The role of *TMPRSS6* gene variants in different types of iron deficiency anaemia – from the rare severe hereditary IRIDA to the common mild acquired IDA

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Introduction & Objectives

Iron-refractory iron-deficiency anaemia (IRIDA) is a rare autosomal-recessive disease characterized by severe hypochromic microcytic anaemia, low serum iron and transferrin saturation, normal-high ferritin and inappropriate high levels of the hormone hepcidin. Patients are unresponsive to iron oral treatment and present a slow persistent response to intravenous iron injections [1].

The disease is caused by loss-of-function mutations in the *TMPRSS6* gene which encodes the matriptase-2 (MT2), a negative regulator of hepcidin transcription. In those patients, high hepcidin levels prevent iron absorption in the duodenum and iron recycling by macrophages (Fig. 1). Furthermore, it has been suggested that common variants in *TMPRSS6* might modulate haematological phenotype and iron status [2].

Therefore, the objective of this work was to search for severe genetic variants in *TMPRSS6* in order to elucidate IRIDA-like phenotypes in some patients and to evaluate whether the SNP rs855791 influences **iron deficiency anaemia (IDA)** susceptibility in Portuguese women.

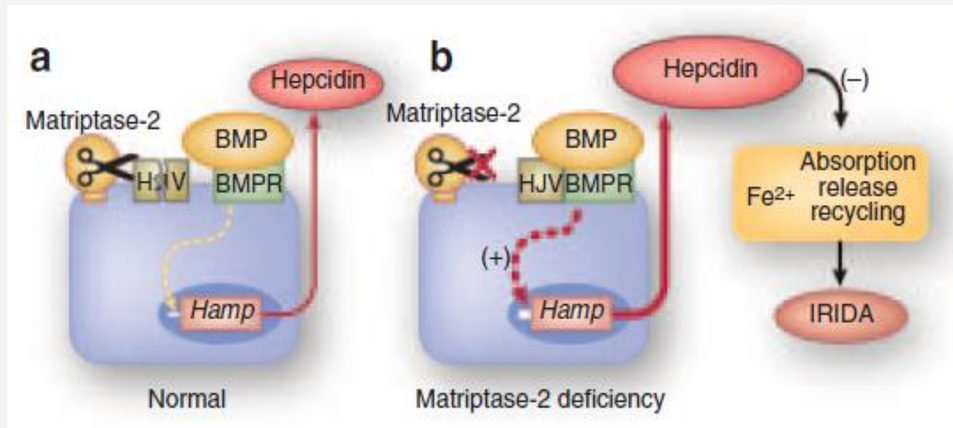

Fig.1. Regulation of hepcidin expression by matriptase-2.
(a) Matriptase-2 prevents hepcidin overexpression by degrading hemojuvelin (HJV), which acts as a co-receptor for bone morphogenetic protein (BMP) to promote *HAMP* gene expression.
(b) In matriptase-2 deficiency high levels of HJV enhances the BMP signaling pathway, leading to overexpression of hepcidin. Hepcidin inhibits iron absorption, release, and recycling, thereby causing IRIDA [3].

Patients & Methods

Coding regions and intron/exon junctions of *TMPRSS6* gene were amplified by PCR and sequenced in 6 cases presenting an IRIDA-like phenotype (microcytic and hypochromic anaemia, low transferrin saturation, refractory to iron oral treatment).

The SNP rs855791 (c.2321C>T, p.A736V) within *TMPRSS6* gene was characterized, using an *Allele Specific Amplification* approach, in 25 women presenting IDA and in 89 women normal controls.

Results

TMPRSS6 variants and IRIDA-like phenotypes

Table I – Genetic variants found in IRIDA-like patients

TMPRSS6 genetic variants	Gene localization	Consequences
c.57 A>G; p.K253E rs2235324	Exon 7	Causes IRIDA
c.1369+4 A>T	Intron 11	Novel splicing variant?
c.1563 C>T; p.D521D rs4820268	Exon 13	SNP associted to IDA
c.2207 C>T; p.A736V rs855791	Exon 17	SNP associated to IDA
c.2217 T>C; p.Y739Y rs2235321	Exon 17	SNP associated to IDA

Sequencing analyses of *TMPRSS6* gene in IRIDA-suspected cases revealed the presence of a previously described pathogenic variant, c.57 A>G; p.K253E (Fig. 2) [4], a novel putative splicing variant, c.1369+4 A>T, whose functional effect is under study, and 3 common variants (SNPs) previously associated with iron deficiency anaemia (Table I) [2].

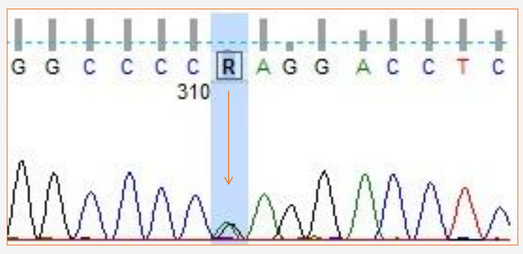

Fig. 2. Partial sequence of exon 7 of the *TMPRSS6* gene in one patient presenting an IRIDA-like phenotype.
Arrow shows the c.57 A>G alteration in heterozigosity.
This pathogenic missense mutation causes an alteration from lysine to acid glutamic in position nr. 253 of matriptase-2.

TMPRSS6 SNP rs855791 and IDA susceptibility

Molecular characterization of SNP rs855791, c.2207C>T; p.A736V, was performed in a group of women presenting IDA and in a group of normal control women. The genotypic and allelic frequency determination revealed that the CC genotype and the C allele (736A) are significantly less frequent in the group presenting IDA. In agreement, the frequency of the TT genotype and of the T allele (736V) is significantly higher in the group presenting IDA (Table II).

The rs855791 genotypes showed a statistically association with haemoglobin, serum iron level and transferrin saturation (Table III and Fig. 3).

Table II – Genotypic and allelic frequencies of SNP rs855791 in normal and IDA women

SNP rs855791	Normal group (n=89)	IDA group (n=25)	*p value*[1]
Genotypic frequencies			
CC	38 (42.7%)	8 (32%)	
CT	47 (52.8%)	12 (48%)	0.037*
TT	4 (4.5%)	5 (20%)	
Allelic frequencies			
C	123 (69.1%)	28 (56%)	0.08
T	55 (30.9%)	22 (44%)	

* Statistical significance considered at p<0.05.
[1] Chi-Square test of independance

Table III – Significant association between SNP rs855791 genotype, haemoglobin and iron status parameters

SNP rs 855791 genotype	Hb (g/dL)			Serum Iron (µg/dL)			TS (%)		
	Mean	Median	*p value*[1]	Mean	Median	*p value*[2]	Mean	Median	*p value*[2]
CC	13.05	12.88		108.60	106.00		30.66	28.57	
CT	12.70	12.53	0.0362*	87.04	89.00	0.0091*	23.94	24.24	0.0154*
TT	11.68	11.50		61.89	66.00		17.03	18.13	

* Statistical significance considered at p<0.05; [1] Kruskal-Wallis test; [2] One-way analysis of variance;
Hb = Hemoglobin; TS = transferrin saturation

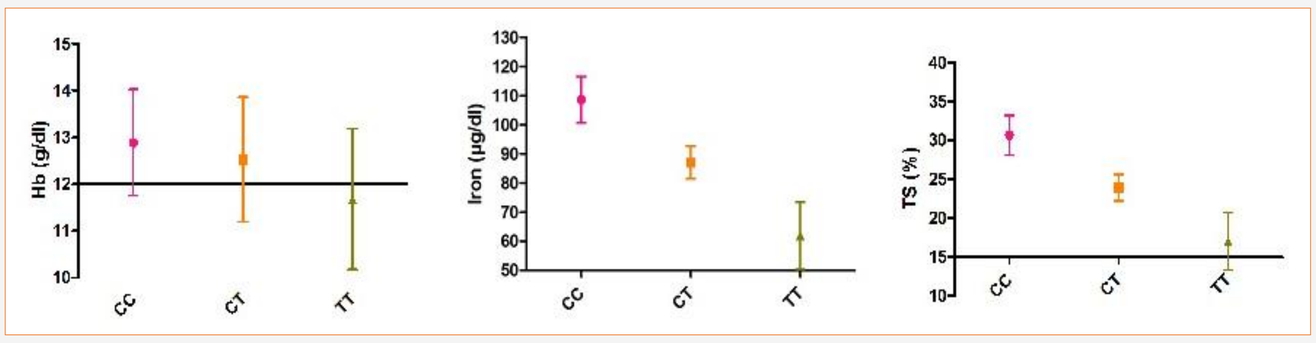

Fig. 3. Significant association between SNP rs855791 genotypes and haemoglobin, serum iron level and transferrin saturation.

Conclusions

✓ **IRIDA is an autosomal-recessive genetic disease, consequently, both *TMPRSS6* alleles must be severely affected in order to the pathology be manifested. Thus, *TMPRSS6* gene can be affected by two different severe mutations, in compound heterozygosity, or by the same mutation in the homozygous form. However, these conditions were not verified in our IRIDA-like patients. Nevertheless, a severe iron deficiency anaemia phenotype can be attributed to the presence, in heterozygosity, of one *TMPRSS6* pathogenic mutation together with the occurrence of SNPs already reported as risk factors to develop iron deficiency anaemia (e.g. the allele T of SNP rs855791). This situation was observed in some of our patients.**

✓ **Our study allowed to conclude that, in Portuguese women, there is an association between the allele T and the genotype TT of SNP rs855791 and IDA susceptibility. This SNP gives rise to partial impairment of Matriptase-2 and consequently is able to perturb iron absorption in the duodenum as well iron recycling by macrophages. It is considered a risk factor to develop iron deficiency anaemia.**

✓ **This study also suggests that *TMPRSS6* gene has a role in iron-related common disorders in which it may act as a gene modifier.**

References

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