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 result 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
  - Fe^{2+} + H_2O_2 → Fe^{3+} + HO• + OH⁻
  - Fe^{3+} + H_2O_2 → Fe^{2+} + HOO• + H⁺
  - That’s a bad thing... 😞
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

When the body needs 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

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 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 enteroctye 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

Hint....our patient has an adequate diet even for a growing toddler

Hint....our patient is not bleeding excessively or hemolyzing
Details of iron regulation

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 & 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

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
There are two pathways to hepcidin production in hepatocytes

1. Ligand with information about how much iron is circulating
2. Membrane receptors receive the message and transfer the information inside the cell = TR2 associates with HFE and hemojuelin associates with BMPR to initiate the internal signal
3. Second messengers carry information into the nucleus
4. The hepcidin gene is up-regulated

Increased plasma hepcidin and diminished ferroportin activity

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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

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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

Iron = Hepcidin = Ferroportin

So iron absorption and recycling slows

Iron = Hepcidin = Ferroportin

So iron absorption and recycling rise

Iron = Hepcidin = Ferroportin

So iron absorption and recycling rise
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

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### Type of Hemochromatosis

<table>
<thead>
<tr>
<th>Type of Hemochromatosis</th>
<th>Type 1</th>
<th>Type 2A Juvenile</th>
<th>Type 2B Juvenile</th>
<th>Type 3</th>
<th>Type 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Affected gene</td>
<td>HFE</td>
<td>HFE2 (HJV)</td>
<td>HAMP</td>
<td>TFR2</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>
</tr>
<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>
</tr>
<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>

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### 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.
Back to regulation of iron absorption

Iron Homeostasis

How can hepcidin production be suppressed temporarily?

Matriptase-2 (MTP-2) is a protein in the membrane that cleaves hemojuvelin (HJV). MTP-2 responds to hypoxia and how much iron is in storage. When stored iron is low, matriptase-2 is active. Decreased plasma haptoglobin and active ferroportin lead to increased 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 HEMOCHROMATOSIS?

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 decreased iron absorption and recycling

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

Matriptase = Hepcidin = Ferroportin

So iron absorption and recycling slows

Iron Refractory Iron Deficiency Anemia (IRIDA)

Did you ever expect that we’d discover a hereditary version of iron deficiency?

Iron Refractory Iron Deficiency Anemia (IRIDA)

- Hereditary mutations (lots of different ones are know) of matriptase-2
  - Usually autosomal recessive
- Persistent activation of HJV 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

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

Atransferrinemia
Deficiency of Divalent Metal Transporter-1 (DMT-1)

Remember transferrin?

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

Remember transferrin as the ligand for hepatocyte iron sensing?

Without transferrin:

- 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 in RBCs

Case of Atransferrinemia (Shamsian, et al)
• 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)

### Atransferrinemia

• 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 to mitochondria
  – Especially in red cells
  – Hepatocyte
  – Macrophage
• Iron transport in the placenta, to some degree

### Case of DMT-1 deficiency (Priwitzerova, 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 case

• HB = 7.4 gm/dL (Ref: 12-15.5)
• MCV = 54 (Ref: 80-90)
• MCH = 15 (Ref: 26-31)
  – Calculated MCHC = 28.5% (Ref: 32-36)
• 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)
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

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

<table>
<thead>
<tr>
<th>Clinical</th>
<th>IRIDA</th>
<th>ATf-emia</th>
<th>DMT-1 deficiency</th>
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</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>*Assumes mother was not iron deficient during pregnancy</td>
<td></td>
<td></td>
<td></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>

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

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Iron Deficiency</th>
<th>Anemia</th>
<th>Thalassemia</th>
<th>IRIDA</th>
<th>Atransferrinemia</th>
<th>DMT-1 deficiency</th>
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<tbody>
<tr>
<td>HB/MCV</td>
<td>↓/↑</td>
<td>↓/↓</td>
<td>↓/↓</td>
<td>↓/↓</td>
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<td>Serum iron</td>
<td>↓</td>
<td>N/↑</td>
<td>↓</td>
<td>N/↑</td>
<td>↑</td>
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<td>TIBC (TI)</td>
<td>↑</td>
<td>N</td>
<td>N</td>
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<tr>
<td>% Sat</td>
<td>↓/↑/↑</td>
<td>N/↑</td>
<td>↓</td>
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<td>sTfR</td>
<td>↑</td>
<td>N</td>
<td>↑?</td>
<td>↑</td>
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<td></td>
</tr>
<tr>
<td>Hepcidin</td>
<td>↓</td>
<td>↓</td>
<td>↑</td>
<td>↓/N</td>
<td>↓?</td>
<td></td>
</tr>
</tbody>
</table>

? when no literature confirmation found

Iron transfer, not regulatory, proteins
Why is hepcidin low in DMT-1 deficiency and ATf-emia? Kathy’s hypothesis.

• Polychromatic normoblasts sense iron
• When they are iron-starved, NRBCs produce a hormone called erythroferrone (Kautz, 2014)
• 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 (Kautz, 2016)

https://www.memorangapp.com/flashcards/81368/Hematology/

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)

Sources and References

• Anderson GJ. Ironing out diseases: inherited disorders of iron homeostasis. IUBMB Life. 2001; 51: 11-17.


• 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.
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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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