, although laboratory findings of iron-restricted erythropoiesis (low mean corpuscular volume, low mean corpuscular hemoglobin, low serum iron, low transferrin saturation) persisted. In addition to a relative amelioration of anemia into adulthood, serum ferritin levels also appeared to rise with age in this kindred. The investigators suggested that

the increased severity of the anemia during childhood could indicate the greater iron demands for body growth and for the accompanying expansion of the red cell mass that occurs during this period; in adulthood, however, a larger proportion of the limited iron available could be used in erythropoiesis.[14]

GENETICS

Strong evidence that the IRIDA phenotype has an inherited basis was obtained through genetic characterization of a large, consanguineous kindred from Sardinia. In this kindred, in which disease in affected individuals could be attributed to homozygosity for a mutation arising in a common ancestor, the IRIDA phenotype mapped to the long arm of chromosome 22 (22q12.3–13.1) under a model of recessive inheritance.[18] IRIDA subsequently was shown to be caused by mutations in the gene *TMPRSS6*,[12,14] which resides within this critical region of chromosome 22q and for which a key role in iron balance had recently been revealed through study of the orthologous gene in mice (see later in this article).[19] Notably, patients with the IRIDA phenotype showed levels of hepcidin in their serum, plasma, and/or urine that were indicative of impaired hepcidin regulation.[12,14,20] Although hepcidin levels are normally reduced in response to systemic iron deficiency (an adaptive response to promote absorption of dietary iron),[21,22] patients with IRIDA displayed hepcidin levels that were either within or above the reference range. Given the known ability of hepcidin to limit ferroportin-dependent iron export from enterocytes and macrophages,[4] the inappropriately elevated hepcidin levels in IRIDA provide insight into the iron refractory features of the disorder. Specifically, the inappropriate hepcidin excess in IRIDA can explain (1) the development of systemic iron deficiency as a result of impaired absorption of dietary iron, (2) the failure to achieve a hematological response to oral iron therapies, and (3) the sluggish and incomplete utilization of parental iron formulations, which consist of iron-carbohydrate complexes that require processing by macrophages before the iron can be used in erythropoiesis. In many respects, IRIDA can be considered the pathophysiologic and phenotypic opposite of hereditary hemochromatosis, in which the "uncoupling" of appropriate hepcidin expression from the sensing of iron stores results in an inappropriate hepcidin "deficiency" (see the article by Brissot, elsewhere in this issue).

TMPRSS6 (transmembrane protease, serine 6) encodes matriptase-2, a membrane-spanning protease that is primarily expressed by the liver.[23] Matriptase-2 is a member of the type II transmembrane serine protease (TTSP) family, a group that is anchored to the membrane at their amino termini. The protein name, matriptase-2, reflects structural homology to another TTSP, matriptase-1. Matriptase-2 contains a large extracellular region containing several structural domains, including an SEA (sea urchin sperm protein, enteropeptidase, agrin) domain, 2 CUB (C1r/C1s, urchin embryonic growth factor, bone morphogenetic protein 1) domains, 3 LDLRA (low-density lipoprotein receptor class A) domains, and a C-terminal catalytic domain containing a classic catalytic triad of serine, histidine, and aspartic acid residues (**Fig. 1**). Matriptase-2 is believed to be synthesized as an inactive, membrane-bound, single-chain polypeptide that undergoes a complex series of proteolytic cleavage events during zymogen activation.[24] When overexpressed in cultured cells, matriptase-2 localizes to the plasma membrane[23] and is shed from the cell surface as an activated, 2-chain form.[25] Recombinant matriptase-2 has been shown in vitro to degrade components of the extracellular matrix and basement membrane, such as fibrinogen, fibronectin, and type I collagen.[23] Interestingly, overexpression of matriptase-2 in breast and prostate cancer cell lines can reduce their

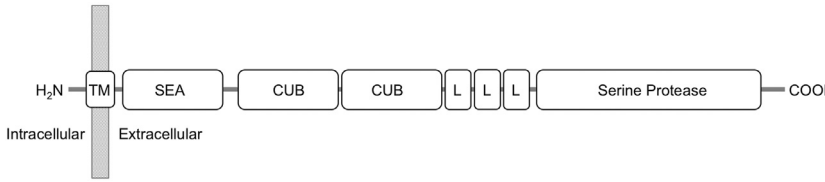

Fig. 1. Schematic representation of the domain structure of matriptase-2. Labeled are the transmembrane (TM), complement factor C1r/C1s, urchin embryonic growth factor, and bone morphogenetic protein (CUB), LDL-receptor class A (L), and serine protease domains.

invasive properties in vitro, suggesting a possible role for matriptase-2 in cancer development and progression.[26]

A key role for *TMPRSS6* in iron homeostasis was first revealed through elucidation of the genetic basis of a chemically induced, recessive mutant mouse phenotype termed *mask*.[19] The *mask* mutant received its name because it showed progressive loss of truncal hair but retained hair on the head. Notably, mice with the *mask* phenotype also exhibited microcytic anemia, low plasma iron levels, and low iron stores when raised on a standard rodent laboratory diet. Furthermore, *mask* mice showed evidence of defective hepcidin regulation in the setting of iron deficiency. Although control mice suppressed hepatic hepcidin production in response to a low-iron diet (an appropriate physiologic response to promote iron absorption), hepcidin messenger RNA (mRNA) levels in livers of *mask* mutants were inappropriately elevated. Genetic mapping of the underlying mutation revealed that *mask* mice were homozygous for a mutation that resulted in defective splicing of the *Tmprss6* transcript, which eliminated the proteolytic domain of matriptase-2. The elevated hepcidin mRNA levels detected in the livers of the *mask* mutants suggest that the normal function of the *Tmprss6* gene product, matriptase-2, is to lower hepcidin expression by the liver.

A *Tmprss6* knockout (*Tmprss6*$^{-/-}$) mouse, generated by standard gene-targeting techniques, exhibited a phenotype very similar to the *mask Tmprss6* splicing mutant, including the key feature of hepatic hepcidin overexpression.[27] Notably, the anemia and alopecia phenotypes of both the engineered and the chemically induced *Tmprss6* mutants could be rescued by iron administration.[19,27] Consistent with the known ability of hepcidin to promote ferroportin internalization and degradation, duodenal enterocytes of *Tmprss6*$^{-/-}$ mice showed decreased ferroportin protein expression in the basolateral membrane that was accompanied by histologic evidence of iron retention within these cells.[27] Thus, in the setting of hepcidin elevation, when basolateral export of iron into the plasma is restricted, iron accumulates within duodenal enterocytes and is ultimately lost from the body when these cells are shed into the gut lumen.

Studies conducted in tissue culture systems and transgenic models have begun to shed insight into the mechanism by which matriptase-2 regulates hepcidin production. This mechanism appears to involve modulation of bone morphogenetic protein (BMP)/SMAD signaling, a key signal transduction pathway that promotes hepcidin transcription in hepatocytes (**Fig. 2**). BMPs are secreted ligands of the transforming growth factor β superfamily that interact with type 1 and type 2 BMP receptors at the cell membrane to trigger the phosphorylation of multiple receptor-associated SMAD proteins (SMAD1, SMAD5, SMAD8). Once phosphorylated, these receptor-associated SMADs bind to a common mediator, SMAD4, forming heterodimeric complexes that translocate to the nucleus to regulate transcription of BMP target genes, including the gene encoding hepcidin, by binding to specific elements in the promoters of genes.[28] Of note, signaling through the BMP pathway is modulated in

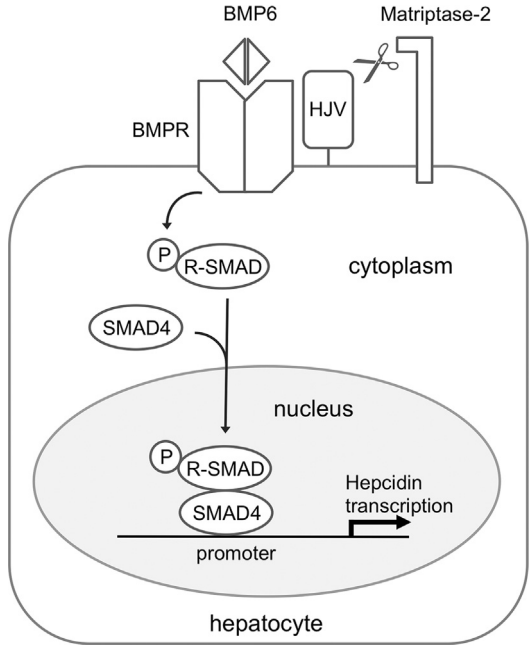

Fig. 2. Model of hepcidin regulation by matriptase-2. The binding of BMP ligands to BMP receptor complexes (BMPRs) at the hepatocyte plasma membrane initiates an intracellular signaling cascade that promotes hepcidin transcription. BMP6 appears to be the particular BMP family ligand that plays a key role in initiating this signaling in vivo. BMP6 binding to BMPRs induces phosphorylation (P) of receptor-associated SMAD proteins (R-SMADs). Once phosphorylated, R-SMADs form a complex with the common mediator SMAD4. This SMAD complex then translocates to the nucleus to bind specific target elements in the promoter of the hepcidin gene to increase hepcidin expression. The *TMPRSS6* gene product, matriptase-2, dampens signal transduction through this pathway by cleaving hemojuvelin (HJV), a BMP co-receptor, from the plasma membrane.

response to hepatic iron stores. When local iron stores increase, the liver raises expression of BMP6, the particular BMP ligand that appears to play a key role in promoting hepcidin transcription. This leads to increased hepcidin expression, an adaptive response to limit further iron absorption from the diet.[29–31]

Matriptase-2 has been shown to inhibit BMP signaling, and thus hepcidin transcription, by cleaving a glycosylphosphatidylinositol-linked protein termed hemojuvelin from the cell membrane.[32] Hemojuvelin, which functions as a co-receptor for BMP ligands,[33] plays a key role in promoting hepatic BMP signaling, as evidenced by the fact that loss-of-function mutations in the hemojuvelin gene result in juvenile hemochromatosis, a severe form of hereditary iron overload that is associated with inappropriately low levels of hepcidin.[34] In cultured cells, the ability of recombinant matriptase-2 to cleave hemojuvelin has been shown to be impaired by missense mutation of the matriptase-2 catalytic domain and to be abolished by a matriptase-2 truncating mutation that eliminates the catalytic domain entirely.[32] Additionally, mice with genetic disruption of *Tmprss6* show low hepatic iron stores accompanied by up-regulated hepatic expression of Bmp target genes, phenotypic features that are dependent on the presence of both the Bmp6 ligand and the Bmp co-receptor hemojuvelin.[35–37] Collectively, these findings suggest that the hepcidin elevation observed in patents with the

IRIDA phenotype results from an inability to appropriately down-regulate hepatic BMP signaling in the context of low hepatic iron stores.

Given that the presence of functional matriptase-2 appears to prevent hepcidin overexpression, it has been proposed that changes in matriptase-2 protein levels or protein activity may serve as a means to regulate hepcidin production. Indeed, in studies conducted in cultured cells and/or animal models, a variety of stimuli with known capacity to modulate hepcidin expression have been found capable of modulating *TMPRSS6* mRNA and/or matriptase-2 protein levels. The stimuli include hypoxia,[38,39] acute dietary iron restriction,[40] chronic dietary iron loading,[41] BMP6 injection,[41] and inflammation.[42] Future studies may elucidate how these various stimuli interact to collectively orchestrate matriptase-2 expression.

To date, at least 45 different *TMPRSS6* mutations have reported in individuals with the IRIDA phenotype. These include 20 missense mutations, 5 nonsense mutations, 10 frameshift mutations, 1 large in-frame deletion, and 9 intronic mutations predicted to disrupt normal splicing.[12,14,15,20,43–56] Most of the reported mutations are unique to single families, whereas a small number have been found to recur in 2 or more kindreds. Mutations have been identified in kindreds from a range of ethnic backgrounds, without evidence for a significant founder effect. Many of the *TMPRSS6* mutations detected in patients with the IRIDA phenotype are predicted to impair matriptase-2 proteolytic activity. For example, some pathogenic mutations generate truncated or aberrantly spliced *TMPRSS6* transcripts, whereas others introduce missense substitutions in the catalytic domain. *TMPRSS6* missense mutations are not restricted to the proteolytic domain, however, and functional analyses have revealed how missense substitutions in other matriptase-2 domains can also ultimately result in impaired hemojuvelin cleavage activity. For example, missense mutations in the second LDLRA domain have been shown to impair matriptase-2 trafficking to the plasma membrane,[43] whereas a missense mutation in the SEA domain has been shown to impair activation of the protease.[15]

TMPRSS6 mutations are routinely sought by polymerase chain reaction–based DNA sequencing, an approach that typically examines all coding regions (ie, exons and intron/exon boundaries) of a gene. To date, most individuals who exhibit the IRIDA phenotype have been found to possess either 2 different *TMPRSS6* mutations in compound heterozygosity (ie, inherited from different parents) or a single mutation in homozygous form. However, affected individuals from several unrelated kindreds also have been reported who have each been found to harbor only a single, heterozygous *TMPRSS6* mutation.[12,53] It is possible that such individuals may harbor a second mutation on the other *TMPRSS6* allele in an unanalyzed noncoding region that is important for *TMPRSS6* gene regulation (such as an intronic or promoter region), but this has yet to be demonstrated. In some reported kindreds, microcytic anemia has been observed in the parent of a child exhibiting the classic IRIDA phenotype, raising the possibility that heterozygosity for *TMPRSS6* mutation may increase the susceptibility to iron deficiency anemia in some settings.[52] Indeed, in mice that are heterozygous for *Tmprss6* mutation, systemic iron homeostasis is mildly compromised.[36,57]

To date, *TMPRSS6* is the only gene in which mutations are known to result in the IRIDA phenotype. Although *TMPRSS6* genotype-phenotype correlations in IRIDA have not yet been extensively studied, some investigators have noted a tendency toward lower hemoglobin, lower erythrocyte mean corpuscular volume, and lower serum transferrin saturation in affected individuals harboring 2 nonsense mutations compared with those harboring either 2 missense mutations or 1 missense and 1 nonsense mutation.[13] Notably, although the IRIDA phenotype associated with *TMPRSS6* mutations was originally defined to include the inability to respond to oral

iron therapy,[12] several individuals with biallelic *TMPRSS6* mutations who have been reported subsequently have shown a partial correction of anemia with prolonged or sustained administration of oral iron.[43,51,54,55] In one of these kindreds, the affected siblings presented with microcytic anemia, hypoferremia, and, interestingly, hyperferritinemia before the initiation of oral iron therapy.[54] Thus, it is becoming evident that the phenotypic spectrum of disease associated with *TMPRSS6* mutations extends beyond the classic IRIDA phenotype, and this spectrum of presentations should be recognized during the clinical evaluation of iron deficiency anemia.

In addition to the rare pathogenic *TMPRSS6* mutations that have been associated with IRIDA, several common variants (ie, single nucleotide polymorphisms [SNPs]) in *TMPRSS6* have also been described in multiple global populations.[58,59] Genome-wide association studies conducted in several large populations have correlated these SNPs at the *TMPRSS6* locus with several laboratory parameters related to iron status, such as hemoglobin level, mean corpuscular volume, mean corpuscular hemoglobin, serum iron level, and serum transferrin saturation.[60–65] One of the *TMPRSS6* SNPs showing the strongest associations to these parameters, rs855791, encodes an alanine-to-valine substitution at position 736 within the matriptase-2 serine protease domain (p.Ala736Val). Compared with the alanine-containing variant, matriptase-2 possessing a valine at position 736 was found to be less effective in suppressing hepcidin levels in vitro, and p.Ala736Val was also shown to associate with serum hepcidin levels in a large Italian population from which subjects with iron deficiency and inflammation had been excluded.[66] Although these findings suggest that the association of *TMPRSS6* polymorphisms with laboratory parameters of iron status may result from an intermediate effect of these polymorphisms on hepcidin expression, 2 population-based studies interestingly have found that the associations of *TMPRSS6* SNPs with iron and erythrocyte parameters are at least partly independent of hepcidin levels.[67,68]

The key role of matriptase-2 in dampening hepcidin production through the BMP/SMAD pathway has raised the possibility that inhibition of *TMPRSS6* activity could be used as a therapeutic strategy to increase hepcidin expression, and therefore reduce iron loading, in certain clinical disorders in which iron loading results from hepcidin insufficiency. In proof-of-principle studies, genetic disruption of *Tmprss6* has been shown to reduce iron loading in mouse models of *HFE*-associated hereditary hemochromatosis[69] and β-thalassemia intermedia.[70] In humans, milder changes in matriptase-2 activity resulting from *TMPRSS6* polymorphisms have been suggested to influence the phenotypic expression of several clinical disorders associated with abnormalities of iron homeostasis. For example, the *TMPRSS6* p.Ala736Val variant has been found to correlate with serum transferrin saturation and serum ferritin levels in patients with hereditary hemochromatosis,[71] with serum hepcidin levels and erythropoietin requirements in patients undergoing chronic hemodialysis,[72] and with hepatic iron accumulation in patients with nonalcoholic fatty liver disease.[73]

DIFFERENTIAL DIAGNOSIS

The differential diagnosis of microcytic hypochromic anemia is dominated by acquired iron deficiency resulting from either poor dietary intake or ongoing losses. Similarly, for congenital microcytic hypochromic anemias, the differential diagnosis is dominated by the thalassemia syndromes. The approach to the diagnosis of rarer forms of congenital microcytic anemias has been recently reviewed.[74] In addition to the congenital defect in iron absorption that underlies IRIDA, these rarer congenital microcytic anemias result from defects in iron transport, iron uptake, and mitochondrial iron utilization (see the article about sideroblastic anemia by Bottomley, elsewhere in this issue).

The prevalence of the rare congenital microcytic anemias is not easily determined. However, the recent increase in published IRIDA cases and affected families suggests that IRIDA may be the most common form. An approach to the diagnosis of IRIDA is shown in Fig. 3. Once iron deficiency is confirmed, the algorithm must start with a rigorous exclusion of the acquired causes of iron deficiency (eg, blood loss, iron-poor diet, and long-standing inflammatory conditions). Clues for an IRIDA diagnosis

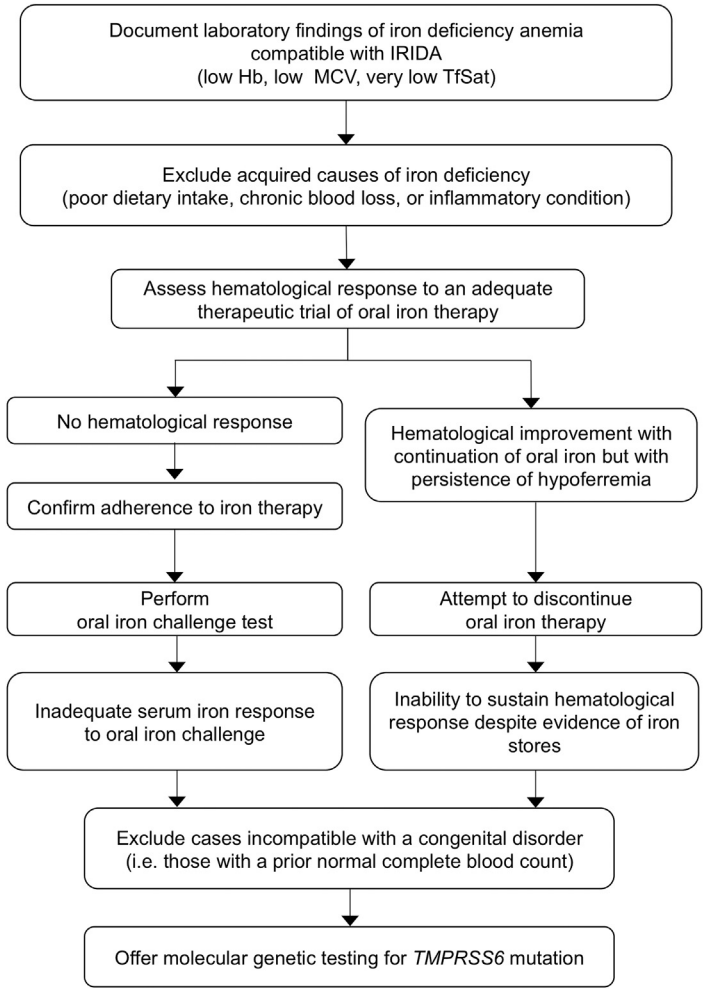

Fig. 3. Diagnostic algorithm for the clinical evaluation of IRIDA. Suspicion for IRIDA should arise in a subject with a lifelong significant microcytic hypochromic anemia and biochemical evidence of iron deficiency without a history of inadequate iron intake or ongoing iron/blood loss. If the iron deficiency anemia does not respond to oral iron supplementation and/or has an incomplete/transient response to parenteral iron therapy, an oral iron challenge will assess for impaired intestinal iron absorption from either intestinal pathology or an inappropriately high hepcidin state. Hb, hemoglobin; IDA, iron deficiency anemia; IRIDA, Iron-Refractory Iron Deficiency Anemia; MCV, mean corpuscular volume; TfSat, transferrin saturation.

from the initial assessment of iron status in an untreated subject include 2 patterns: (1) the degree of microcytosis (mean corpuscular volume [MCV] 45–65 fL range) relative to the anemia (hemoglobin [Hb] 6–8 g/dL range); and (2) a profound hypoferremia and low transferrin saturation (usually <5%) relative to a slightly low or even normal ferritin. Most commonly, subjects with iron deficiency will be treated with an empiric course of adequate iron supplementation. Poor or absent response to oral iron supplementation is most commonly associated with poor adherence to therapy, inadequate dosing, or inadequate duration of therapy. If these common pitfalls can be avoided and an adequate oral trial fails to produce a hematologic benefit, one must consider the possibility of impaired intestinal iron absorption.

Efficient intestinal iron absorption requires an acidic duodenal environment and a functioning duodenal epithelium. Common reasons for poor iron absorption include achlorhydria due to chronic proton pump inhibition and damage to the duodenum (eg, celiac sprue). States of elevated hepcidin, which include anemia of chronic inflammation, as well as IRIDA, result in impaired export of iron from the duodenal enterocyte into the plasma. In the hypoferremic patient, an oral iron challenge (**Box 2**) can identify inadequate iron absorption; however, the test does not distinguish the etiology and may prompt more aggressive gastrointestinal evaluation.

If the oral iron challenge suggests inadequate absorption and the iron deficiency truly appears to have onset in infancy or childhood, IRIDA is a more likely diagnosis. The only current diagnostic test for IRIDA is sequencing of the *TMPRSS6* gene. Ideally, clinicians could determine the plasma or urinary hepcidin and easily distinguish true iron deficiency (based on a finding of low hepcidin) from IRIDA (in which hepcidin would be inappropriately high). However, although more than a decade has passed since the discovery of hepcidin, there is yet no hepcidin assay approved by the Food and Drug Administration available for clinical use. Once a hepcidin assay becomes available, it may serve as a useful aid in the diagnosis of IRIDA.

Box 2
Oral iron challenge

In the hypoferremic patient, this simple and minimally invasive test distinguishes an intestinal iron absorption defect from other causes of chronic iron deficiency. There are no systematically validated, published procedures or expected response criteria for an oral iron challenge test. The procedure that follows is largely based on our clinical experience and data published in individual case reports.[77,78]

Procedure:

A. Ideally the subject should be fasting for at least 6 hours.

B. Draw blood samples for serum iron, transferrin (TIBC), and ferritin.

C. Ferrous sulfate (eg, Fer-in-Sol) 4–6 mg/kg of elemental iron PO.

D. Redraw blood samples for serum iron at 90 minutes post dose (some perform repeated sampling every 30 minutes for up to 3 hours post oral dose).

Interpretation:

In a hypoferremic subject capable of absorbing iron from the intestine, the serum iron level is expected to increase by at least 50 μg/dL 90 minutes after the oral iron challenge. Failure of a fasting subject to achieve an appropriate increase in serum iron level is indicative of a defect in intestinal iron absorption. For nonfasting subjects, an equivocal rise in serum iron would not be readily interpretable; however, the test results would remain interpretable if either a substantial increase in the serum iron level or no change in the serum iron level whatsoever were observed.

TREATMENT

The mainstay of therapy for IRIDA is intermittent parenteral iron supplementation. In many case reports and series, parenteral iron has been demonstrated to improve the anemia in the IRIDA phenotype. However, the hemoglobin response to parenteral iron is usually not completely corrective and is of shorter duration than expected in most cases. Depending on the formulation and dose limitations, repeated dosing is usually required and can become onerous. Although many parenteral iron formulations have been used with efficacy, the optimal formulation and frequency of dosing has not been determined. Although not yet described, the concern with repeated parenteral iron dosing would be for iron overload; however, given the inappropriately high hepcidin levels in IRIDA, one would expect a hemosiderosis pattern of reticuloendothelial macrophage loading rather than parenchymal loading.

Given that the classic IRIDA phenotype includes absent/minimal response to an oral iron challenge, there does not seem to be a significant role for oral iron supplementation in IRIDA. However, Cau and colleagues[75] recently described a child with homozygous *TMPRSS6* splice site mutation (IVS6+1 G>C) who demonstrated the classical unresponsiveness to oral iron therapy and partial response to parenteral therapy, yet had a remarkable response with the addition of ascorbic acid to the ferrous sulfate oral supplement. The investigators noted that the addition of ascorbic acid was not effective in the affected adults in the family, and they hypothesized that the increased iron needs of the rapidly growing child explained the differential benefit observed between age groups.

The addition of recombinant erythropoietin has been described by several groups but has not shown significant benefit in IRIDA.[15,56] The rationale for erythropoietin supplementation was that in high doses it can provide some benefit in the high hepcidin state of anemia of chronic inflammation. However, as shown by Lehmberg and colleagues,[56] administration of recombinant human erythropoietin up to 273 U/kg per week alone did not improve anemia.

In a recent case report, a hematologic response to glucocorticoid therapy was reported in a child with hypochromic microcytic anemia who had shown little response to oral therapy and who also had an elevated hepcidin level. However, given that sequencing of the *TMPRSS6* gene revealed only common polymorphisms (but not pathogenic *TMPRSS6* mutations) in this child, the relevance of this therapy for patients with IRIDA due to *TMPRSS6* mutations remains uncertain.[76]

Ultimately, optimally effective therapies may require manipulation of the inappropriately elevated hepcidin levels. There are currently several experimental agents in clinical trials for anemia of chronic inflammation that also could have benefit in treating the pathophysiology of IRIDA (see the article by Ganz, elsewhere in this issue).

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