The protein Matriptase-2 damaged by a novel missense mutation in the *TMPRSS6* gene originates an IRIDA-like phenotype in an African child

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Introduction

Iron-refractory iron deficiency anemia (IRIDA) is a rare autosomal recessive anemia usually unresponsive to oral iron intake and partially responsive to parenteral iron treatment [1].

This disease is the result of mutations in *TMPRSS6* gene, encoding Matriptase-2, a transmembrane serine protease that plays a key role in down-regulating hepcidin, allowing iron bioavailability for erythropoiesis (Figure 1) [1,2].

Once *TMPRSS6* is mutated, the corresponding protein is absent or inactive at the hepatocyte membrane leading to uncontrolled high levels of hepcidin and impaired iron absorption [3].

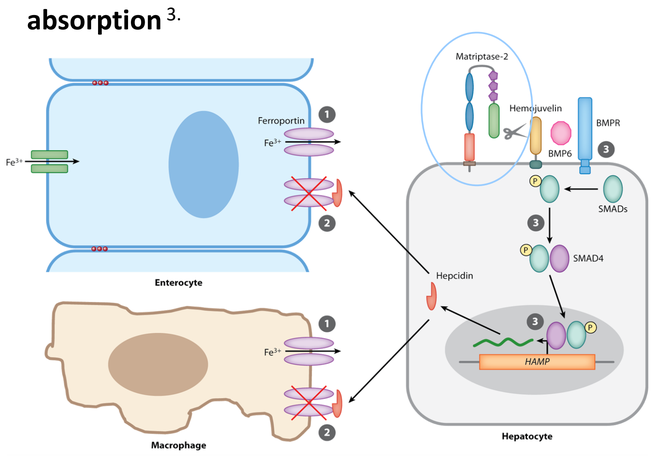


Figure 1: **Matriptase-2** (circled in blue) **regulates cellular iron export**:
(1) Dietary iron and iron recycled from erythrocytes are stored in enterocytes and splenic macrophages and are released to the circulation through ferroportin;
(2) Hepcidin produced by hepatocytes binds ferroportin and targets the channel for degradation.
(3) Hepcidin gene (*HAMP*) expression is positively regulated by bone morphogenic protein (BMP)6, which signals through the BMP receptor (BMPR)-SMAD pathway in a hemojuvelin-dependent manner. Matriptase-2 increases cellular iron export by degrading hemojuvelin to reduce hepcidin production and thus increase ferroportin[2].

Aims

This study aimed to investigate a 4-year-old boy of sub-Saharan ancestry (Mozambique/Angola), presenting with microcytic hypochromic anemia, low transferrin saturation, normal ferritin, and having a partial response to intravenous iron treatment (Table 1).

Material and Methods

The subject is a -$\alpha^{3.7kb}$-thalassemia carrier. *TMPRSS6* gene was screened for variants by Next-Generation Sequencing using Nextera XT libraries in a MiSeq platform (Illumina). Genetic variants found were validated by Sanger sequencing. *In silico* analyses were performed in HSF, SIFT, Poly-Phen2 and Missense3D softwares. In order to run a Missense3D analysis, we predicted a 3D Structure for matriptase-2 in the software Phyre2.

Table 1: **Hematologic parameters and iron status of the patient** before and after intravenous iron treatment in comparison to reference values

Hematologic and biochemical parameters	1-4 years old Reference	Patient Iron treatment Before	After
RBC (x10^12/L)	3.50–5.30	4.51	4.38
Hb (g/dL)	10.7–15.1	**9.8**	**10.2**
Ht (%)	31.0–45.0	31.7	33.1
MCV (fL)	72.0–100.0	70.3	75.6
MCH (pg)	23.8–34.2	**21.7**	**23.3**
MCHC (g/dL)	31.6–34.9	**30.9**	**30.8**
RDW (%)	11.6–13.9	**17.7**	**16.6**
Fe (µg/dL)	62-68	**19**	**51**
TIBC (µg/dL)	228-428	313	273
TSAT (%)	16.0-45.0	**6.07**	18.68
Ft (µg/L)	30.0-300.0	33.0	242.3

Results and Discussion

- ✓ DNA analysis revealed a **novel missense mutation c.871G>A** in heterozygosity, in ***TMPRSS6* exon 8.** Additionally, 3 SNPs previously associated with a greater risk of developing iron deficiency anemia (**K253E**, **V736A** and **Y739Y**) were also identified (**Figure 2**). At protein level, the novel variant gives rise to the G291S mutation, located in the first CUB1 domain, which suggest it may affect the enzyme activation and substrate recognition (**Figure 2**).
- ✓ *In silico* analysis indicates the conserved amino acid G291 change may be damaging to the protein stability (**Figure 3**).

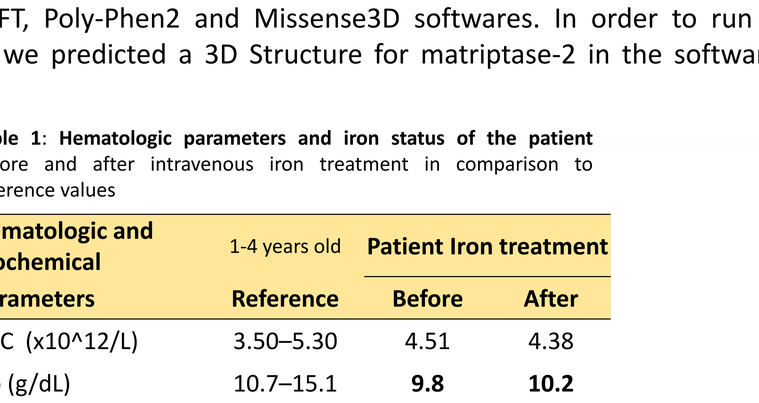
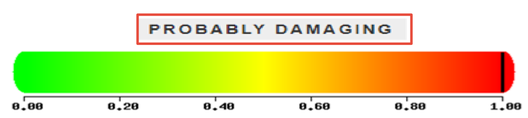


Figure 2: Schematic model of matriptase-2. a Structurally, matriptase-2 contains a short N-terminal cytoplasmic domain, a membrane-spanning region (TM), an SEA domain, 2 CUB domains, three LDLa domains, and a trypsin-like serine protease domain. Coding region mutations are indicated by arrows. The novel G291S mutation is highlighted in yellow. Adapted from Lee *et al*. 2009 [4]

(a)



(b)

dbSNP	# CHR	POS	REF_ ALLELE	ALT_ ALLELE	AA_ CHANGE	SIFT_ SCORE	SIFT_ MEDIAN	#_SEQS_AT_ POSITION	SIFT_ PREDICTION
rs145053404	22	37482452	G	A	G291S	0	3.01	22	DELETERIOUS

(c)

#	Variants	Analysis Results
1	final (-) | 291 GLY > SER	Cavity altered · Gly in a bend

(d)

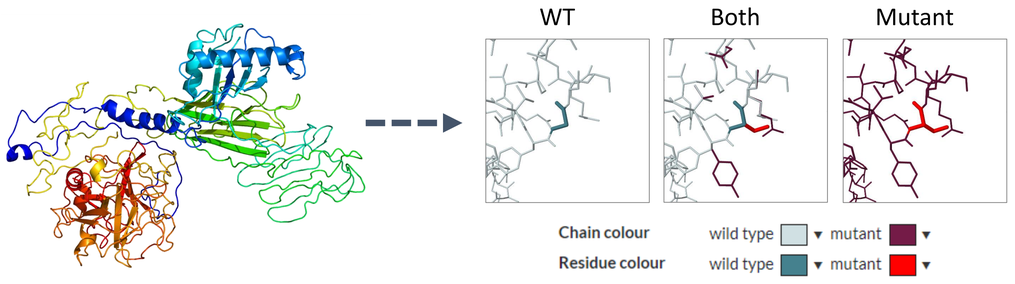


Figure 3: Results from *in silico* analysis showing potential damage to the protein stability. Results from:
(a) Poly-Phen2 that takes into consideration differences between human proteins and homologous proteins in other mammals (score = 1.000);
(b) SIFT, that assumes the importance of one amino acid based on its level of conservation within protein the family;
(c) Missense3D, that perceives the importance of a amino acid based on 3D structure of the whole protein;
(d) Also in **Missense3D** we can compare, based on the predicted structure for matriptase-2 obtained in the software Phyre2, with 90% of confidence, the amino acids alteration consequence in the protein structure.

Conclusions

Although IRIDA is noted as an autosomal recessive disease, we infer that, in this case, the result of a digenic inheritance of the novel damaging mutation (c.871G>A; G291S) and 3 common modulating SNPs in the same gene in addition to the co-inheritance of the α-thalassemia allele, may add up to an IRIDA-like phenotype. Further functional studies of the mutated protein as well as family studies should be conducted.

References

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The authors declare no conflict of interest.