Impossible is nothing...
Kabuki syndrome: A potentially treatable cause of intellectual disability.

Hans Tómas Björnsson MD PhD
What is DNA?

• DNA is the language of life (how the cells know what to do);
• The entire content of DNA = “Book of life” = the human genome;
• If the human genome is a book, the “genes” would be words;
• We’ve had the entire “book of life” for 15 years but we don’t know how to read it yet;
Same text, >300 different meanings

“Book of life”

Sperm

Neuron

How can you get multiple separate meanings from a single text?
Highlighting genes for cell type specific functions

Gene 1, 2, 3, 4, 5……..genome

Cell type 1
Gene 1, 3, 5
Open chromatin

Cell type 2
Gene 2, 4
Closed chromatin

But how are these achieved and maintained in cells?
Epigenetic machinery: the genome's “highlighter”

• Epigenetic marks are modifications of DNA or associated proteins, other than the DNA sequence itself, that are heritable through cell division (mitosis).

• Reversible and affected by the environment.

• Add to information content of DNA
  » DNA methylation
  » Histone tail modifications
Histone acetylation is seen in open chromatin

Acetylation, Methylation, and more.
Emerging links between metabolic pathways and histone modifications

Lu et al. Cell Metab. 2012
Summary (1):

• Epigenetic modifications are thought to help establish and maintain cell type specific identity;
• Many of the donors for epigenetic modifications are critical intermediates of cellular metabolism, linking gene expression with cellular metabolic states;

What are the components of the histone machinery?
Histone machinery

1. Writers

2. Erasers

3. Readers

4. Remodeler

Fahrner JA and Bjornsson HT. Annual Review of Human Genetics and Genomics. 2014.
Histone marks in open and closed chromatin

Fahrner JA and Bjornsson HT. Annual Review of Human Genetics and Genomics. 2014.
Acetylation is an open chromatin mark

\[ \Delta = \text{binary system (0,1)} \]
\[ \bigcirc = \text{quaternary system (0, 1, 2, 3)} \]

Fahrner JA and Bjornsson HT. Annual Review of Human Genetics and Genomics. 2014.
H3K4me3 is an open chromatin mark

Fahrner JA and Bjornsson HT. Annual Review of Human Genetics and Genomics. 2014.
H3K27me3 is a closed chromatin mark

△ = binary system (0,1)
○ = quaternary system (0, 1, 2, 3)

Fahrner JA and Bjornsson HT. Annual Review of Human Genetics and Genomics. 2014.
Summary (2):

• The histone machinery consists of writers (highlighters), erasers, readers and remodelers;

• There are many different histone modifications, and certain combinations of marks are seen in open chromatin (H3K4me3, H4ac) and other combinations in closed chromatin (H3K9me3, H3K27me3);

But what happens when epigenetic modifications or machinery are disrupted?
Epigenetics and Chromatin Clinic

- Classical epigenetic disorders
  - Beckwith Wiedemann syndrome
- Disorders of the DNA methylation machinery
  - Rett syndrome
- Disorders of the histone machinery
  - Kabuki syndrome

Genetic disorders with epigenetic consequences
Ideas:

1. Build expertise;

2. Learn from patients and families;

3. Educate health professional about Kabuki syndrome and related disorders
An ever growing number of dysfunctional histone enzymes

Fahrner JA and Bjornsson HT. Annual Review of Human Genetics and Genomics. 2014.
An emerging cause of intellectual disability

All thought to be caused by haplo-insufficiency!

Baylor: WES with ID/DD 19% had mutation in histone machinery
Summary (3):

• Deficiencies of histone writers and erasers are Mendelian disorders with epigenetic consequences;

• Even though these disorders all involve enzymes, they uniformly are caused by the loss of a single allele;

• Despite known redundancy of the histone machinery, other components with overlapping function are not able to compensate for the loss of a single allele, indicating tight regulation of the levels of these enzymes and the marks they affect.
Kabuki syndrome: An imbalance of open and closed chromatin?

<table>
<thead>
<tr>
<th>Disorder of histone methylation</th>
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<tbody>
<tr>
<td>Autosomal dominant/ X linked (escapes)</td>
</tr>
<tr>
<td>Mutations in MLL2 or KDM6A (UTX)</td>
</tr>
<tr>
<td>Variable intellectual disability, postnatal growth retardation</td>
</tr>
<tr>
<td>1/30,000</td>
</tr>
</tbody>
</table>


Kabuki syndrome (KS): A treatable cause of intellectual disability?
**Mll2+/βGeo** mice: Knock-out of the SET methyltransferase domain from *Mll2*

- **Bjornsson et al.** Sci Transl Med. 2014
$MII2^{+/βGeo}$ mice: hippocampal memory defects in NOR and MWM testing


Bjornsson et al. Sci Transl Med. 2014
**MII2⁺/β<sub>Geo</sub>** mice: decreased H3K4me3 activity

<table>
<thead>
<tr>
<th>H3K4me3 indicator</th>
<th>C-EGFP</th>
<th>L-N-EGFP</th>
<th>TAFIII BD</th>
<th>3xNLS</th>
</tr>
</thead>
<tbody>
<tr>
<td>H3 (40 AA)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>K4, K9, K27</td>
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</table>

H3K4me3 Methylation Indicator, Mouse embryonic fibroblasts (transient)

- **MII2⁺⁺⁺**
- **MII2⁺/β<sub>Geo</sub>**

Bar graph showing GFP positive cells with AR-42 concentrations:
- 0 μM: MII2⁺⁺⁺ (blue) = 4.0, MII2⁺/β<sub>Geo</sub> (yellow) = 1.0
- P < 0.001

Bjornsson *et al.* Sci Transl Med. 2014
H3K4me3 deficiency is improved *in vivo* with HDACi AR-42

Summary (4):

• The two types of Kabuki syndrome suggest that an imbalance between open and closed chromatin states may play a role;

• Our mouse model of Kabuki syndrome has overlapping phenotypic features with patients with Kabuki syndrome as well as hippocampal memory defects, a phenotype that can be monitored during therapeutic trials.

• *Mll2*+/β*Geo* mice have a deficiency of both H3K4me3 activity and genome-wide H3K4me3 levels and either can be manipulated with histone deacetylase inhibition;
*Mll2* is highly expressed in Granule Cell Layer (GCL) of the dentate gyrus

Dentate gyrus is part of hippocampus;

Strongest expression in Granule Cell Layer (GCL);

Adult neurogenesis occurs in subgranular zone (SGZ);

Defects of adult neurogenesis lead to hippocampal memory defects.

Immunofluorescence against *Mll2* (WT)
$Mll2^{+/βGeo}$ mice have decreased H3K4me3 in the GCL of the dentate gyrus

**Bjornsson et al. Sci Transl Med. 2014**
Mll2+/βGeo mice have a thinner GCL layer

Bjornsson et al. Sci Transl Med. 2014
Mll2+/βGeo mice have a deficiency of neurogenesis

Bjornsson et al. Sci Transl Med. 2014
Impaired neurogenesis in $Mll2^{+/βGeo}$ mice is improved with 2 weeks of HDAC inhibitor AR-42

Five month old cohort

Bjornsson et al. Sci Transl Med. 2014
Hippocampal memory defect in $\textit{Mll2}^{+/\beta\text{Geo}}$ mice is improved with 2 weeks of HDAC inhibitor AR-42.

\textit{Bjornsson et al. Sci Transl Med. 2014}
Summary (5):

• A mouse model of KS reveals defective H3K4me3 in the GCL layer of dentate gyrus, thinning of this cell layer caused by impaired neurogenesis;

• Defects in the dentate gyrus can be reversed using drugs that target the epigenetic machinery, suggesting that the intellectual disability seen in KS (and perhaps other disorders of epigenome homeostasis) may be treatable;

Moving a cancer therapeutic drug to kids with ID will be a challenge. Alternative options?
Beta-hydroxybutyrate as a therapeutic agent for Kabuki syndrome?

Ketogenic used for many therapeutic applications including seizures

Shimazu et al. Science. 2013
In vitro beta-hydroxybutyrate increases histone acetylation (less potent than AR-42)
Mice tolerate a modified ketogenic diet for months without any ill effect

- **Ingredients:** Lard, Butter, Corn Oil, Casein, Cellulose, Mineral Mix, Vitamin Mix, Dextrose (trace)

Ketogenic Diet AIN-76A-Modified, High Fat, Paste

- 6:1 fat to protein ratio
- 4:1 fat to protein ratio

(Dr. Hilary Vernon)

Joel Benjamin
Giovanni Carosso
BHB increases on a ketogenic diet in both $Mll2^{+/+}$ and $Mll2^{+/-\beta GeO}$ mice.
H3K4me3 deficiency in GCL of dentate gyrus improves on a ketogenic diet

Average normalized H3k4me3 intensity/average DAPI intensity

- Regular Diet
- Ketogenic diet

- $Mll2^{+/+}$
- $Mll2^{+/βGeo}$

P<0.05
P<0.005

- DAPI
- H3K4me3
A deficiency of neurogenesis seen in $Mll2^{+/\beta\text{Geo}}$ mice improves on ketogenic diet.
A deficiency of neurogenesis seen in $Mll2^{+/\beta Geo}$ mice improves on ketogenic diet

Reg. Diet

$Mll2^{+/+}$

$Mll2^{+}/\beta Geo$

EdU positive cells

7 day period (proliferation)

Average EdU positive cell count per slice

$P < 0.001$

$P < 0.001$
Hippocampal memory defect improves on a ketogenic diet

Platform Crossings

Regular Diet  Ketogenic Diet

Mll2+/+
Mll2+-

N=100
19-31 per group
Tester blinded to genotype.
Summary (6):

- A modified ketogenic diet can increase beta-hydroxybutyrate in our mice;
- A modified ketogenic diet increases open chromatin marks (H3Ac, H3K4me3) and normalized gene expression in relevant neurons;
- Defects of neurogenesis in dentate gyrus and hippocampal memory defects in mouse model of Kabuki syndrome normalize after 2 weeks of ketogenic diet;
Is Kabuki syndrome a treatable cause of intellectual disability?

- Proof-of-principle
- AR-42
- MKD
- Neurogenesis
Q1: Do our findings apply to humans?

- If the neurogenesis defect in Kabuki syndrome is a major component of the ID one should be able to demonstrate this studying patients (specific tasks linked to dentate gyrus);

- Recruited Jacqui Weissman MD, and Rebecca Vaurio PhD from the KKI. Jacqui is a neurobehavioral fellow who is conducting a prospective study at JHH/KKI to ask what tasks are abnormal in kids with KS (30-40 minutes for test);

- This will also help us understand strengths/weaknesses in general, form a baseline for any clinical trial and perhaps give some general educational recommendations.
Q2: What could we potentially treat?

What we may be **possibly** able to target with a medical treatment

- Memory defect to some extent (if proven in patients);
- Hypotonia (early in life);
- Immune dysregulation (immunodeficiency, and/or autoimmune phenomena);

What we will **never** be able to target with a medical treatment

- Anatomical defects (heart, kidneys, ortho, ophtho, fingerpads);
- Facial features (eyes, ears, and nose);

Remember: these results need to be verified in other labs and in humans before we can get to optimistic
How can we work together?

- **Participate in research:** samples, studies, results (Jacqui Weissman study), Send us MRI’s;
- **Fundraise:** sometimes a small amount of money can help a lab get preliminary data so can get NIH funding: thank you to all the families attending today fundraising for our lab;
- **Organize meetings** (great for patients and family interaction, great for providers to interact), great to push the research (East Coast Gathering organized by Dana Levinson);
- **Lobby for Kabuki syndrome,** help increase awareness of Kabuki syndrome among public, among lawmakers, among funders;

*BeHeard Research Challenge by the Rare Genomics Institute to help raise awareness about Kabuki syndrome (2013). 12,000 votes!!!*
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