Published in final edited form as:
Blood Cells Mol Dis. 2010 January 15; 44(1): 16. doi:10.1016/j.bcmd.2009.09.001.

Polymorphisms and Mutations of Human *TMPRSS6* in Iron Deficiency Anemia

E. Beutler[1], **C. Van Geet**[2], **D.M.W.M. te Loo**[3], **T. Gelbart**[1], **K. Crain**[1], **J Truksa**[1], and **P.L Lee**[1]

[1]Department of Molecular and Experimental Medicine, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA, 92037, USA [2] Department of Pediatric Hemato-Oncology, University Hospital KULeuven, Herestraat 49, B-3000 Leuven, Belgium [3] Department of Pediatrics Hemato-Oncology, Radboud University Hospital Nijmegen, 6500 HB Nijmegen, The Netherlands.

Abstract

Male subjects with iron deficiency from the general population were examined for polymorphisms or sporadic mutations in *TMPRSS6* to identify genetic risk factors for iron deficiency anemia. Three uncommon non-synonymous polymorphisms were identified, G228D, R446W, and V795I (allele frequencies 0.0074, 0.023 and 0.0074 respectively), of which the R446W polymorphism appeared to be overrepresented in the anemic population. In addition, three children with iron refractory iron deficiency anemia, and one sibling with iron responsive iron deficiency anemia were also examined for polymorphisms or sporadic mutations in *TMPRSS6*. Two children (family 1) were compound heterozygotes for a L674F mutation and a previously described splicing defect predicted to cause skipping of exon 13 (IVS13+1 G>A). One child from the second family was homozygous for a deletion (497T) causing a frameshift (L166X+36) and premature termination. The sibling and mother from the second family were compound heterozygotes for the L166X mutation and the uncommon R446W polymorphism. Although in vitro expression studies demonstrated that the R446W isoform was biologically similar to wildtype Tmprss6, clinical data indicate that the R446W produces a milder disease when carried in trans with severe mutation in *Tmprss6*. The four children carrying mutations in *TMPRSS6* all exhibited inappropriately high urinary hepcidin levels for the degree of iron deficiency.

Keywords

hepcidin; iron deficiency; anemia; TMPRSS6; matriptase

Corresponding author: Pauline Lee, Department of Molecular and Experimental Medicine, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA, 92037, USA, phone (858)-784-8042; fax (858) 784-2083, plee@scripps.edu.

Authorship

Dr. Pauline Lee coordinated the project, analyzed the data and contributed to the writing of the manuscript. Dr. Christel Van Geet provided the clinical findings of Family 1. Dr. D. Maroeska te Loo provided the clinical findings of Family 2. Terri Gelbart and Karen Crain performed the sequencing analyses for the patients and contributed to the writing of the manuscript. Dr. Jaroslav Truksa performed the mutation analyses and contributed to the writing of the manuscript.

Disclosures

The authors have no conflicts of interest to declare.

INTRODUCTION

Iron deficiency is one of the most common maladies in humans. Many risk factors are well established. These include bleeding, multiple parturition, celiac sprue and other intestinal disorders that prevent normal iron absorption, and a diet poor in iron. Except for genetically determined causes of bleeding or intestinal malabsorption, hereditary risk factors have not been identified.

Iron deficiency is also common in children, largely because of the demands for a positive iron balance that is imposed by growth and partly because diets rich in milk are not a good source of iron. There are, however, rare instances of children who develop iron deficiency anemia that appears to resist treatment with oral iron. The first such patient was probably described in 1981 by Buchanan and Sheehan (1). Three sibs manifested iron deficiency anemia that was unresponsive to oral iron administration but did respond to the injection of parenteral iron dextran. Oral iron absorption test suggested decreased iron absorption. Subsequently, two siblings with a similar disorder were described by Hartman and Barker(2). Again the oral administration produced only a minimum increase in serum iron levels and no therapeutic response, and the response to parenteral iron dextran was incomplete.

Previously, the microcytic anemia of *mk* mice (3) and Belgrade rats (4) have been shown to be the result of a mutation in the bivalent metal transporter 1 (*DMT1*) gene. Surprisingly, however, mutations that have been found in *DMT1* in humans have been associated with iron overload, not iron deficiency (5-8).

We have recently shown in mice that an ENU-mutagenesis induced mutation in *Tmprss6*, a gene encoding a membrane serine protease, results in loss of body hair giving the “mask” phenotype and severe iron deficiency in mice fed a normal laboratory diet (9). The anemia is corrected, however, by the feeding of large amounts of oral iron or the injection of iron dextran. This discovery led to the rapid identification of *TMPRSS6* mutations in patients with iron refractory iron deficiency anemia (10;11). Furthermore, it led us to investigate the role of polymorphisms and sporadic mutations of the *TMPRSS6* gene in iron deficient adults and children with hereditary iron deficiency. Polymorphisms of *TMPRSS6* might be risk factors for common forms of iron deficiency anemia and rare mutations might produce familial forms of iron deficiency, a syndrome that has been described only a very few times (1;2;10;12;13). We have therefore undertaken the study of patients with iron deficiency anemia, both as it occurs commonly in the adult general population and in unusual childhood cases, in which there appears to be a moderate degree of resistance to iron therapy and a positive family history.

MATERIALS AND METHODS

Patients

Adults—Two groups of white adult iron deficient patients who had participated in the Scripps/Kaiser hemochromatosis study (14) and provided informed consent were studied. The first group of iron deficient adults were 69 males of European origin who had serum ferritin levels <20 ng/ml, transferrin saturations of <16% and who did not have a history of bleeding or of malignant disease. Forty-five of these men had hemoglobin levels below 13.7 g/dL(15). These patients represent all such males among 18,897 subjects in the Scripps/Kaiser study for whom both transferrin saturation and serum ferritin levels were available, and thus represent a highly selected sample, chosen to represent a population in which iron deficiency is very uncommon. Their average age was 61.9 years (S.D.=13.5 years). Complete sequencing of the *TMPRSS6* gene of these individuals was performed.

The second group of adults consisted of 61 menstruating women of European origin who had serum ferritin levels of <20 ng/ml and transferrin saturation of <16%. Twenty-six of these women had hemoglobin levels of <12.0/dL.

The control population for these two iron deficient cohorts consisted of 39 non-anemic white men and 52 menstruating non-anemic white women with serum ferritin levels >19 ng/ml and serum transferrin saturations of >15%. An additional 207 controls were used to obtain the frequency of the rare 1366 T>C polymorphism.

Children

Family 1: Two affected brothers, patient 1 and 2, non-consanguineous individuals of Belgian origin, were followed in the pediatric hematology department of the University Hospital of Leuven, Belgium with a large requirement for oral iron intake from childhood onwards until adulthood. They have a non-affected sister and a brother and both parents are normal (Figure 1).

The two patients presented at the age of 8 years and 9 months respectively with a microcytic hypochromic anemia due to iron deficiency (Tables 1 and 2). The second patient was already on prophylactic iron treatment at presentation, receiving 1 mg/kg elemental iron/day. Extensive examination to exclude occult gastro-intestinal blood loss was performed including gastroscopy and meckelscan. Moreover intestinal biopsy revealed normal architecture of the intestinal mucosa thereby excluding gluten enteropathy. Hematological and bone marrow examination eliminated other causes of anemia. Iron absorption was studied as described previously (16) and was considered deficient (Table 3).

With an oral treatment consisting of 7 mg/kg elemental iron (in addition to the normal amount of dietary iron), both patients reached an acceptable hemoglobin level but remained microcytic with low serum iron, normal total iron binding capacity (TIBC), and very decreased transferrin saturation (<10%) (Tables 1 and 2).

Family 2: Patient 3 is a Dutch boy from the region of Nijmegen presented at the age of six with severe anemia not responding to oral iron therapy (Figure 1). Although both parents came from the Southern part of Holland, they are not from the same village and there was no known consanguinity. At presentation the hemoglobin level was 6.3 g/dL and the MCV 57 fl. The patient had a normal diet and there were no signs of bleeding. Serum iron was diminished to 2.0 micromol/L and ferritin was normal at 88 microgram/L. There was no thalassemia, or hemolytic anemia. Bone marrow aspiration showed no abnormal erythropoeisis and no ring sideroblasts. Intramuscular iron therapy was started with parenteral iron-sorbitol-citric-acid complex, dextrin stabilized, 1mg/kg once every week and a normal hemoglobin level was reached. Laboratory values are shown in Table 4. The patient now age 15 continues to receive iron supplementation. The sister of patient 3 (patient 4) has a mild form of anemia, improving with oral iron therapy (Table 5). Her hemoglobin level is around 7.3 g/dL without treatment and with oral iron therapy she reaches levels of 10.5- 12.0 g/dL. The patient's mother has a history of iron deficiency as a child and during pregnancy. She received iron supplements as a child and during pregnancy. Currently her hemoglobin level is 12.4 g/dL, with normal ferritin levels.

DNA sequencing and data analysis

Male iron deficient subjects—The PCR primers for the human *TMPRSS6* gene were designed in the genomic level using a perl script embedded with prime program in GCG package. After the genomic sequence was masked with RepeatMasker http://www.repeatmasker.org/, the PCR primers were designed to cover every coding exon as

well as at least 40 nucleotide flanking sequences on both sides. The sequencing primers were then designed according to the amplicon sequences. All primer designs were then set up in a 96-well plate format and purchased through Sigma-Genosys.

The PCR and sequencing reaction preparations were carried out robotically on Biomek FX (Beckman Coulter). For each PCR reaction, the final concentrations of 0.4 μmol of each primer, 1X of ReadyMix (Sigma, Cat#P1107), and 20 ng of sample DNA were mixed, and cycled for 40 rounds with 95°C for 30 seconds, 56°C for 30 seconds, and 72°C for 2 minutes. The PCR products were cleaned with Agencourt AMPure following manufacture's protocol. The cleaned PCR products were normalized according to the UV absorptions, and 15 pmol of each product was sequenced using ABI BigDye following the manufacture's protocol. The sequencing reaction products were then separated on ABI 3730 sequencer after cleaned with Agencourt CleanSeq.

The sequencing data was analyzed using a perl script embedded with phred, phrap, and polyphred programs http://www.phrap.org/phredphrapconsed.html and http://www.codoncode.com/polyphred/. For each amplicon, the sequencing reads from all 69 patients were assembled, and the mutations and polymorphisms were called using polyphred default parameters. After confirmation with the consed visual tool, the results as well as the gene annotations were organized using a perl script.

Iron refractory iron deficient children and family members—Direct sequencing of patient samples with iron refractory iron deficiency and their family members was performed following amplification with the primers described in Supplemental Table 1. Amplified DNA was purified for sequencing using Qiaquick PCR purification kit (Qiagen) and sequenced using an ABI 3100 Genetic Analyzer.

Allele-specific oligonucleotides analysis (ASOH)

In order to determine allele frequencies of the single nucleotide polymorphisms (SNPs) analysis was performed by ASOH. DNA fragments containing the SNP sites were amplified using the polymerase chain reaction (PCR) with the primers indicated in Supplemental Table 2. Five microliters of each PCR product was spotted on duplicate nylon membranes. The membranes were denatured with 0.5M NaOH, 1.5M NaCl, and then neutralized with 1.5M Tris-HCl, pH 7.5, 1.5M NaCl followed by ultraviolet crosslinking. The membranes were hybridized overnight at 42C with ^{32}P labeled oligonucleotide probes that corresponded to the polymorphic sequences shown in Supplemental Table 2. After hybridization the membranes were washed at a temperature at which the unmatched probe melted from the template. The radioactive signals from the membranes were visualized on Kodak X-ray film.

Urinary and plasma hepcidin levels

Urinary and plasma hepcidin levels were determined by Intrinsic Life Sciences (La Jolla, CA). In vitro assay of wildtype and mutant Tmprss6 activity by Dual Luciferase Assay.

Murine *Tmprss6* cDNA clone was described previously (9). Site directed mutagenesis was performed using the QuikChange mutagenesis kit (Stratagene) and verified by direct sequencing.

HepG2 cells (5×10^4 per well) from ATCC were plated onto 24-well plates (Corning, NY). The next day, 100 ng of selected plasmid constructs containing the firefly luciferase gene under the control of the murine 2.5 Kb *Hamp1* and 2.5 ng per well of the normalization plasmid pGL4.73 containing the renilla luciferase gene (Promega, Madison, WI) were transfected into the cells using HyFect transfection reagent (Denville Scientific, Metuchen, NJ) according to

manufacturer's instructions. As indicated, wildtype murine *Tmprss6*, mutant *Tmprss6* Q446R, V795I or L674F were cotransfected. The final ratio of DNA (μg):Hyfect reagent (μl) was 1:1.6. Transfected cells were lysed using 150 μl of the passive lysis buffer (Promega, Madison, WI). Ten μl of the cell lysate was injected with 35 μl of LARII solution (Promega) and the light output was measured using a LB 96V microplate luminometer (Berthold Technologies GmbH&Co.KG, Bad Wildbad, Germany). Next, 35 μl of Stop & Glow solution (Promega) was injected and the renilla luciferase luminescence was measured to permit normalization. Results are expressed as firefly (*luc*)/renilla luminescence ratio where the value for the full length *Hamp1* 2.5 Kb promoter was set as 1.

RESULTS

Complete sequencing of 69 males with iron deficiency, defined as serum ferritin <20 ng/ml and transferrin saturation <16% was performed in order to determine if sporadic mutations or polymorphisms in *TMPRSS6* (Reference Sequence NM_153609) were associated with the observed iron deficiency. The allele frequencies of the SNPs observed in this group are summarized in Supplemental Table 3. Two common non-synonymous polymorphisms, nt. 757A>G (K253E, rs2235324) and nt. 2207T>C (V736A, rs855791), were found; One uncommon, nt. 1336C>T (R446W), and two rare non-synonymous polymorphisms, nt. 683G>A (G228D) and nt. 2383 G>A (V795I) were observed in this group (Table 6). Coding coordinates for both nucleotides and amino acids are numbered from the start of translation. None of the less common polymorphisms occurred in the same individual. Allele frequencies of these polymorphisms were measured in populations of normal controls (plasma ferritin >19 ng/ml, transferrin saturation >15%) and iron-deficient men and women (plasma ferritin <20 ng/ml, transferrin saturation <16%), the latter with and without anemia, defined as hemoglobin <13.7 gm/dL for males and 12.0 gm/dL for females. The common polymorphisms were in linkage equilibrium and therefore are considered separately. The data obtained from iron deficient men and women are presented separately in Table 6 because iron deficiency due to bleeding is so common in women that genetic risk factors might be less apparent in women than in men, in whom iron deficiency in the absence of overt bleeding is quite uncommon. Of the three less common polymorphisms, the R446W mutation appeared to occur at somewhat higher frequency in the anemic population than controls, although not statistically significant.

Complete sequencing of the female subject who exhibited iron deficiency anemia and the uncommon R446W polymorphism was performed on the entire coding region, in order to determine if additional polymorphisms or mutations might be identified that might explain her iron deficiency anemia. No additional mutations or novel polymorphisms were found.

Two of the uncommon polymorphisms, the R446W (murine equivalent Q446W) and the V795I were introduced by site directed mutagenesis into murine *Tmprss6* and tested for their ability to inhibit murine *Hamp1* promoter driven reporter activity. *Tmprss6* mutants Q446W and V795I were able to repress *Hamp1* promoter driven luciferase activity whereas the *Mask* mutation could not (Figure 2). Nevertheless, the *Tmprss6* R446W polymorphism was slightly less effective than wildtype *Tmprss6* at higher levels of expression (Figure 2).

We examined subjects from two families with iron refractory iron deficiency anemia for mutations of *TMPRSS6* (Figure 1). In family 1, a Belgian family, both patient 1 and 2 were compound heterozygotes for a splicing defect that is predicted to cause skipping of exon 13 (IVS13+1 G>A) and a point mutation in exon 16 specifying L674F (Figure 1). The exon 13 (IVS13+1 G>A) mutation has been previously described in a kindred from northern Europe (10). Although the leucine at amino acid position 674 is highly conserved among species down to zebrafish, testing of the L674F mutation for biological activity demonstrated that it was not associated with a loss in ability to inhibit *Hamp1* reporter expression (Figure 2). In family 2,

a Dutch family, patient 3 was homozygous for the deletion of coding nucleotide 497T of leucine 166 (L166X) leading to a frame-shift error in exon 5 and termination after 36 additional amino acids (Figure 1). His sister (patient 4) and mother, who exhibited iron deficiency but responded to oral iron therapy, were compound heterozygotes for the frame-shift error in exon 5 and the uncommon R446W polymorphism in exon 11. In each case, iron deficiency was associated with high levels of urinary hepcidin (Figure 1) and serum hepcidin (data not shown) indicating failure of hepcidin suppression that normally occurs as a physiologic response to iron deficiency.

DISCUSSION

While we did not find any of the common polymorphisms of *TMPRSS6* to be risk factors for iron deficiency, we identified three rare polymorphisms G228D, R446W and V795R of which R446W allele occurred in slight excess in the anemic population. In addition we identified rare mutations of *TMPRSS6* in two families associated with refractory iron deficiency anemia. In a Belgian family, compound heterozygosity for a previously described splicing defect (IVS13 +1 G>A) (10), and a point mutation in exon 16 specifying L674F were found to be associated with disease. The splicing defect would result in an in frame deletion of exon 13 leading to V490G and loss of amino acids 491-528 encompassing the entire second LDLa domain. Although the leucine corresponding to the L674F mutation is highly conserved among species, our experiments did not demonstrate a loss of function associated with the L674F mutation, suggesting that this mutation is possibly in linkage disequilibrium with an unidentified non-coding mutation in this family. In a Dutch family, homozygosity for a single nucleotide deletion (497T del) causing a frameshift at leucine 166 (L166X) in exon 5 was associated with disease. In addition, compound heterozygosity for the frameshift error L166X and a R446W mutation in exon 11 was associated with iron deficiency anemia that was responsive to oral iron therapy. In each case, iron deficiency is associated with high levels of urinary hepcidin, indicating failure of the hepcidin suppression that normally occurs as a physiologic response to iron deficiency.

The hypomorphic status of the R446W isoform of TMPRSS6 is suggested by two observations. First, it is over-represented (although not significantly so) in a population of iron deficient individuals. Second, it is associated with anemia and iron deficiency when carried in trans with the exon 5 frame-shift error present the Dutch family. Nevertheless, in vitro, the 446W isoform seems to have near normal biological activity, although we cannot rule out that the 446W isoform might be less stable. Our data indicate that simple heterozygosity of R446W may not produce a phenotype but compound heterozygosity with a severe mutation is associated with a milder disease.

In conclusion, the common polymorphisms in *TMPRSS6* are not likely to be associated with iron deficiency anemia observed in the general population. On the other hand, sporadic mutations in *TMPRSS6* are more likely associated with iron refractory iron deficiency anemia. The uncommon R446W polymorphism may contribute to iron deficiency anemia when carried in trans with a severe *TMPRSS6* mutation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors would like to acknowledge the immense contribution of Dr. Ernest Beutler for the conception of the project, data analysis and the writing of the manuscript before his untimely passing.

This is manuscript number 19931-MEM from The Scripps Research Institute. This work was supported by grants from the National Institutes of Health (DK53505-10), the Stein Endowment Fund and The Skaggs Scholars in Clinical Science Program from The Scripps Research Institute. The authors would also like to thank Yu Xia, Kevin Khovananth and Bruce Beutler for their help with DNA sequencing.

References

1. Buchanan GR, Sheehan RG. Malabsorption and defective utilization of iron in three siblings. J Pediatr 1981;98:723–728. [PubMed: 7229750]
2. Hartman KR, Barker JA. Microcytic anemia with iron malabsorption: An inherited disorder of iron metabolism. Am J Hematol 1996;51:269–275. [PubMed: 8602626]
3. Fleming MD, et al. Microcytic anaemia mice have a mutation in Nramp2, a candidate iron transporter gene. Nat Genet 1997;16:383–386. [PubMed: 9241278]
4. Fleming MD, Romano MA, Su MA, Garrick LM, Garrick MD, Andrews NC. Nramp2 is mutated in the anemic Belgrade (b) rat: evidence of a role for nramp2 in endosomal iron transport. Proc Natl Acad Sci USA 1998;95:1148–1153. [PubMed: 9448300]
5. Priwitzerova M, et al. Severe hypochromic microcytic anemia caused by a congenital defect of the iron transport pathway in erythroid cells. Blood 2004;103:3991–3992. [PubMed: 15121718]
6. Mims MP, et al. Identification of a human mutation of DMT1 in a patient with microcytic anemia and iron overload. Blood 2005;105:1337–1342. [PubMed: 15459009]
7. Iolascon A, d'Apolito M, Servedio V, Cimmino F, Piga A, Camaschella C. Microcytic anemia and hepatic iron overload in a child with compound heterozygous mutations in DMT1. Blood 2006;107:349–354. [PubMed: 16160008]
8. Lam-Yuk-Tseung S, Camaschella C, Iolascon A, Gros P. A novel R416C mutation in human DMT1 (SLC11A2) displays pleiotropic effects on function and causes microcytic anemia and hepatic iron overload. Blood Cells Mol Dis 2006;36:347–354. [PubMed: 16584902]
9. Du X, et al. The serine protease TMPRSS6 is required to sense iron deficiency. Science 2008;320:1088–1092. [PubMed: 18451267]
10. Finberg KE, et al. Mutations in TMPRSS6 cause iron-refractory iron deficiency anemia (IRIDA). Nat Genet 2008;40:569–571. [PubMed: 18408718]
11. Guillem F, Lawson S, Kannengiesser C, Westerman M, Beaumont C, Grandchamp B. Two nonsense mutations in the TMPRSS6 gene in a patient with microcytic anemia and iron deficiency. Blood 2008;112:2089–2091. [PubMed: 18596229]
12. Mayo MM, Samuel SM. Iron deficiency anemia due to a defect in iron metabolism: a case report. Clin Lab Sci 2001;14:135–138. [PubMed: 11517621]
13. Andrews NC. Iron deficiency: lessons from anemic mice. Yale J Biol Med 1997;70:219–226. [PubMed: 9544492]
14. Beutler E, Felitti VJ, Koziol JA, Ho NJ, Gelbart T. Penetrance of the 845G->A (C282Y) *HFE* hereditary haemochromatosis mutation in the USA. Lancet 2002;359:211–218. [PubMed: 11812557]
15. Beutler E, Waalen J. The definition of anemia: What *is* the lower limit of normal of the blood hemoglobin concentration? Blood 2006;107:1747–1750. [PubMed: 16189263]
16. Molla AM, Verpoorten C, Eggermont E. The intestinal mucosa of children with iron deficiency. Acta Paediatr Belg 1973;27:5–12. [PubMed: 4732831]

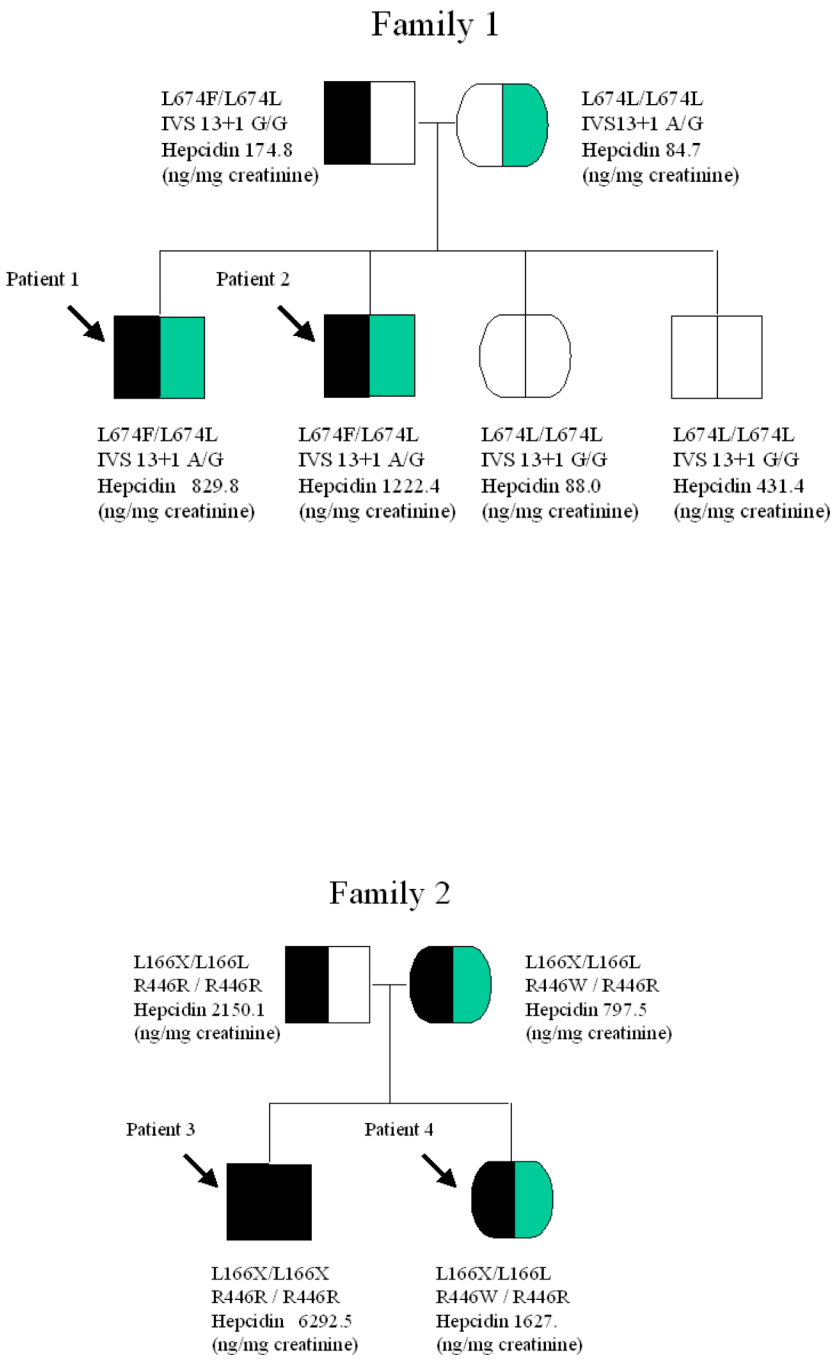

Figure 1.
The pedigrees of two families with iron refractory iron deficiency anemia. The *TMPRSS6* genotypes are indicated as well as the urinary hepcidin levels (ng/mg creatinine). Arrows indicate the patients studied.

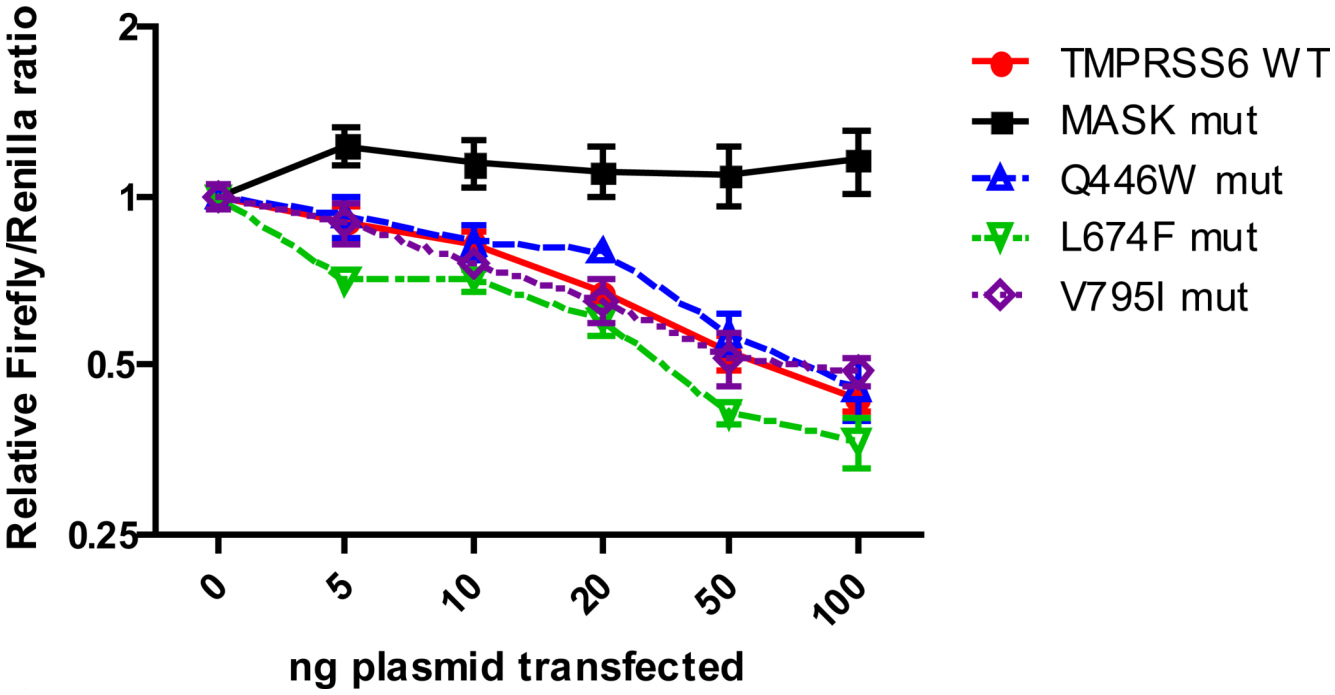

Figure 2.

Inhibition of *Hamp1* promoter driven expression of luciferase by wildtype murine Tmprss6, or the Tmprss6 mutants, Mask, Q446W, V795I, and L674F. HepG2 cells were transfected with 2.5 Kb *Hamp1* promoter driven luciferase constructs along with murine *Tmprss6* wildtype or mutant constructs. The data are presented as mean values and SEM. The *Tmprss6* mutants Q446W, V795I and L674F all inhibited expression of *Hamp1* promoter driven luciferase activity but only Q446W inhibited to a slightly lesser degree than wildtype *Tmprss6* at high plasmid concentrations.

Table 1

Laboratory findings on Patient 1, family 1

Patient 1	**without extra iron**	**with 7 mg/kg elemental iron**	
	at the age of 8 years	**for 6 months**	**for 8 years**
Hb (g/dl)	9	10.1	10.5
MCV (fl)	59	66.7	69.6
Fe (μg/dl)	5	11	15
TIBC (μg/dl)	280	247	211
Transferrin saturation (%)	1.8	4.4	7
Ferritin (ng/ml)	20	65	70

Table 2

Laboratory findings on Patient 2, family 1

Patient 2	1 mg/kg elemental iron	with 7 mg/kg elemental iron	
	at the age of 9 months	for 3 years	for 15 years
Hb (g/dl)	8.2	9.4	10.8
MCV (fl)	63	60	64
Fe (μg/dl)	19	25	24
TIBC (μg/dl)	228	242	258
Transferrin saturation (%)	8	10	7
Ferritin (ng/ml)	41	79	33

Table 3

Iron absorption test with 2 mg/kg elemental iron on Patient 1, family 1

Patient 1	Pre iron-load	after 30’	60’	90’	120’	180’
Fe (μg/dl)	14	20	21	26	19	21
TIBC (μg/dl)	260	243	249	265	239	242
Transferrin saturation (%)	5	8	8	9	7	8
Ferritin (ng/ml)	57	57	61	55	55	54

Blood Cells Mol Dis. Author manuscript; available in PMC 2011 January 15.

Table 4

Laboratory Data on Patient 3, Family 2

Patient 3	Apr-97	Apr-97	Mar-98	Nov-01	Nov-04	Jun-05	Feb-06	Nov-06	Mar-07	Jan-08	Jun-08
Hb (g/L)	62.8	103.1	95.1	95.1	119.2	124.1	120.9	99.9	96.6	108	101.5
Ferritin (ng/ml)	16	66	59	113	300	323	230	130	99	78	86
MCV (fl)	57	64	62	61	67	68	69	65	63	63	64
Reticulocytes × 10^9	27	nd	13	107	nd	nd	nd	nd	49	nd	nd

Table 5

Laboratory Data on Patient 4, Family 2

Patient 4	Nov-01	Oct-02	Nov-04	Feb-06	Nov-06	Mar-07	Jan-08
Hb (g/L)	104.7	101.5	120.9	120.9	108	101.5	117.6
Ferritin (ng/ml)	42	19	7	45	nd	53	73
MCV (fl)	77	77	80	79	78	78	81
Reticulocytes × 10^9	20	nd	nd	nd	nd	49	nd

Table 6

Allele frequencies of the nt. 683 G>A (G228D), nt. 757 A>G (K253E), nt. 1336C> T (R446W), c. 2207 C>T (A736V), and the c. 2383 G>A (V795I) polymorphisms in iron deficient and control white adults. The frequencies of the minor alleles are shown. The numbers in parentheses are the number of minor alleles/total number of alleles). Coding coordinates numbering from the start of translation are given for both nucleotides and amino acids.

Group	683A (228D)	757G (253E)	1336T (446W)	2207T (736V)	2383A (795 I)
Controls	0 (0/100)	0.443 (78/176)	0.007 (4/592)	0.324 (59/182)	0.0056 (1/178)
Iron deficient men	0.0074 (1/136)	0.337 (37/110)	0.017 (2/118)	0.467 (56/120)	0.0074 (1/136)
Iron deficiency anemia men	0.0074 (1/136)	0.286 (24/84)	0.023 (2/88)	0.489 (44/90)	0.0074 (1/136)
Iron deficient women	0 (0/124)	0.402 (49/122)	0.007(1/136)	0.500 (69/138)	0.0152(2/132)
Iron deficiency anemia women	0 (0/52)	0.375 (18/48)	0.019 (1/52)	0.385 (2/52)	0 (0/50)