Disorders of Erythrocyte Volume

Patrick G Gallagher MD
Professor of Pediatrics, Pathology and Genetics
Director, Yale Center for Blood Diseases
patrick.gallagher@yale.edu

“Water is the only drink for a wise man.”
Henry David Thoreau
Nothing to declare

Learning Objectives

• Review of mechanisms of erythrocyte volume control
• Review recent advances in understanding inherited anemias associated with altered volume homeostasis
• Evaluation of advances in relation to our understanding the pathophysiology of erythrocyte disorders with secondary alterations in volume control including sickle cell disease, thalassemia, and hereditary spherocytosis
The Mature Erythrocyte (Not Your Average Cell)

- Lacks a nucleus, DNA, RNA, and ribosomes
- It cannot synthesize RNA, DNA, or protein
- It cannot divide
- It has no mitochondria
- It cannot perform the Krebs cycle
- It lacks an electron transport system for oxidative phosphorylation
- After enucleation, the reticulocyte leaves the marrow and enters the circulation equipped with full complement of enzymes, transporters, signaling molecules, and all other proteins necessary to perform the essential functions of the red cell during its life span
The Erythrocyte: Paradigm for Cellular Hydration

- Classic model system for studying how ion, nutrients, and other solutes cross the plasma membrane
- “Pump – Leak Hypothesis” - Tosteson and Hoffman
- “Aquaporins” - Preston, Miller, and Agre
Transport Systems of the Erythrocyte Membrane

**Pumps**
- $3Na^+ / 2K^+$
- $Ca^{2+} / (2H^+)$

**Co-transporters**
- $K^+ – Cl^-$
- $Na^+ – 2Cl^- – K^+$

**Exchangers**
- $Na^+ / H^+$
- $Cl^- / HCO_3^-$
- $K^+ (Na^+)/H^+$

**Carriers**
- glucose
- nucleosides
- choline
- lactate
- amino acids
- urea

**Aquaporins**

**Homeostasis & Hydration**
- Volume
- Elastic properties

**ATP**

**ADP + P_i**
Principal Ion Transport Pathways of the Human Erythrocyte

- Gradient-driven, passive transporters
  - Na-K-Cl cotransport
  - K-Cl cotransport
  - B3 anion exchanger
  - Na-H exchanger

- Active transporters
  - Na-K-ATPase
  - Ca ATPase

- Channels
  - AQP1 water channel
  - SK1-Gardos channel
Abnormalities of Erythrocyte Hydration

- Overhydration
  - Primary
    - Hydrocytosis (Overhydrated Stomatocytosis)
- Dehydration
  - Primary
    - Hereditary Xerocytosis
  - Secondary
    - Hereditary Spherocytosis
    - Sickle Cell Disease
    - Thalassemia
    - Hemoglobin SC, Hemoglobin CC
Hereditary Xerocytosis (HX)

- Defect of cellular hydration
- Red blood cells are dehydrated
  - Xerocytosis (Greek ξηρό, xero – dry)
  - Desicctosis (Latin dessicatus – to dry up, shrivel)
- Potassium, sodium and water are decreased
- Dehydrated red cells lack flexibility and cannot squeeze through small blood vessels of spleen
- Genetic linkage to long arm of chromosome 16
Hereditary Xerocytosis

- Dominantly inherited hemolytic anemia characterized by:
  - dehydrated red blood cells
  - decreased osmotic fragility
- Clinically, biochemically, and genetically heterogeneous
- Complications include NIHF, aplastic crises, thrombosis, gallstones
- Pleiotropic, allelic with NIHF and pseudohyperkalemia in some cases
- Propensity for severe iron overload as adult
Hereditary Xerocytosis
Laboratory Characteristics

• Decreased RBC potassium and sodium
• Compensated anemia with reticulocytosis
• Elevated MCV (>95%)
• Increased MCHC (>35 %)
• Abnormal peripheral blood smear
• Decreased osmotic fragility
• Abnormal ektacytometry
HX Erythrocytes Are Not Macrocytic

- ↑ MCV of HX RBCs despite dehydration
- This is partially an artifact of cellular stiffness
- In electronic counters, conversion of pulse height (from the resistance of a cell passing through an electric field) to cellular volume dependent on cell shape.
- Xerocytes have decreased deformability, causing electronically measured MCV to be ~10% too high
- Reticulocytosis also contributes to elevated MCV
Morphology on Peripheral Blood Smear
Hereditary Xerocytosis

Hereditary Xerocytosis Complications

- Fatigue
- Pallor
- Splenomegaly
- Jaundice
  - Hemolysis
  - Gallstones
- Crises
  - Hemolytic
  - Aplastic
  - Megaloblastic
- Iron Overload
  - Not transfusion
  - Not linked to HFE, etc.
  - May require chelation

Hereditary Xerocytosis Treatment

• Treatment supportive
  – E.g. folic acid if hemolytic
  – Monitor for complications of hemolysis
• Chelation if iron overload
• Splenectomy deleterious
  – Life threatening thrombosis and/or pulmonary hypertension reported
  – Post splenectomy HX RBCs and reticulocytes exhibit increased phosphatidylserine (PS) membrane exposure, indicating altered membrane phospholipid asymmetry.
Hereditary Xerocytosis Pedigrees
Rochester/Sodus, NY - Central PA
And Winnipeg, MN

Family A  Upstate/Rochester, NY

Family B  Winnipeg, MN

* Only known (presumed) homozygotes.
Hereditary Xerocytosis: Research Plan

Stage I – Use genetics to identify chromosome where HX gene is found using DNA from a large German-Swiss family from Rochester (Sodus), NY and central PA.

Stage II - Narrow down and delineate candidate regions where HX gene is found.

Stage III - Fine mapping these candidate regions using positional cloning approaches.

Stage IV – Search candidate genes for mutations.

Stage V - Analyses consequences of HX gene mutations on the structural and/or functional roles of the HX protein in the red blood cell.
Several recombinations were detected with marker D16S511, which defined the centromeric limit for the HX region; no recombinants were detected on the telomeric side. Thus, the HX locus is \(~\)20 cM, from D16S511 to 16qter.
Linkage Analysis: Winnipeg Kindred

• 137 family members studied
• Critical recombination event between \( D16S2621 \) and HX, defines centromeric boundary, places HX telomeric to \( D16S2621 \).
• HX region *not* located between 16q23–16q24, as previously reported
• Reduces size of \( D16S511 \)–16qter candidate HX interval to \( D16S2621 \)–16qter
  – 2.4 cM region
  – 51 known and predicted genes

Candidate Gene Identification: *FAM38A* Encoding PIEZO1

- Model assuming dominant inheritance
- Search whole exome sequence data, variants in 16q24.2-16qter candidate region, present in HX patients, not controls
- New York Kindred:
  - Two genes: *FAM38A/PIEZO1* and *TPSAB1*
- Canadian HX kindred:
  - Five genes: *FAM38A/PIEZO1*, *ACSM2A*, *E4F1 CENPBD1* and *C16orf55*, were identified in the candidate region.
- Novel, nonsynonymous variants in the *FAM38A/PIEZO1* gene, which encodes the protein PIEZO1, were present in both kindreds.

Vince Schulz
Co-segregation of *FAM38A* Gene Mutations with Hereditary Xerocytosis Phenotype

Amino Acids Mutated in Hereditary Xerocytosis are Conserved Across Species


<table>
<thead>
<tr>
<th>Species</th>
<th>Amino Acid Sequence</th>
<th>M2225R</th>
<th>R2456H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human FAM38A</td>
<td>PIFTMSAQQPSIPF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family A</td>
<td>PIFTMSAQQPSIPF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family B</td>
<td>PIFTMSAQQPSIPF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human FAM38B</td>
<td>PIFTMSAQQSQLKV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dog FAM38A</td>
<td>PIFTMSAQQPSIPF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dog FAM38B</td>
<td>PIFTMSAQQSQLKV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elephant FAM38A</td>
<td>PIFTMSAQQPSIPF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elephant FAM38B</td>
<td>PIFTMSAQQSQLKV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Armadillo FAM38B</td>
<td>PIFTMSAQQSQLKV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platypus FAM38A</td>
<td>PIFTMSAQQPSVPF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anole lizard FAM38A</td>
<td>PIFTMSAQQPSVPF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anole lizard FAM38B</td>
<td>PIFTMSAQQSQLRN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X tropicalis FAM38</td>
<td>PIFTMSAQQNOLOQQ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zebrafish FAM38</td>
<td>PIFTMSAQQNHLKSS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zebrafish FAM38</td>
<td>PIFTMSAQQNQLKEL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>consensus</td>
<td>PIFTMSAQQ</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2004 2004 1964 1480 1455 1482 256 488 1021 1456 318 319 553 546 551 1532
Xerocytosis-Associated Mutations in FAM38A/PIEZO1

PIEZO Proteins

- Recently identified pore-forming subunits of channels that mediate mechanotransduction in mammalian cells
- Critical for development of the embryo, touch, pain, and hearing, control of blood pressure, kidney function, muscle and tendon stretch, vascular development
- Primary mechanism of mechanosensation linked to calcium permeable, stretch activated cation channels.
- Acts as potassium channels
PIEZ01 is HX Disease Locus: Additional Kindreds

HX mutants generate mechanically activated currents with slower inactivation kinetics than wild type, increasing cation flux, suggesting increased cation permeability likely leads to cellular dehydration.
What is Role of PIEZO1 in RBC?

• Provide a mechanism for mechanosensory feedback in response to the cell’s mechanical environment, perhaps by regulating its volume and deformability?

• Does it regulate a stretch-activated calcium pathway? Upon deformation, calcium enters the erythrocyte through an as yet unidentified pathway.

• Similar to other nascent erythrocyte channels that are activated primarily in pathologic conditions in mature erythrocytes, such as sickle cell disease (SCD)?
Overhydrated Hereditary Stomatocytosis Kindred (Hydrocytosis)
Hereditary Stomatocytosis/Hydrocytosis

- Prominent stomatocytosis, marked hemolysis
- Elevated erythrocyte sodium concentration, reduced potassium concentration, and increased total Na\(^+\) and K\(^+\) content
- Macrocytosis (110 to 150 fL)
- Excess cations elevate cell water, producing large, osmotically fragile cells with a low MCHC (24 to 30%).
## Comparison of Hereditary Xerocytosis (HX) Hereditary Hydrocytosis (OHSt) and Hereditary Spherocytosis (HS)

<table>
<thead>
<tr>
<th></th>
<th>HX</th>
<th>OHSt</th>
<th>HS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inheritance</strong></td>
<td>AD</td>
<td>AD</td>
<td>AD, AR</td>
</tr>
<tr>
<td><strong>Hb (g/dl)</strong></td>
<td>11-15</td>
<td>8-12</td>
<td>9-12</td>
</tr>
<tr>
<td><strong>Retic (%)</strong></td>
<td>4-8</td>
<td>Elevated</td>
<td>Variable</td>
</tr>
<tr>
<td><strong>MCV</strong></td>
<td>High</td>
<td>V High</td>
<td>Low</td>
</tr>
<tr>
<td><strong>MCHC</strong></td>
<td>Elevated</td>
<td>Low</td>
<td>Elevated</td>
</tr>
<tr>
<td><strong>Stomatocytes</strong></td>
<td>Few</td>
<td>Many</td>
<td>None</td>
</tr>
<tr>
<td><strong>Spherocytes</strong></td>
<td>Few</td>
<td>None</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Incubated OF</strong></td>
<td>Decreased</td>
<td>Increased</td>
<td>Increased</td>
</tr>
<tr>
<td><strong>Splenectomy</strong></td>
<td>No!</td>
<td>No!</td>
<td>Beneficial</td>
</tr>
</tbody>
</table>
Sickle Cell Disease

- Single mutation in $\beta$-globin, Glu6Val, in all homozygous Hb SS patients
- Vaso-occlusion leads to tissue ischemia and infarction, and on to progressive tissue damage
- Variable clinical severity in SCD
- Modifier genes and environmental factors influence clinical severity
The Vicious Cycle of SCD

Hb S → Inciting event
  → Hypoxia
  → Dehydration

Sickle RBC → Endothelial Injury

Endothelial Injury → Thrombosis

Thrombosis → Inflammation

Inflammation → Endothelial Injury
Cation Transport Pathways Responsible for Sickle Cell Dehydration

KCl Cotransport

K+ Cl- → - O2

Steady state

Ca++- dependent K+ channel (Gardos channel)

Sickling-induced permeability (Psickle)

RBC volume depends on [Na + K]
Strategies for Treatment of SCD

- Transfusion
- Transplantation
- Gene and Cell-Based Therapies
- Modulation of Hb F
- **Modify Cellular Hydration**
  - Lower plasma osmolarity (↑water, ↓salt, desmopressin)
  - Block pathways of cation & H₂O transport leading to cellular dehydration.
- Anti-inflammatory Strategies
- Allosteric Effectors of Hemoglobin
- Adhesion Modulators
- Nitric Oxide Pathway Modulation
- Etc…
Erythrocyte Dehydration in SCD (I)

- Hemoglobin polymerization (& sickling) critically dependent on intracellular Hb SS concentration, i.e. RBC hydration status
- Dehydrated RBCs, dense cells, exhibit decreased deformability, increased fragility, and are prone to Hb S polymerization, sickling and vaso-occlusion.
- Dense cells trapped in microcirculation, rapidly removed.
- Number of circulating dense cells positively correlated with severity of hemolysis in SCD patients
Erythrocyte Dehydration in SCD (II)

- Complex interactions between HbS polymerization and activity of water and solute transport systems leads to cellular dehydration.
- Variable clinical severity: not completely explained, Influenced by:
  - Modifier genes (common or rare),
  - Environment
  - Other factors
There is a Genetic Contribution to Erythrocyte Hydration

- Genome wide association studies demonstrate significant components of erythrocyte indices are genetically determined in normal subjects.
- Variation in indices of erythrocyte hydration, including cell volume (MCV) and hemoglobin concentration (MCHC) are strongly influenced by genetic factors.
- In multivariate analysis, one model can account for 30% variability of dense cells in HbSS and 57% of variability of dense cells in HbS-beta thalassemia.
Hypotheses

- Rare, independent mutations in genes associated with erythrocyte hydration contribute to the development of hemolytic-related complications in SCD.
- These variants alter the cotransporter/channel activation or function, leading to a change in hydration status, influencing Hb polymerization and clinical phenotype.
- These variants will be useful to guide therapy and predict response to treatment in selected sickle patients.
Selection for an Advantageous Mutant Allele: *Plasmodium falciparum* Malaria

- Sickle cell disease
- Thalassemia
- HbC
- Elliptocytosis
- Duffy (-) phenotype
- SE Asian ovalocytosis
- G-6-PD deficiency
Friends and Collaborators

**New Haven:** Edyta Glogowska, Yelena Maksimova, Vince Schulz, Kim Lezon-Geyda, Jesse Rinehart, Joe Hoffman, Slav Bagriantsev, Elena Gracheva, Eve Schneider and group, Shrikant Mane and YCGA staff

**Roscoff:** Serge Thomas, Stefan Egee

**Atlanta:** Clint Joiner  **Rochester:** Brian Smith

**Bethesda:** Dave Bodine

**Various/Oakland:** Frans Kuypers, Carolyn Hoppe

**Winnipeg:** Brett Houston, Ryan Zarychanski, Don Houston

Special Thanks: Physicians and families

Supported by NIH, NIDDK and Doris Duke Research Foundation