When iron regulatory proteins go bad!

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Upon completion of this session (and perhaps some private review), you should be able to:

• List the proteins involved in body iron regulation.
• Describe the function of each protein involved in iron regulation
• Explain how mutations to iron regulatory proteins results in hereditary anemias and hemochromatoses.

Outline

• Case study introduction
• Overview of iron regulation
• Review of nutritional iron deficiency
• Details of iron regulation and dysregulation
  – Hemochromatosis
  – Hereditary iron deficiency anemia
• Laboratory assessment of iron restricted erythropoiesis – sometimes it isn’t iron deficiency at all

Case study

• 2 year old girl seen during a well-child visit and identified with anemia
• Microcytic, hypochromic
• Low serum iron
• Treated with iron supplements and was non-responsive after expected interval with adequate treatment
Overview of iron regulation

Iron is a highly reactive molecule

- This allows it to bind and release oxygen in hemoglobin
  - That's a good thing 😊
- Iron’s reactivity also makes it toxic by causing oxidation of proteins, lipids, DNA via the Fenton reaction
  - \[ \text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{HO}• + \text{OH}^- \]
  - \[ \text{Fe}^{3+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{2+} + \text{HOO}• + \text{H}^+ \]
  - That's a bad thing.... 😞

Free radicals = oxidizing agents

So the body has to keep iron tightly regulated

Iron is hard to acquire from the environment/diet

- Iron excess was unlikely for our Paleolithic ancestors
  - So an excretion mechanism for iron did not develop
- The only mechanism for iron regulation is via intestinal absorption from the diet
  - Increase absorption when the body needs iron
  - Decrease absorption when the body has adequate iron and needs to avoid having too much
Overview of iron kinetics

This salvage and recycling of iron is an important continuing source of iron.

Iron is carried into the enterocyte by a luminal membrane protein – Divalent metal transporter 1 (DMT-1)

Iron is transported out the other side of the cell and into the blood by a membrane protein – ferroportin

Enterocyte Development

Absorptive cells for nearly everything we absorb from our intestines

When the body's need for iron has been satisfied, this system needs to be shut down temporarily so that iron excess does not develop – this is where the liver comes in.

When the body needs iron

GI Lumen

Blood

Liver

Cross Section of Intestine
When the liver senses that there is enough iron in the body, it produces a protein called **hepcidin**.

Hepcidin will block the absorption of iron from the enterocyte into the blood by causing the degradation of ferroportin.

Once the level of body iron begins to decline again, hepcidin production will decline and ferroportin will be active again.

Hepcidin also regulates release of iron from macrophages and hepatocytes via ferroportin in their membranes.
Quick review of how iron deficiency can develop

Iron deficiency develops when intake doesn’t keep up with need

- When the diet is inadequate or not bioavailable
- When need is increased
  - Infants and children need more for growth
  - Women need more during pregnancy because they need to support the growth of the fetus
- When iron is lost in excess
  - Hemorrhage, especially slow, chronic bleeding from GI or renal tracts
  - Hemoglobinemia resulting in hemoglobinuria
  - Menstruation and delivery/lactation
- When absorption is impaired
  - Celiac disease or gastric bypass

We need to take a closer look at how hepatocytes respond to iron status and produce hepcidin
But first – a bit of background on pertinent cell biology

How do cells receive and respond to messages from outside themselves?

1. The ligand (signal) binds to a receptor on the cell membrane that extends into the cytoplasm

2. Ligand binding causes a conformational change to the cytoplasmic domain of the receptor so it can catalyze cytoplasmic reactions creating second messengers.

3. The second messenger may produce a cellular response OR transport into the nucleus to affect gene expression

4. If target gene codes for a protein and the gene gets turned on (up regulated) by the second messenger, then more of the protein will be produced

Production of hepcidin is the expression of the hepcidin gene as a result of signal transduction in hepatocytes

Let’s look at the signal transduction process leading to hepcidin production

Because remember, when hepcidin rises, ferroportin activity decreased and iron absorption decreases

Got an idea about what is happening for our patient…shhhh….don’t tell
1. Ligand with information about how much iron is circulating
2. Membrane receptors receive the message and transfer the information inside the cell = TFR2 associates with HFE and hemouvelin associates with BMPR to initiate the internal signal

There are two pathways to hepcidin production in hepatocytes

- Diferic TF
- Haptoglobin
- Ferritin

4. The hepcidin gene is upregulated. Increased plasma hepcidin and diminished ferroportin activity

When the liver senses that there is enough iron in the body, it produces hepcidin – which decreases ferroportin activity in enterocytes, macrophages, liver

Iron = Hepcidin = Ferroportin
So iron absorption and recycling slows

Iron = Hepcidin = Ferroportin
So iron absorption and recycling rise
When iron regulatory proteins go bad!

What happens if any of these proteins’ genes are mutated and the proteins are non-functional?

Hepcidin cannot be produced and ferroportin is constantly active

Fe is continuously absorbed

That’s hemochromatosis = Iron overload

In most forms of hemochromatosis, mutations of iron regulatory proteins prevent production of hepcidin

Iron absorption is continuous due to ferroportin activity in the intestine

Iron Overload Phenotype

- Liver dysfunction – cirrhosis – carcinoma
- Cardiomyopathy with heart failure
- Diabetes
- Skin discoloration – “bronzed diabetes”
- Hypothyroidism
- Hypogonadism

http://www.merckmanuals.com/professional/hematology-and-oncology/iron-overload/hereditary-hemochromatosis
## Type of Hemochromatosis

<table>
<thead>
<tr>
<th>Type</th>
<th>1</th>
<th>2A</th>
<th>2B Juvenile</th>
<th>3</th>
<th>4</th>
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</thead>
<tbody>
<tr>
<td>Affected gene</td>
<td>HFE</td>
<td>HFE2 (HJV)</td>
<td>MAMP</td>
<td>TTR2</td>
<td>SLC40A1</td>
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<tr>
<td>Mutated protein</td>
<td>Hereditary hemochromatosis protein</td>
<td>Hemojuvelin</td>
<td>Hepcidin</td>
<td>Transferrin receptor protein 2</td>
<td>Ferroportin 1</td>
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<tr>
<td>Type of mutations</td>
<td>Loss of function</td>
<td>Loss of function</td>
<td>Loss of function</td>
<td>Loss of function</td>
<td>Gain of function</td>
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<tr>
<td>Inheritance pattern of most common alleles</td>
<td>Autosomal recessive</td>
<td>Autosomal recessive</td>
<td>Autosomal recessive</td>
<td>Autosomal recessive</td>
<td>Autosomal dominant</td>
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<tr>
<td>Age of onset</td>
<td>Adulthood; Men earlier</td>
<td>Childhood</td>
<td>Childhood</td>
<td>In between</td>
<td>Childhood</td>
</tr>
</tbody>
</table>

**Hepcidin-regulatory proteins in hepatocytes**

What happens if any of these proteins’ genes are mutated and the proteins are non-functional?

- Hepcidin cannot be produced and ferroportin is constantly active
- Fe is continuously absorbed

**That’s hemochromatosis = Iron overload**

### Ferroportin mutation

The hepatocyte functions normally to produce hepcidin trying to reduce iron absorption.

But the enterocyte ignores hepcidin due to its mutation and continues to absorb iron.
How can hepcidin production be suppressed temporarily?

Matriptase-2 (MTP-2) is a protein in the membrane that cleaves hemjuvelin HJV. MTP-2 responds to hypoxia and how much iron is in storage. When stored iron is low, matriptase-2 is active.

- Decreased plasma hepcidin → Active ferroportin → Increased iron absorption

You must have normal matriptase-2 for normal iron absorption.

So normal regulation of hepcidin production turns on and off – depending on how much iron is present in the body, in part by regulating the activity of matriptase 2 – to create iron homeostasis.
REMEMBER HEMOCROMATOSIS?

Most cases of hemochromatosis are due to a mutation of HFE.

If matriptase-2 can be suppressed in the other pathway, then HJV will be active, and more hepcidin can be produced to reduce iron absorption.

So mechanisms of diminishing matriptase-2 activity could be a way to treat some forms of hemochromatosis.

Now...we get to talk about iron deficiency.

And our patient....

What happens if there is a mutation in matriptase-2 gene???

Matriptase-2 never cleaves hemojuvelin so it is always active.

Persistently increased plasma hepcidin

Persistently ineffective ferroportin

Persistently decreased iron absorption and recycling.

This is known as Iron Refractory Iron Deficiency Anemia (IRIDA).
Iron Refractory Iron Deficiency Anemia (IRIDA)

- Hereditary mutations (lots of different ones are known) of matriptase-2
  - Usually autosomal recessive
- Persistent activation of HIV leads to persistent production of hepcidin, so ferroportin is never active
  - GI ferroportin seems to be most sensitive to hepcidin; retain some macrophage ferroportin activity
- GI iron absorption is impaired and iron deficiency anemia (IDA) results
- Called "iron refractory" because it is IDA that does not usually respond to oral iron supplements since GI ferroportin is inactivated by hepcidin

IRIDA – laboratory picture untreated

- Marked microcytic, hypochromic anemia
- Low serum iron (hypoferremia)
- Usually normal TIBC
- Low transferrin saturation
- Serum ferritin is usually normal/elevated – hyperferritinemia
- Low reticulocyte count
Special testing

- High urinary hepcidin
  - In typical iron deficiency, hepcidin is VERY low so that ferroportin is active as the body tries to absorb all it can

More on IRIDA

- Anemia not present at birth
  - The transfer of iron from mom to baby is like giving the baby IV iron; gi absorption is not needed
  - Shows up shortly after that though, because of impaired absorption
  - The delay in onset is an important clinical finding to differentiate IRIDA from inherited mutations of other iron related proteins

Treating IRIDA

- Most patients require parenteral iron
  - Macrophages take up the iron-sucrose and then export iron into the plasma via ferroportin
  - So this is the same process that macrophages use when recycling iron
  - Response is slower than in typical iron deficiency probably because macrophage ferroportin is also affected by high hepcidin levels – just not as much as enterocytes
- Anemia is improved
  - Typically does not fully correct it
  - Microcytosis often remains
- Serum ferritin levels remain normal or slightly increased
  - Probably because macrophages are converting their excess iron to ferritin and slowly releasing it
- FUTURE – anti-hepcidin antibodies or hepcidin gene suppression

True iron deficiency – regulation and recycling are working normally. Any iron from senescent cells gets returned to the plasma quickly.
IRIDA– recycling is impaired but not eliminated. Any iron from senescent cells is retained in macrophages for a time and gets converted to ferritin before export. Ferritin levels can rise but red cells are still starved for iron because they cannot use ferritin as an iron source.

IRIDA trivia

- The gene for matriptase-2 is called *TMPRSS6*
- The gene is located on chromosome 22q12-q13
- 40 different mutations have been reported; some double heterozygotes
- In 2013, 32 families of varying ethnic heritages with 50 identified individuals had been reported
  — Likely it is underdiagnosed

Back to microcytic anemias and other causes of hereditary iron deficiency anemia

A transferrinemia
Deficiency of Divalent Metal Transporter-1 (DMT-1)
TF is the ligand with information about how much iron is circulating.

**Without transferrin:**
- Iron absorption into enterocytes is normal
- Export into plasma is normal
- BUT...Plasma iron is not attached to Tf so it is in ionic form and then is absorbable by most cells
  - But acquisition is not regulated
  - As a result, massive iron overload in tissues = hemochromatosis phenotype
  - But RBCs cannot absorb ionic iron, thus iron deficiency anemia

**Case of Atransferrinemia** (Shamsian, et al)
- 3 mo old girl seen for gastroenteritis
- Healthy parents and sib
- CBC – HB = 4 g/dL (Ref: 11-14)
  - MCV = 71 fl (Ref: 80-100)
  - MCH = 23 pg (Ref: 28-32)
    - Calculated MCHC = 33% (Ref: 32-36)
    - Retics = 0.5% (Ref: 0.5-2)
- Bone marrow – erythroid hyperplasia (ineffective erythropoiesis), absent iron
• At 6 mo, after transfusions and iron and folate supplements, HB= 9.4 g/dL, normal iron studies, normal HB electrophoresis
• At 3 yr, Tf was measured = 24 mg/dL (Ref: 200-300)
• Parents’ Tf levels were:
  – Father = 109 mg/dL
  – Mother = 169 mg/dL
• At 9 yr, HB = 4.5 g/dL, MCV = 62 fl, MCH = 17 pg, [MCHC (calculated) = 28%], retics = 0.5%, ferritin= 837 ng/mL (Ref: 9-90)

A transferrinemia
• Disease develops related to iron accumulation like hemochromatosis that can affect life span
• Autosomal recessive inheritance
• In 2013, 16 cases reported from 14 families
• Treated with phlebotomies to remove iron via RBCs and plasma transfusion to provide Tf or purified apotransferrin

DMT-1 is important in other places too
• Intracellular iron trafficking
  – Especially in red cells
  – Hepatocyte
  – Macrophage
• Iron transport in the placenta, to some degree
Case of DMT-1 deficiency (Privitzerova, et al)

- 20 year old woman
- Transfused shortly after birth and then 8 transfusions in infancy; after that, whenever her HB dropped below 7 gm/dL
- BM = erythroid hyperplasia, decreased hemoglobinization of erythroid precursors, no sideroblasts

DMT-1 deficiency

- Autosomal recessive
- 3 affected families – different mutations
- Anemia is present at birth because DMT-1 is also present in the placenta and because RBCs need DMT-1 to make hemoglobin
- Hypo, micro, anemia with polychromasia
- High serum iron, normal TIBC, increased % sat, elevated ferritin and increased soluble TFR

DMT-1 deficiency case

- HB= 7.4 gm/dL (Ref: 12-15.5)
- MCV = 54 (Ref: 80-90)
- MCH = 15 (Ref: 26-31)
  - Calculated MCHC = 28.5%
- Retics = 2.1% (Ref: 0.5-3%)
  - Estimated reticulocyte production index = 0.6%
- Serum iron increased, TIBC = normal, Ferritin high normal, stfR= 41.5 mg/L (Ref: 1.9-4.4)

More on DMT-1 deficiency

- Treated with transfusions and EPO
  - EPO doesn’t improve iron utilization, just increases the number of poorly hemoglobinized RBCs
  - Oral and IV iron are ineffective due to the other roles for DMT-1
- Iron overload in tissues because iron can get into cells but cannot be used properly
- Normal to low hepcidin levels leads to increased absorption of any iron that enters the enterocyte
  - explanation to follow
Deficiency of DMT-1

- Mutations can be of different types in different individuals
- The same mutation can affect DMT-1 differently in one body location vs others in the same individual

<table>
<thead>
<tr>
<th>Clinical</th>
<th>IRIDA</th>
<th>AT-emia</th>
<th>DMT-1 deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inheritance</td>
<td>Autosomal recessive</td>
<td>Autosomal recessive</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td>Affected protein</td>
<td>Matriptase-2</td>
<td>(Apo)transferrin</td>
<td>DMT-1</td>
</tr>
<tr>
<td>Mutation impact</td>
<td>↑ hepcidin; ↓ ferroportin = decreased iron absorption/recycling</td>
<td>Unregulated iron delivery to cells but not to erythroblasts</td>
<td>Decreased iron absorption and impaired intracellular use including RBCs</td>
</tr>
<tr>
<td>Anemia at birth*</td>
<td>No</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Iron overload phenotype</td>
<td>No</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Treatment</td>
<td>IV iron</td>
<td>IV plasma/Tf</td>
<td>EPO+Tx</td>
</tr>
</tbody>
</table>

*Assumes mother was not iron deficient during pregnancy

Tests of iron restricted erythropoiesis

Differentiating iron deficiency, IRIDA, and other microcytic anemias

Useful analyses in the differential diagnosis

- Classic iron studies
- Ferritin
- Soluble (serum) transferrin receptor (sTfR)
  - Iron deficient cells make more transferrin receptors than normal cells
  - Some of the receptors slough into the plasma and can be measured
  - Increased amounts of sTfR are consistent with iron deficiency in CELLS
- Hepcidin...rarely
**Why is hepcidin low in DMT-1 deficiency and ATf-emia? Kathy’s hypothesis.**

- Polychromatoc normoblasts sense iron
  - When they are iron-starved, NRBCs produce a hormone called erythroferrone
  - It acts on the liver to decrease hepcidin in hopes of stimulating iron absorption by increasing ferroportin activity (mechanism still not clear)
- Erythroferrone rises in any condition in which there is ineffective erythropoiesis and elevated EPO e.g. thal

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**To summarize**

- Iron regulation is heavily dependent on proteins
  - Whenever proteins are involved in a process, mutations can be expected to affect function
- Elucidation of the iron regulatory proteins in the hepatocyte membrane has led to recognition of mutations that cause decreases in hepcidin production and over absorption of iron = hemochromatosis (iron overload)
- Mutations of matriptase-2 lead to increased hepcidin production and iron refractory iron deficiency anemia (IRIDA)

- IRIDA is refractory to oral supplements but can be treated with IV iron
- IRIDA is rare, but also likely underdiagnosed so more cases are expected to be identified in the future by the use of molecular testing
- Atransferrinemia and DMT-1 deficiency are SUPER RARE micro, hypo anemias due to iron transport protein mutations. They are treated with apotf and transfusion/EPO, respectively. Iron accumulations must be managed.
Sources and References


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website: www.ASCLS.org

Dr. Doig received an honorarium for authoring the monograph but receives no compensation for sales.
Questions?

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