The *Mask* Mutation Identifies TMPRSS6 as an Essential Suppressor of Hepcidin Gene Expression, Required for Normal Uptake of Dietary Iron
Iron Regulation 101*

* partially adapted from ASH 2007 Educational Session on Iron Metabolism

**Diagram:**
- **Storage:** Liver
- **Recycling:** Spleen, RBC
- **Flow:** Plasma, Duodenum, Bone Marrow
- **Iron Levels:**
  - Plasma: 4mg
  - Duodenum: 1-2mg
  - Bone Marrow: 20mg/day
  - Spleen: 2mg
  - Recycling: 20mg/day
  - Losses: 1-2mg
Hepcidin and Ferroportin

• Hepcidin - 25 aa peptide made in hepatocytes
  - iron flow regulation hormone

• Ferroportin - cellular iron exporter to plasma
  (duodenal, hepatic, macrophage, placental cells)

• Hepcidin binds ferroportin and causes internalization and degradation of ferroportin
Iron Exporting Cell

duodenal hepatocyte macrophage

Fe

iron pump

Fe

ferroportin

Fe

plasma side

the beyond
Iron Exporting Cell

duodenal hepatocyte macrophage

Iron pump

ferroportin

lysosome

hepcidin

plasma side

the beyond
Hepcidin and Iron Flow

- **HEPCIDIN**
- **PLASMA Fe**
- **LIVER**
- **SPLEEN**
- **DUODENUM**
- **RBC**
- **BONE MARROW**
Regulation of Hepcidin

- Fe and inflammation (IL1, IL6) $\uparrow$ hepcidin
- anemia, hypoxia, erythropoeisis $\downarrow$ hepcidin

Transcriptional control of hepcidin*

Extracellular:
- TfR2
- HFE
- HJV
- BMP
- BMPR
- IL6 R

Intracellular:
- SMAD4
- STAT3

5' $\cdots$ HEPcidin
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What is the *Mask Mouse*?! 

- mice with homozygous recessive gene causing chronic iron deficiency (genetic abN caused by ethylnitrosourea, ENU, mutagenesis)

- phenotypic truncal alopecia, hence the name “mask”
Hepcidin and the *Mask* Mouse

- both normal and *Mask* mice were fed Fe\(^{59}\) diets
- 24hr clearance of Fe\(^{59}\) monitored with \(\gamma\) counter

![Graph showing clearance over time.](image)

at plateau of clearance, total body counts stable, so no evidence of bleeding
Hepcidin and the *Mask* Mouse ...2

Mask mice found to have high liver levels of hepcidin mRNA (in Fe def state, should normally have low hepcidin mRNA)
Finding the Gene

positional cloning of the \textit{Mask} region finds the \textit{Tmprss6} gene

- gene encodes a membrane bound serine protease of previously unknown function

- \textit{Mask} mut is $A \rightarrow G$ transition in 3’ end (splicing error) = premature truncation = loss of serine protease
Proving Loss Causes Phenotype

in vivo

• rescued *Mask* mice with BAC transfection

4/4 mice reversed phenotype
3/4 corrected iron
3/4 corrected anemia
Proving Loss Causes Phenotype

in vitro

- transfected HepG2 cell line to express WT TMPRSS6 and mutated TMPRSS6 (lacking serine protease)
  - WT cells showed marked inhibition of hepcidin despite HJV, BMP, IL1 and IL6 stimulation
  - mutants had diminished inhibitory activity
Conclusions

- TMPRSS6 down regulates hepcidin

- TMPRSS6 is a non-redundant component of a hepcidin suppression pathway

- TMPRSS6 prevents up regulation of hepcidin by all known stimulators (upstream action)
Future Questions

- confirmation in humans

- can TMPRSS6 be new therapeutic target?
  - inhibitors may increase hepcidin levels and potentially relieve iron O/L
  - agonists may decrease hepcidin levels and potentially ameliorate ACI
“Better ask the doctor. I’m just in here because the waiting room’s too full.”