Iron Refractory Iron Deficiency Anemia: Presentation With Hyperferritinemia and Response to Oral Iron Therapy

abstract

Iron-refractory iron-deficiency anemia (IRIDA) is an autosomal recessive disorder caused by mutations in *TMPRSS6*. Patients have hypochromic microcytic anemia refractory to oral iron and are only partially responsive to parenteral iron administration. We report a French-Canadian kindred in which 2 siblings presented in early childhood with severe microcytic anemia, hypoferremia, and hyperferritinemia. Both children have been successfully treated solely with low-dose oral iron since diagnosis. Clinical and biological presentation did not fit any previously described genetic iron-deficiency anemia. Whole exome sequencing identified in both patients compound heterozygous mutations of *TMPRSS6* leading to p.G442R and p.E522K, 2 mutations previously reported to cause classic IRIDA, and no additional mutations in known iron-regulatory genes. Thus, the phenotype associated with the unique combination of mutations uncovered in both patients expands the spectrum of disease associated with *TMPRSS6* mutations to include iron deficiency anemia that is accompanied by hyperferritinemia at initial presentation and is responsive to continued oral iron therapy. Our results have implications for genetic testing in early childhood iron deficiency anemia. Importantly, they emphasize that whole exome sequencing can be used as a diagnostic tool and greatly facilitate the elucidation of the genetic basis of unusual clinical presentations, including hypomorphic mutations or compound heterozygosity leading to different phenotypes in known Mendelian diseases. *Pediatrics* 2013;131:e620–e625

AUTHORS: Dong-Anh Khuong-Quang, MD,[a] Jeremy Schwartzentruber, MSc,[b] Mark Westerman, PhD,[c] Pierre Lepage, PhD,[b] Karin E. Finberg, MD, PhD,[d] Jacek Majewski, PhD,[a,b] and Nada Jabado, MD, PhD[a,e]

Department of [a]Human Genetics, and [e]Pediatrics, McGill University, Montreal, Canada; [b]McGill University and Genome Quebec Innovation Centre, Montreal, Canada; [c]Intrinsic LifeSciences LLC, La Jolla, California; and [d]Department of Pathology, Duke University School of Medicine, Durham, North Carolina

KEY WORDS

iron, *TMPRSS6*, hypomorphic mutations, hepcidin, whole exome sequencing, anemia

ABBREVIATIONS

Hb—hemoglobin
IRIDA—iron refractory iron deficiency anemia
MCV—mean corpuscular volume
WES—whole exome sequencing

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Address Correspondence to Nada Jabado, MD, PhD, Department of Pediatrics, McGill University/McGill University Health Center, 4060 Ste Catherine West, PT-239, Montreal, QC H3Z 2Z3 Canada. E-mail: nada.jabado@mcgill.ca

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The main causes of iron-deficiency anemia are blood loss or an inadequate dietary iron intake. However, several genetic defects can lead to ineffective iron intestinal absorption or impaired iron recycling by macrophages, the 2 main processes that fulfill iron needs in mammals. Iron-refractory iron deficiency anemia (IRIDA; Online Mendelian Inheritance in Man database #206200) is an autosomal recessive disorder caused by loss-of-function mutations in *TMPRSS6*,[1] first described in 1981. Patients exhibit severe, congenital hypochromic, microcytic anemia with low serum iron and transferrin saturation that occurs in infancy and is refractory to oral iron treatment and partially recovered after parenteral iron administration.[2] They also show an inappropriate elevation of hepcidin, a circulating hormone that inhibits iron duodenal absorption and macrophage iron recycling. To date, >30 *TMPRSS6* mutations have been identified in patients without any common ethnic or geographic distribution,[1,3–14] suggesting that *TMPRSS6* mutations might be underestimated in patients with iron-deficiency anemia. Here, we report a family in which whole exome sequencing (WES) identified compound heterozygous *TMPRSS6* mutations in 2 siblings with iron deficiency anemia that differed clinically and biologically from classic IRIDA.

PATIENT PRESENTATION

Written informed consent and assents were obtained from the patients and their guardians for whole exome sequencing and publication of this case report.

In 1999, a 3-year-old boy was referred to our clinic at the Montreal Children's Hospital for investigation of a microcytic hypochromic anemia diagnosed after symptoms of fatigue and abdominal pain. His growth and development were normal, and no anterior hemoglobin (Hb) measurement was available. He was of French Canadian descent with no known parental consanguinity. His parents and older sister had normal complete blood counts, reticulocyte counts, and ferritin levels. Laboratory values, described in Table 1, showed a severe microcytic, hypochromic anemia (Hb of 75 g/L, mean corpuscular volume [MCV] of 64 fL), low serum iron, low fractional transferrin saturation, and normal transferrin levels. Unexpectedly, the serum ferritin level was elevated at 348 μg/L, well above the normal range of 6 to 110 μg/L. Hb electrophoresis did not detect a hemoglobinopathy, there was no clinical or biological evidence of chronic inflammatory state, and gastroenterological investigations provided no evidence for occult blood loss or malabsorption. His diet was well diversified. During a course of oral iron supplementation (6–10 mg/kg/day of elemental iron) for 1 year, the proband's symptoms disappeared, and he experienced a slow rise of Hb up to 119 g/L with normalization of the MCV. The transferrin saturation remained low (0.07), as did the circulating iron level at 4 μmol/L. However, ferritin rose up to 654 μg/L. The time course of hematologic and iron parameters under treatment is summarized in Table 2. After 9 years of follow-up, the patient

TABLE 1 *TMPRSS6* Genotype and Hematologic and Biochemical Parameters in Patients, Their Parents, and Their Unaffected Sister

TMPRSS6 genotype	Pediatric reference range for patients 1 and 2	Proband, 3 y, p.G442R/p.E522K	Affected sister, 2 y, p.G442R/p.E522K	Father, 46 y,[a] WT/p.E522K	Mother, 45 y,[a] WT/p.G442R	Unaffected sister, 17 y,[a] WT/p.E522K
WBC	5.5–15.5 19^9/L	8.6	10.2	6.39	6.05	7.3
RBC	3.90–5.30 10^12/L	4.31	5	4.81	3.91	4.43
Hb	115–135 g/L	75	76	145	121	131
Hct	0.340–0.400	0.276	0.249	0.413	0.352	0.386
MCV	75.0–87.0 fL	64.1	49.7	85.9	90	87
MCH	24.0–30.0 pg/cell	17.4	15.1	30.2	30.9	29.6
MCHC	310–370 g/L	271	304	351	343	341
RDW	11.4% to 14.6%	16.9	22.4	13.2	13.4	14.1
Platelet	140–450 10^9/L	292	616	223	250	271
Reticulocytes	10^9/L	0.024	0.045	0.046	0.025	0.046
Sedimentation rate	3–13 mm/h	8	8	NA	NA	NA
Iron	4–25 μmol/L	<1	1.6	12.3	15.5	19.6
Transferrin	2.00–3.70 g/L	2	2.14	2.34	2.93	3.17
Fractional Transferrin saturation	0.20–0.55	0.02	0.07	0.21	0.21	0.24
Ferritin	6–110 μg/L	348	195	112	36	15
	Reference range	Proband, 15 y	Affected sister, 12 y	Father, 47 y	Mother, 46 y	
Hepcidin	17–260 ng/mL	142.4	198.6	116.1	56.4	
Hb		115	94			
Iron		2.6	1.6			

Parameters at diagnosis are shown in the upper panel. Mutations are described at the protein level. Plasma hepcidin was measured in 2011 while patients were receiving oral iron supplementation (3.5 mg/kg/wk). Results are shown in the lower panel along with Hb and serum iron. Hct, hematocrit; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; NA, not available; RBC, red blood count; RDW, red cell distribution width; WBC, white blood count; WT, wild type.

[a] Results for both parents and their healthy sister were within the normal range.

TABLE 2 Time Course of Hematologic and Biochemical Parameters in Probands Under Oral Iron Supplementation

Date	Tx duration, mo	Patient age, y	OEI suppl	Hb, g/L	MCV, fL	RDW, %	Retic, 10^9/L	Iron, μ mol/L	Ferritin, μg/L	Transf, g/L	Transf saturation
9/16/1999	—	3	6 mg/kg/d	75	64.1	16.9	0.024	<1	348	2.00	0.02
1/19/2000	4	3	↑ up to 10mg/kg/d	100	64	16.4	0.050	3,0	217	2.24	0.05
10/14/2000	12	4	Stop Tx	112	71.9	14.9	ND	ND	ND	ND	ND
11/22/2000	—	4	Restart at 12 mg/kg/d	104	72.8	14.6	ND	ND	325	ND	ND
9/12/2001	9	5	Stop Tx	112	80.3	12.9	ND	ND	ND	ND	ND
5/3/2002	—	5	Restart at 10 mg/kg/d	101	73.4	13.6	ND	2.0	291	1.73	0.05
6/12/2002	1	6	↓ down to 6 mg/kg/d	107	73.3	15.1	0.068	3.0	379	2.16	0.05
9/6/2002	4	6	Stop Tx	113	75.5	13.8	ND	ND	ND	ND	ND
3/28/2003	—	6	Restart at 9 mg/kg/d	102	69.5	16.4	ND	4.0	376	ND	ND
11/3/2003	7	7	Stop Tx	114	75.7	14.6	0.066	4.0	654	2.12	0.07
5/11/2004	—	7	No Tx	109	81.6	14.5	ND	2.0	467	2.14	ND
10/18/2004	—	8	Restart at 4 mg/kg/d	106	73.6	15.3	0.057	2.0	391	2.06	0.04
3/17/2006	16	9	Stop Tx	119	78	14.3	ND	ND	ND	ND	ND
8/18/2006	—	10	Restart at 6 mg/kg/d	103	74.9	15.5	ND	3.1	439	2.07	0.06
10/26/2007	14	11	↓ down to 1.2 mg/kg/d	119	76.1	14.6	ND	5.0	598	ND	ND
5/23/2008	21	12	↓ down to 1.1 mg/kg/d	113	73.1	15.6	0.057	4.1	390	ND	ND
11/20/2009	39	13	↓ down to 0.7 mg/kg/d	119	72.2	17.2	ND	ND	512	ND	ND
12/17/2010	51	14	↓ down to 0.5 mg/kg/d	109	67.2	18.9	0.038	3.2	168	2.24	0.06
12/20/2011	64	15	0.5 mg/kg/d	115	66.8	19.1	ND	2.6	ND	ND	ND

ND, not determined; OEI suppl, oral elemental iron supplementation; RDW, red cell distribution width; Retic, reticulocytes; Transf, transferrin; Tx, treatment; ↑, increase; ↓, decrease.

has had a normal growth curve, normal physical activities, and improved Hb levels. On the basis of the increase in ferritin levels after oral iron therapy, oral iron supplementation has been continued with the dose tapered to 3.5 mg/kg per week, which has maintained ferritin levels similar to those seen at diagnosis and the Hb >110 g/L. The patient never required erythrocyte transfusion or intravenous iron administration.

The proband's youngest sister presented with similar symptoms at age 2 years and also had microcytic anemia, hypoferremia, and high ferritin levels (Table 1). She was initially treated with 6 mg/kg per week of oral iron with elimination of symptoms; the oral iron dose was tapered to 3.5 mg/kg per week, also in response to a rising ferritin level during iron therapy. She is now aged 12 years and, like her brother, has a normal growth curve; she has a normal Hb level when she is compliant with her iron therapy. Notably, several trials to stop oral iron therapy were attempted in both siblings during periods ranging from 2 to 12 months. All resulted in increased fatigue hampering normal daily activities and significant drops in the Hb levels, leading us to resume oral iron therapy.

The pedigree suggested autosomal recessive transmission, and the clinical presentation of microcytic anemia, hypoferremia, and hyperferritinemia, which responded to oral iron supplementation with a concomitant increase in serum ferritin did not suggest any previously described inherited form of iron-deficiency anemia. In congenital hypotransferrinemia, serum transferrin is low.[15] Patients with mutated *SLC11A2* or glutaredoxin 5 deficiency exhibit hyperferremia.[16,17] Patients with IRIDA show no hematologic correction after oral iron. We thus performed WES on genomic DNA from both siblings, a strategy proven by us and others to identify novel genes associated with rare disorders.[18] We identified compound heterozygous mutations in *TMPRSS6* in both patients, causing p.G442R and p.E522K. To see if the unusual clinical presentation could be associated with a coding polymorphism, we searched the exome data for rare variants (allele frequency <1%) in genes known to be involved in iron metabolism (Supplemental Table 4). However, we found no uncommon polymorphisms in the probands for any of these genes. Other family members each carried 1 mutation.

DISCUSSION

We describe here a family in which WES identified compound heterozygous *TMPRSS6* mutations in the 2 siblings affected with iron deficiency anemia. The clinical presentation differed from classic IRIDA in that ferritin levels were high at diagnosis and the anemia in both children has been responsive to low-dose oral iron with >9 years of follow-up.

These 2 missense mutations have been previously reported in patients with the IRIDA phenotype who required parenteral iron.[1,6] To our knowledge, this specific compound heterozygosity is described here for the first time, in association with this atypical clinical and biological presentation of iron-deficiency anemia (Tables 1 and 2). *TMPRSS6* encodes the hepatic transmembrane serine protease matriptase-2.

p.G442R affects the matriptase-2 CUB domain, and p.E522K affects the second low-density lipoprotein receptor class A domain. Matriptase-2 inhibits hepcidin expression by cleaving hemojuvelin, a membrane-associated protein that promotes bone morphogenetic protein signaling in hepatocytes. In vitro, matriptase-2 p.G442R mutants show a partial defect in hemojuvelin cleavage, whereas p.E522K mutants are fully defective.[6]

Reported IRIDA cases show low or normal ferritin levels at diagnosis that increase after parenteral iron (Table 3). In our patients, however, ferritin was high at diagnosis and increased with oral iron treatment. Moreover,

TABLE 3 Literature Review of Previously Reported Cases of IRIDA

Study	Year	Mutation 1	Mutation 2	Ethnic origin	Age,[a] y	Route of iron admin and additional therapies	Hb,[a] g/L	Ferritin,[a] μg/L	Ferritin under Tx, μg/L[b]	Follow-up, y/age at Dx, y
Our study	2012	p.G442R	p.E522K	French Canadian	3	PO	75	348	654	FUP 13
		p.G442R	p.E522K	French Canadian	2	PO	76	195	309	FUP 11
Finberg et al[1]	2008	p.K636fs	p.K636fs	Turkish	6	IV	88	NA[c]	NA	NA
		p.A605fs	p.E527fs	Northern European	1.1	IV	92	NA[c]	NA	NA
		p.G713fs	unknown	Nigerian	1.4	IV	70	NA[c]	NA	NA
		p.G442R	p.D521N	Northern European	11	IV	82	NA[c]	NA	NA
		p.R774C	unknown	African American	7	IV	75	NA[c]	NA	NA
		IVS15-1 G>C	p.D622fs	Nigerian	3	IV	97	NA[c]	NA	NA
		p.Y355X	p.E461fs	African American	1.3	IV	79	NA[c]	NA	NA
Guillem et al[3]	2008	p.Y393X	p.R599X	English	1.5	IV	60	11	109	FUP 12
Melis et al[4]	2008	IVS6+1 G>C	IVS6+1 G>C	Sardinian	0.7–1	IV	100[d]	NA	53	NA
		IVS6+1 G>C	IVS6+1 G>C	Sardinian	14	IV	91[d]	116[d]	413	Dx between 0.7 and 1
		IVS6+1 G>C	IVS6+1 G>C	Sardinian	NA	IV	100[d]	NA	129	NA
		IVS6+1 G>C	IVS6+1 G>C	Sardinian	NA	IV	128[d]	NA	184	NA
		IVS6+1 G>C	IVS6+1 G>C	Sardinian	0.7–1	IV	139[d]	NA	466	
Ramsay et al[5]	2009	p.A118D	p.P686fs	Spanish	15	IV + EPO	98[d]	123[d]	365	Dx at 1
		p.A118D	p.P686fs	Spanish	Twin sister	IV + EPO	1219[d]	388[d]	586	Dx at 1
Silvestri et al[6]	2009	p.D521N	p.E522K	French	0.8	PO (failure) then IV	10	4	180	FUP 6
Edison et al[7]	2009	IVS17(-1)G>C	IVS17(-1)G>C	Indian	35	IV	66[d]	20[d]	NA	Dx at 20
Techou et al[8]	2009	p.S304L	p.K478_ K508del	Swiss	3	IV + fresh-frozen plasma	77[d]	46[d]	310	Dx at 0.3
		IVS15-1 G>C	IVS15-1 G>C	Italian	53	PO (failure)	114[d]	68[d]	NA	Dx at birth
Beutler et al[9]	2010	p.L674F	IVS13+1 G>A	Belgian	8	PO	90	20	70	FUP 8
		p.L674F	IVS13+1 G>A	Belgian	0.8	PO	82[d]	41[d]	33	FUP 15
		p.L166X+36	p.L166X+36	Dutch	6	IM	62.8	16	323	FUP 11
Altamura et al[10]	2010	p.Y141C	p.Y141C	Lebanese	10	PO (failure)	79[d]	86[d]	NA	Dx at 2
De Falco et al[11]	2010	p.Y141C	p.Y141C	Indian	8	IV	91	26[d]	25	Dx at 1.3 y
		p.I212T	p.R271Q	Italian	3	IV	80	25[d]	NA	NA
		p.S304L	p.S304L	Arabian	6	IV	80	112[d]	NA	NA
		p.S304L	p.S304L	Arabian	4	IV	85	32[d]	133	NA
		p.S304L	p.S304L	Arabian	2	IV	80	50[d]	113	NA
		p.L166fs	p.Q229fs	Austrian	3	IV	71	10	74	NA
		p.W247fs	p.W247fs	Greek	2.5	IV	58	8[d]	NA	NA
		p.W247fs	p.W247fs	Greek	2	IV	54	19[d]	NA	NA
		p.S561X	p.S561X	Arabian	7	IV	80	86	NA	NA
		p.S561X	p.S561X	Arabian	5	IV	88	101[d]	NA	Dx at 8
		p.S561X	p.S561X	Arabian	2	IV	79	38	NA	NA
		p.C510S	S570fs	Algerian	9	IV	104	228[d]	NA	Dx at 1.8
Cau et al[12]	2011	IVS6+1 G>C	IVS6+1 G>C	Sardinian	0.4	PO (failure), then IV, then PO with ascorbic acid	75	102	450 (IV)	FUP 2
Sato et al[13]	2011	p.K253E	p.K253E	Japanese	27	IV	108	4.3	35.1	NA
Choi et al[14]	2011	p.G603R	IVS6+1G>T	Korean	2	PO (failure) then IV	70	42[d]	106 (IV)	NA

Mutations are described at the protein level, except for the ones affecting splicing sites. Dx, diagnosis; EPO, erythropoietin; FUP, follow-up; IM, intramuscular; IV, intravenous; NA, not available; PO, per os; Tx, treatment.

[a] At evaluation.

[b] Highest level reported.

[c] In the kindreds reported by Finberg et al[1], ferritin levels were reported to be low-normal, although specific ferritin values were not included.

[d] Hb and/or ferritin levels were measured under iron therapy. Age of patients at the time of diagnosis is included in the comments.

whereas typical IRIDA patients are refractory to oral iron, both patients were effectively treated exclusively by oral supplementation. Review of the literature indicates that the clinical and biological presentations of the siblings we report herein are unique (Table 3). Cau et al[12] report a 5-month-old Sardinian female with IRIDA and homozygous *TMPRSS6* mutation who had normal ferritin at diagnosis. Unlike the patients in our study, this patient failed to respond to oral iron. Interestingly, after showing a partial response to intravenous iron, she responded to a combination of oral iron and ascorbic acid. Notably, other family members carrying the same homozygous TMPRSS6 mutation did not respond to oral iron. Beutler et al[9] described a Belgian family in which the proband with compound *TMPRSS6* heterozygous mutations was diagnosed at age 8 years when the Hb was 90 g/L and the ferritin was 20 ng/mL. The proband and his affected sibling both showed a partial response to oral iron. However, in contrast to the patients in our study, neither developed hyperferritinemia with long-term oral iron therapy.

In these specific cases, iron absorption may be less impaired than reported IRIDA patients, perhaps because of the combined residual function of these particular mutant alleles. These phenotypic differences might also reflect modifier genes that promote iron uptake by enterocytes or reduce hepcidin release by hepatocytes.

Plasma hepcidin was assessed during iron therapy when Hb was improved but hypoferremia persisted. Thus, even if seemingly within normal range for the patients, hepcidin appeared inappropriately elevated relative to serum iron level. Comparison of hepcidin deregulation in our patients to reported IRIDA cases is, however, complicated by likely differences in erythropoiesis and hepatic iron stores, stimuli known to modulate hepcidin transcription. Because macrophages are a major source of serum ferritin,[19] our patients' presenting hyperferritinemia could reflect greater hepcidin sensitivity in macrophages versus enterocytes, in keeping with previous studies.[20]

Recently, a single nucleotide polymorphism encoding V736A in the *TMPRSS6* catalytic domain was found in several genome-wide association studies[21,22] to correlate with decreased Hb and serum iron in healthy populations. Our study suggests that *TMPRSS6* sequence variants lead to a spectrum of matriptase-2 dysfunction, including severe loss-of-function mutations causing classic IRIDA, hypomorphic mutations as seen in our patients and potentially similar atypical ones, and the mild reduction in matriptase-2 activity associated with the common V736A SNP.[23] Accordingly, our results suggest that genetic testing for *TMRSS6* mutation have clinical utility in cases of hypochromic, microcytic anemia with hypoferremia that do not exhibit the classic IRIDA phenotype. We suggest that *TMPRSS6* sequencing should be considered in a subset of patients presenting with iron deficiency anemia of unknown cause in which blood loss, inadequate dietary intake, and chronic inflammatory conditions have been ruled out (see online Supplemental Fig 1 for a diagnostic algorithm). *TMPRSS6* sequencing is available in several CLIA (clinically accredited) certified laboratories. Although hepcidin measurement is not yet widely available as clinical test, we note that the finding of a markedly reduced hepcidin level in the setting of iron deficiency anemia would indicate the anemia is unlikely to be attributed to *TMPRSS6* mutation.

Our results further emphasize that next generation sequencing technologies, particularly WES, greatly facilitate the elucidation of the genetic basis of unusual clinical presentations exhibiting Mendelian inheritance, including hypomorphic mutations leading to different phenotypes. Clinical application of WES in undiagnosed clinical conditions has already been shown to be feasible, yielding an encouraging 50% rate of success in uncovering an underlying genetic defect in select clinical cases in which the probability of a genetic origin is high.[24] There is growing interest in its introduction into the clinic to aid in the diagnosis of conditions for which no genetic cause can be found with targeted testing. Although the mutations identified currently require validation in a Clinical Laboratory Improvement Amendments–certified laboratory, this technology is becoming accessible to clinicians through academic consortia (Finding of Rare Disease Genes in Canada[FORGE Canada], Broad Institute, etc.) or private companies that offer the possibility of sequencing the exome and performing the analysis. The broader use of WES will expand the range of clinical phenotypes associated with mutations in known disease genes.

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