Full spectrum translation in rare disease research

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THE McKUSICK-NATHANS EPIGENETICS AND CHROMATIN CLINIC (ECC)

University of Michigan

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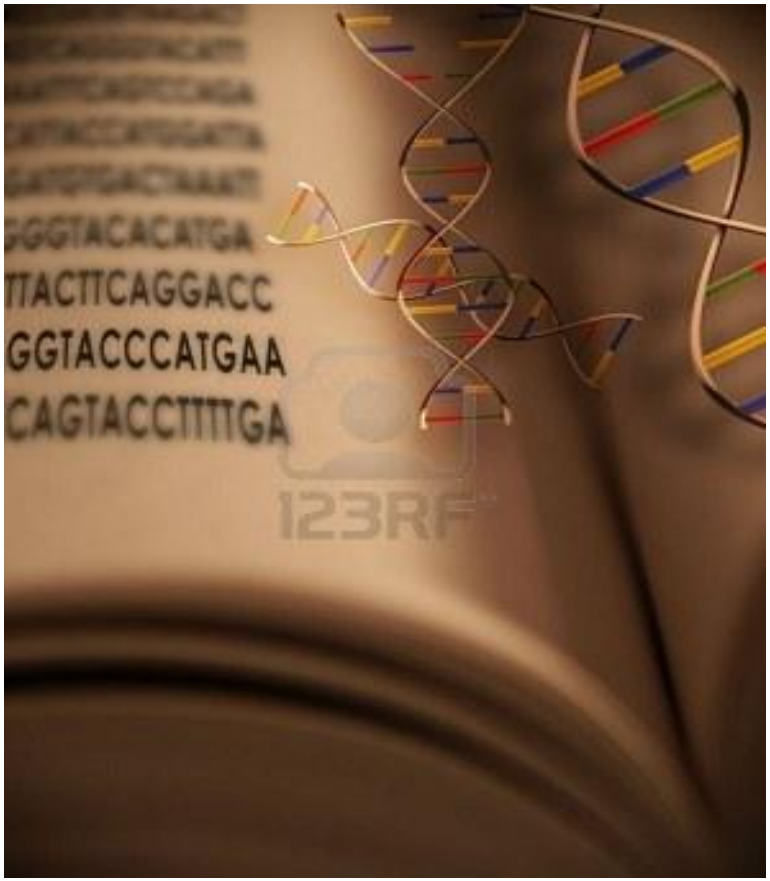
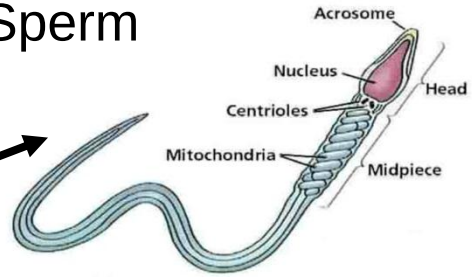
2022

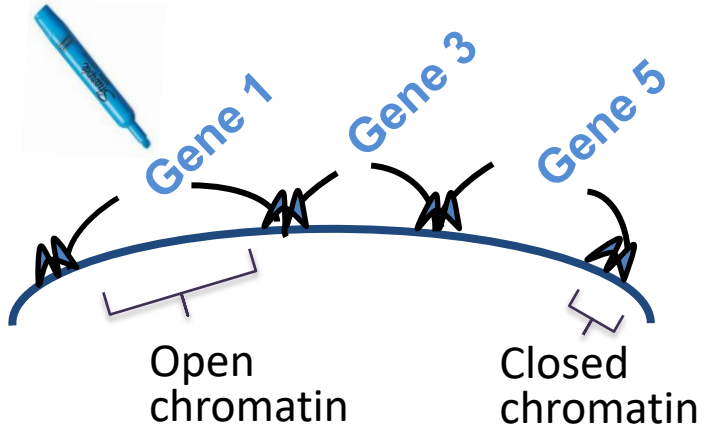
Same text, >300 different meanings

"Book of life"

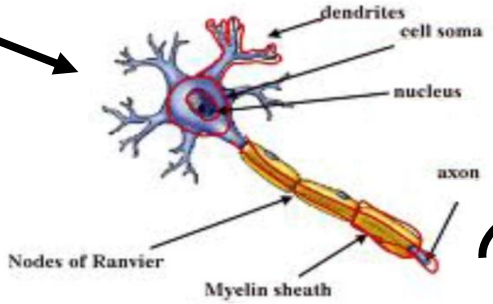


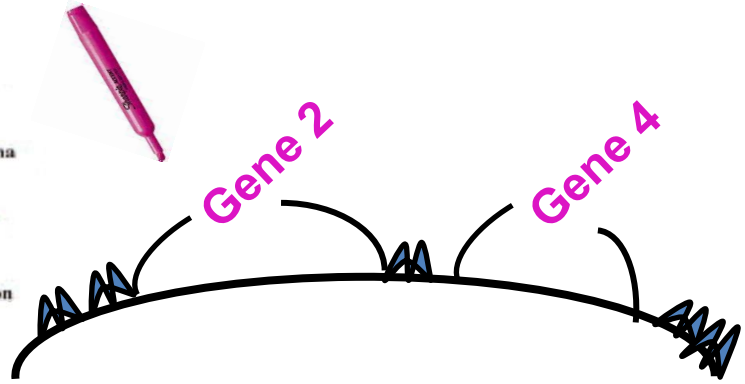
Sperm





Neuron



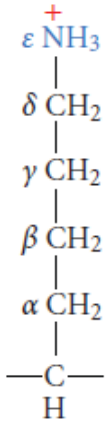


But how are these achieved and maintained in cells?

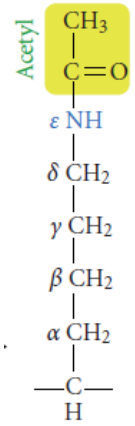
Epigenetic machinery: the genome's “highlighter”

- 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

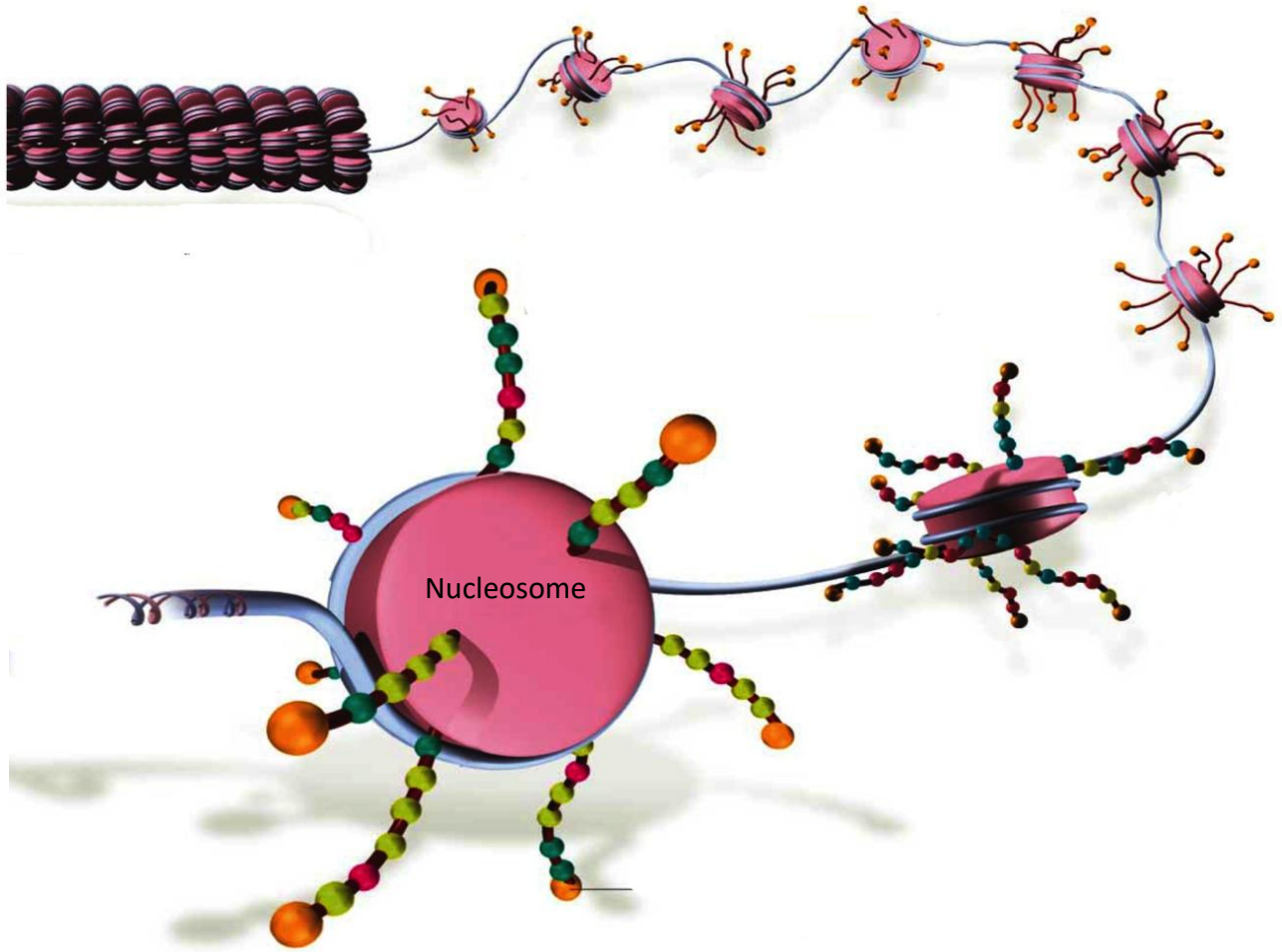
Histone acetylation is seen in open chromatin



Lysine



Acetyl-lysine



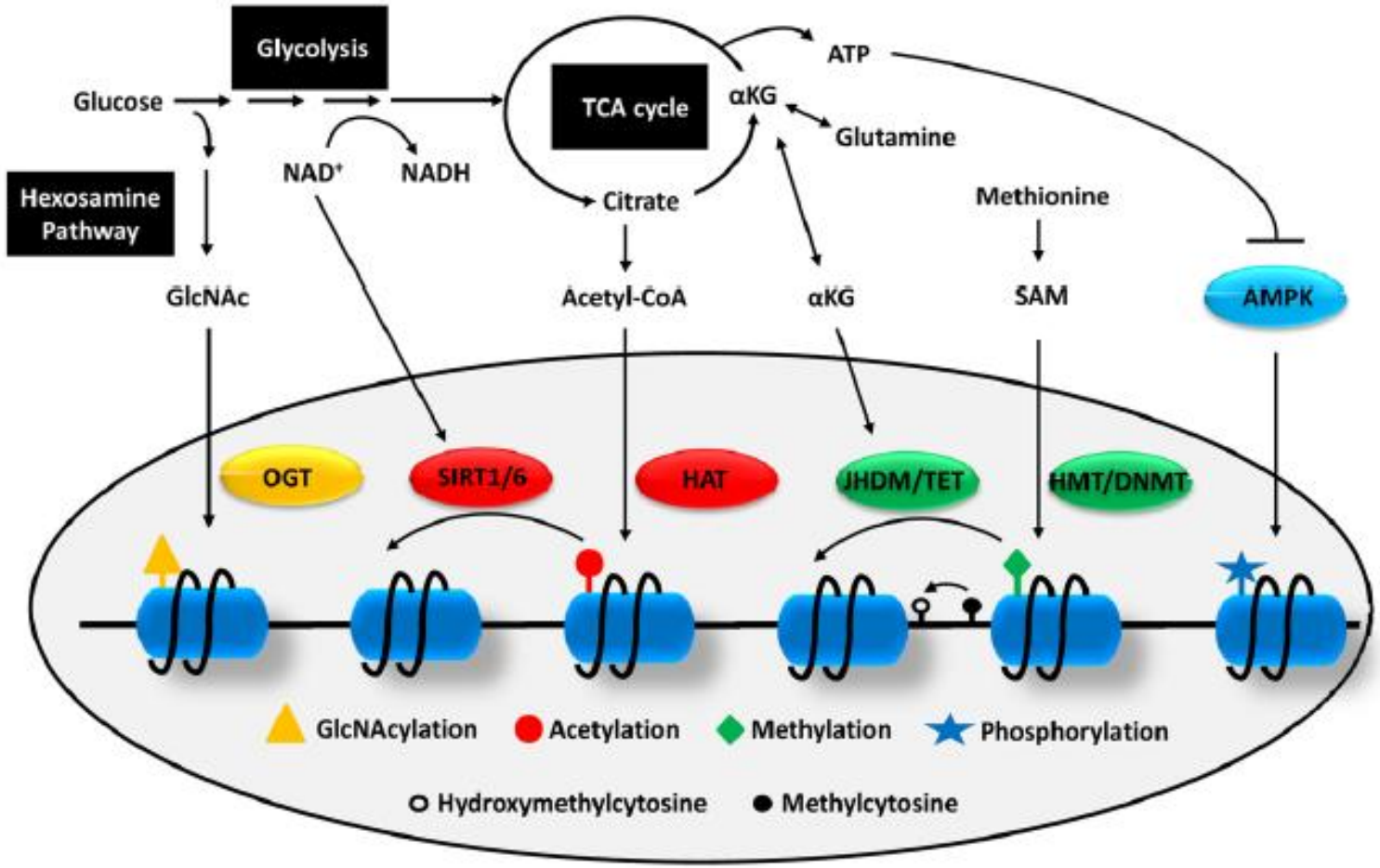
Histone tail modifications

Histone modification types and the interacting domains that "read" them

Modification types
Unmodified lysine
Acetylation
Methylation
Phosphorylation
Ubiquitylation
Sumoylation
ADP-ribosylation
Citrullination
Butyrylation
Propionylation
Glycosylation

Gardner KE, Allis CD, Strahl BD. J. Mol. Bio. 2011

Emerging links between metabolic pathways and histone modifications



Lu et al. Cell Metab. 2012

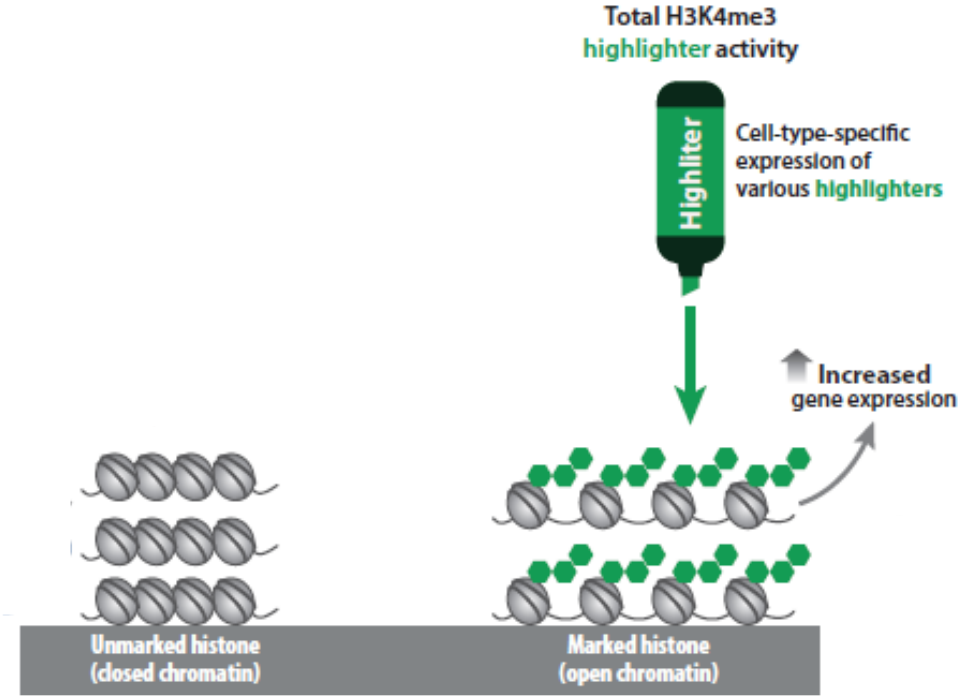
Summary (1):

- 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

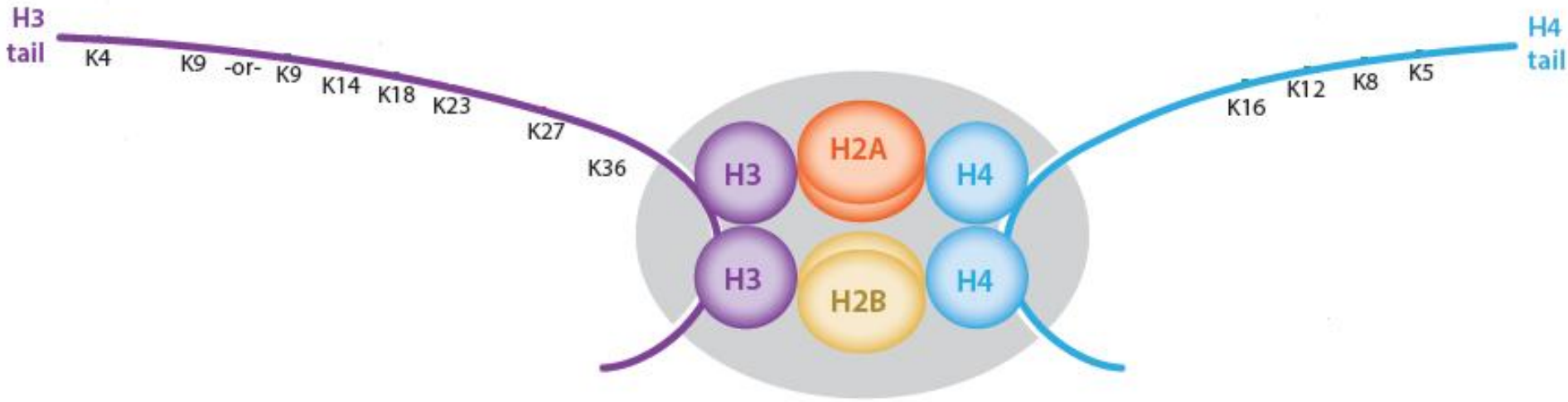
1. Writers



1) Sliding
2) Exchange variants

Fahrner and Bjornsson, Annual Review Gen and Genom, 2014.

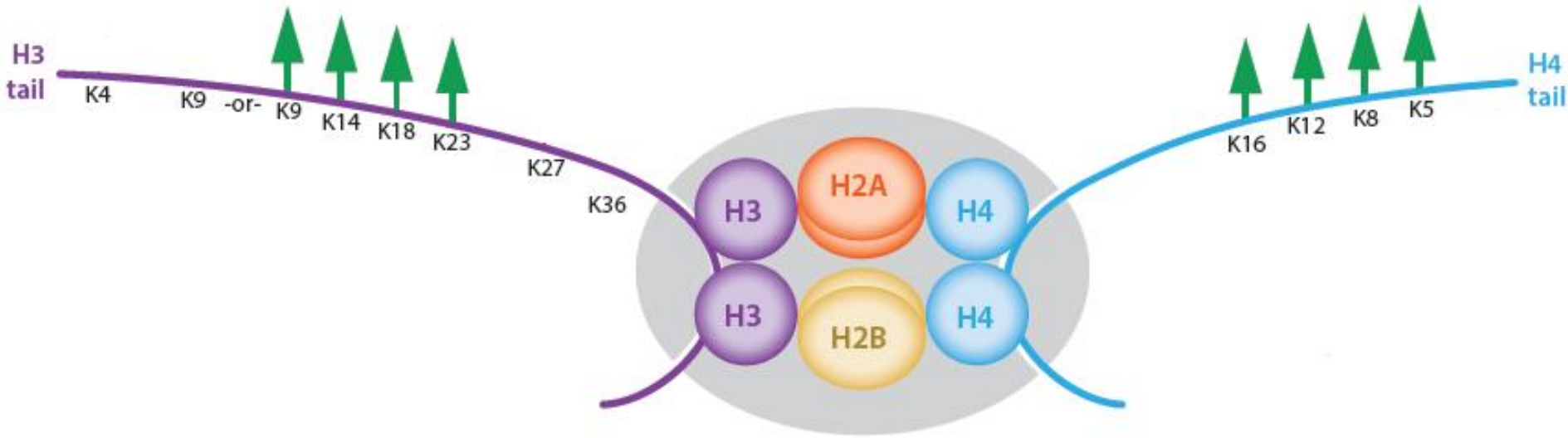
Histone marks in open and closed chromatin



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

Fahrner and Bjornsson, Annual Review Gen and Genom, 2014.

Acetylation is an open chromatin mark



△ = binary system (0,1)
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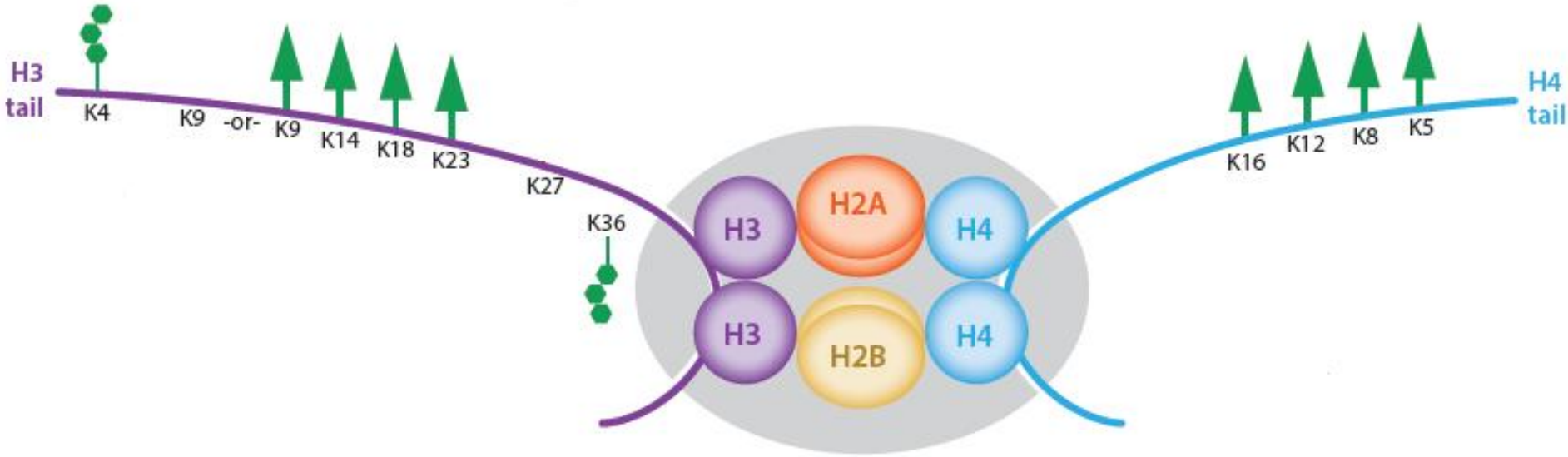
Fahrner and Bjornsson, Annual Review Gen and Genom, 2014.

H3K4me3 is an open chromatin mark



Open, active chromatin

Methyl group

Acetyl group

Closed, inactive chromatin

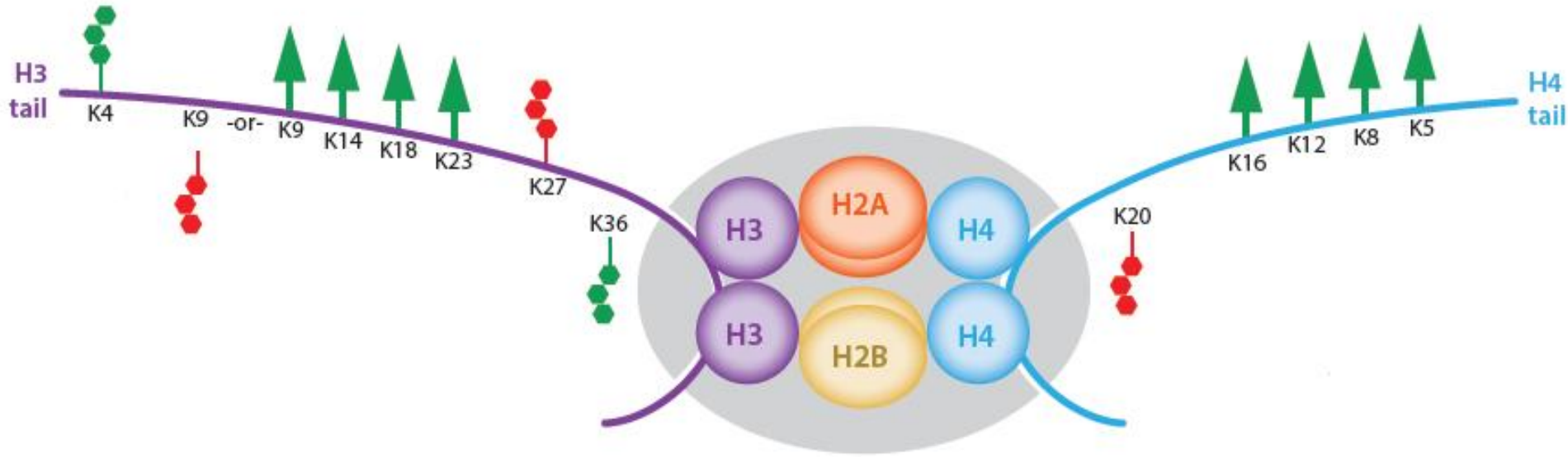
Methyl group

△ = binary system (0,1)

⬡ = quaternary system (0, 1, 2, 3)

Fahrner and Bjornsson, Annual Review Gen and Genom, 2014.

H3K27me3 is a closed chromatin mark



Open, active chromatin

Methyl group

Acetyl group

Closed, inactive chromatin

Methyl group

△ = binary system (0,1)

⬡ = quaternary system (0, 1, 2, 3)

Fahrner and Bjornsson, Annual Review Gen and Genom, 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 (H3K27me3, H3K9me3);

But what happens when epigenetic machinery is disrupted?

The McKusick-Nathans
Epigenetics and Chromatin Clinic

Epigenetics and Chromatin Clinic

- Imprinting disorders
  - » Beckwith Wiedemann syndrome

Mostly CIS

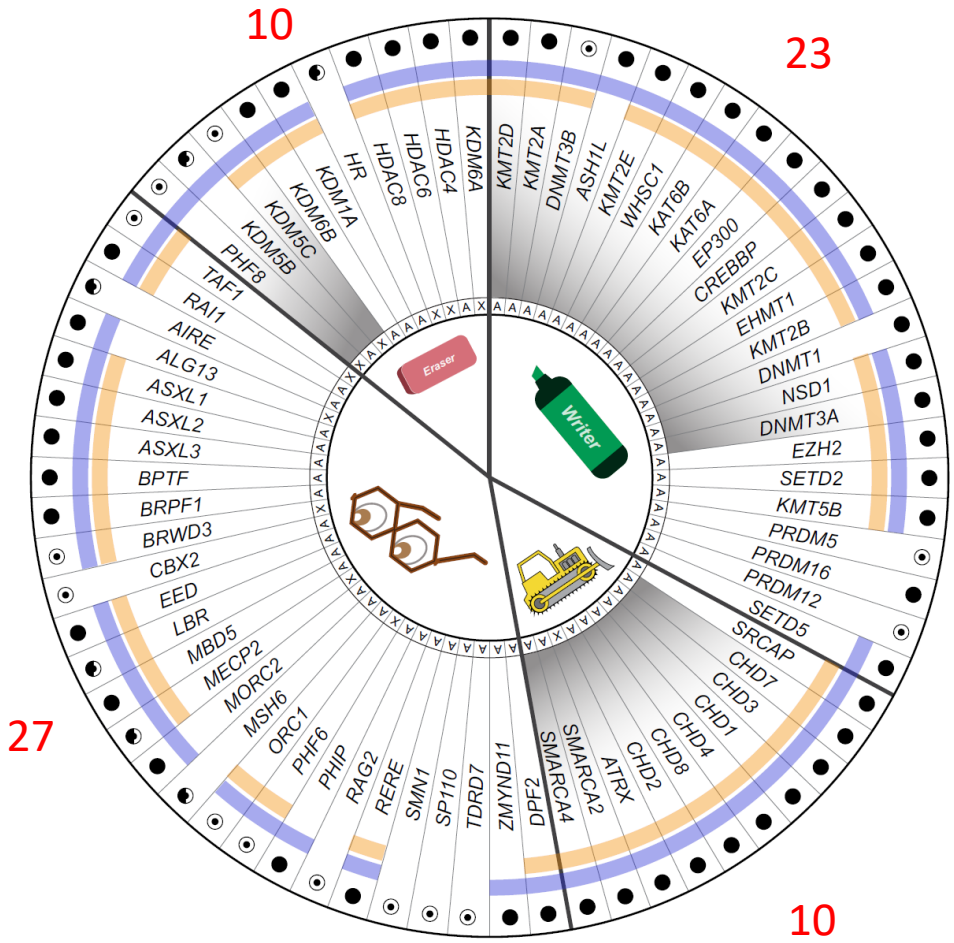
- Disorders of the DNA methylation machinery
  - » Rett syndrome
- Disorders of the histone machinery
  - » Kabuki, Rubinstein-Taybi syndromes

TRANS

Genetic disorders with epigenetic consequences

The Mendelian disorders of the epigenetic machinery: 70 genes

Intellectual disability Growth dysregulation (overgrowth/growth retardation)

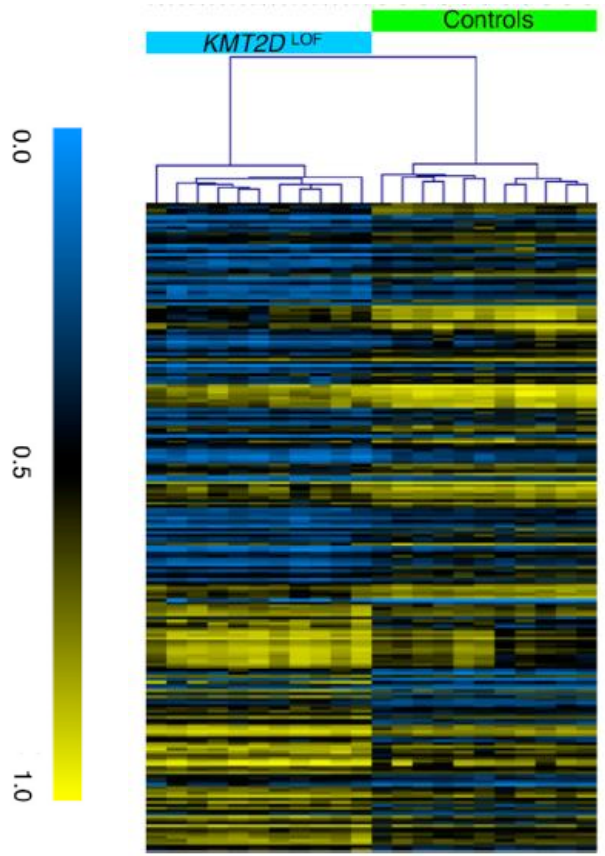


Common themes:

1) <u>Intellectual disability</u> (also growth, immune, limbs);
2) <u>Dosage sensitivity:</u> Uniformly caused by the loss of a single allele;
3) <u>Episignatures;</u>

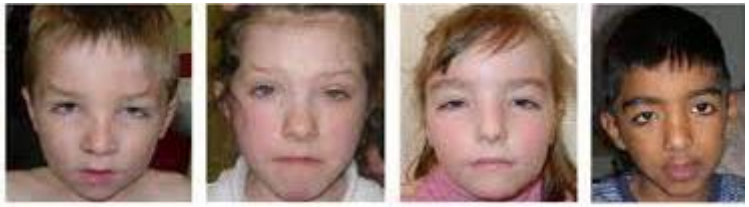
Fahrner et al. Human Mol. Genet. 2019.

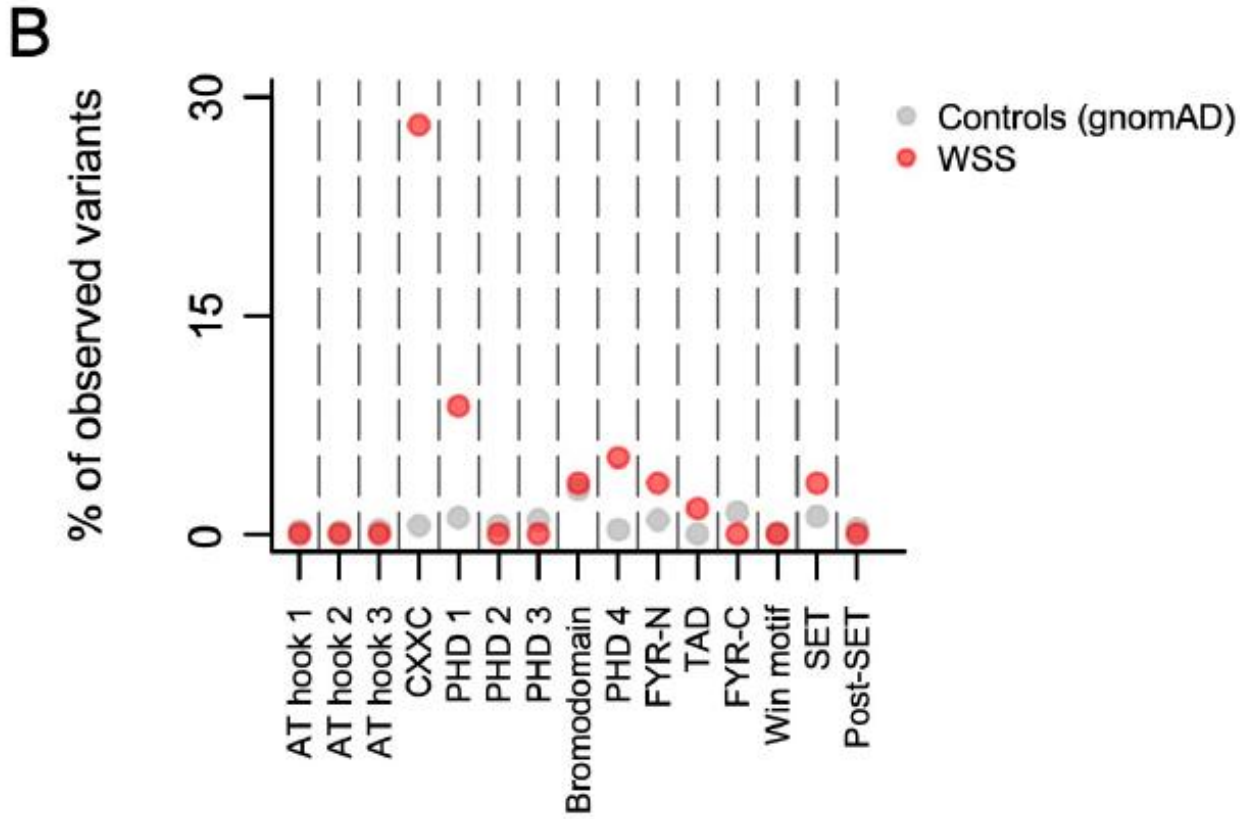
DNA methylation episignatures: interdependence of epigenetic modifications



Butcher et al. AJHG, 2017;
Sobreira et al. EJHG 2017

Caveat: An enzyme domain is not always the most important region for disease state





Jones et al. AJHG 2012

Reynisdottir et al. BioRxiv 2022

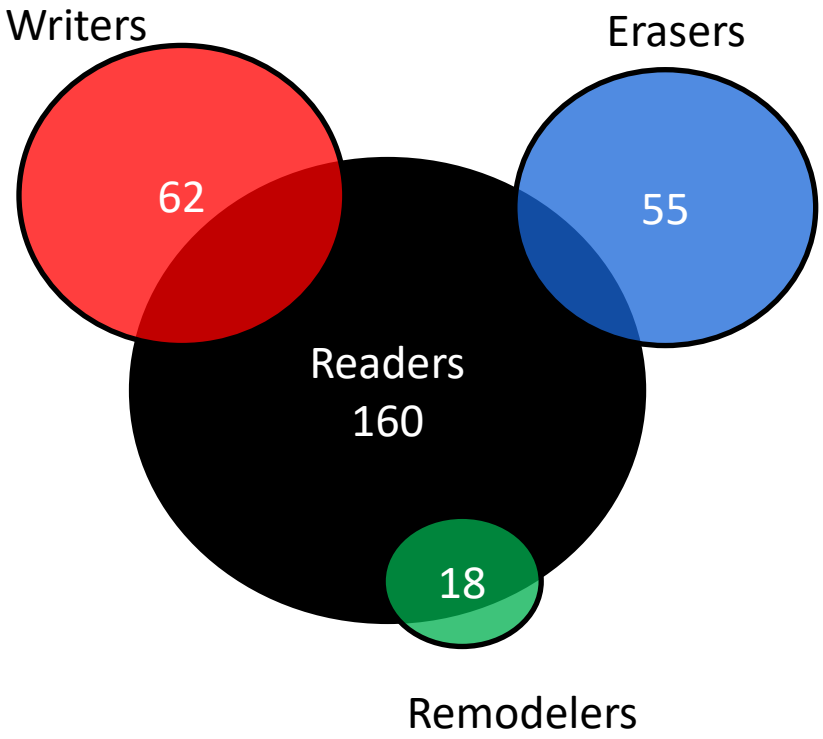
Summary (3):

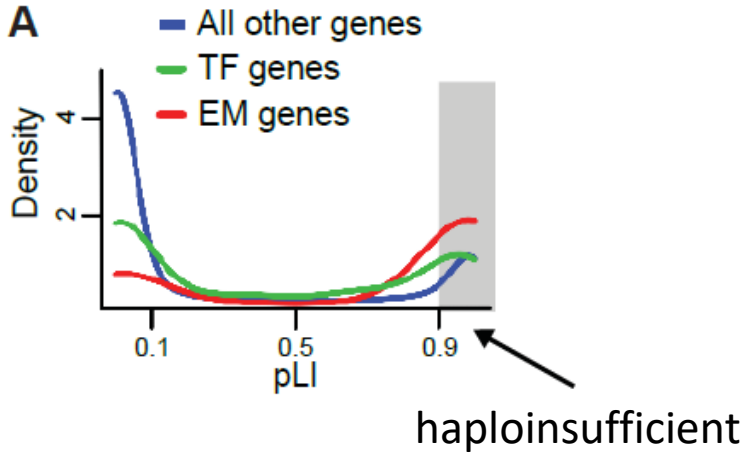
- The Mendelian disorders of the epigenetic machinery (MDEM), are **genetic** disorders that are expected to have **epigenetic consequences**;
- The Mendelian disorders of the epigenetic machinery lead to disruption of **growth** and **intellect**;
- Most of these disorders have unique **DNAm episignatures** that currently can help with evaluation of variants but perhaps in future will help clarify function of various factors;

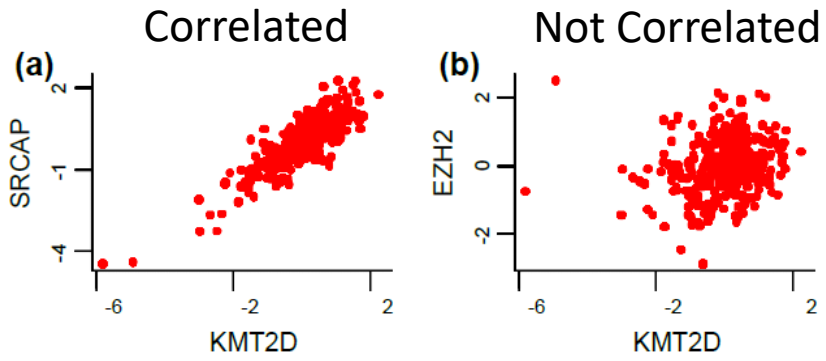
Is dosage sensitivity a general theme in EM factors?

Epigenetic Machinery genes act in a modular way and are intolerant to variation

At least 295 factors have direct function as writers, erasers, reader or remodeler

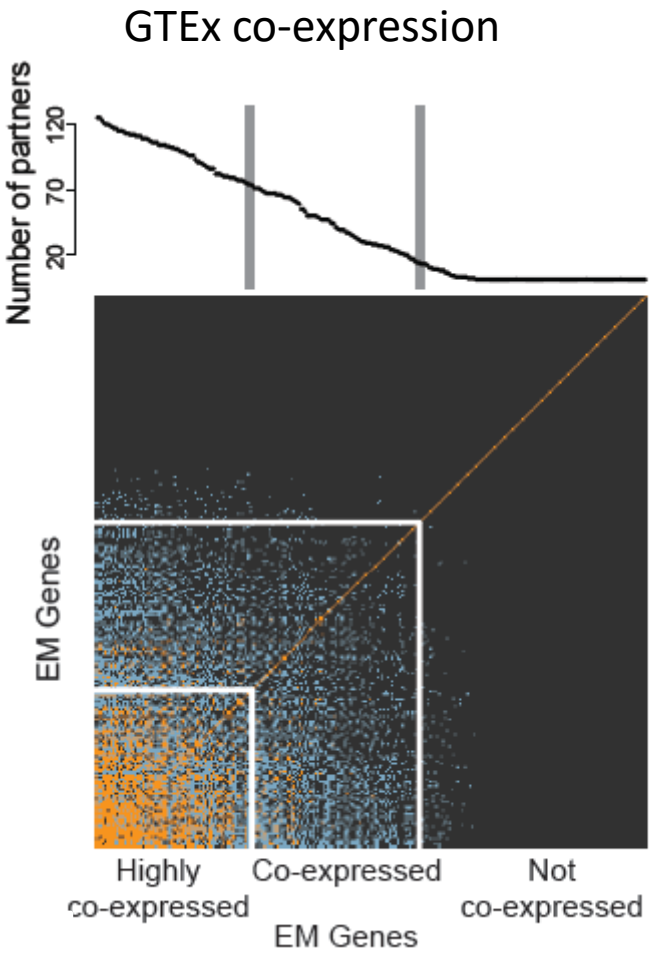


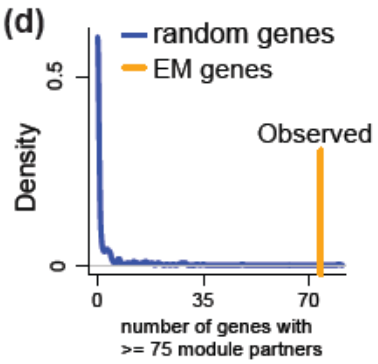


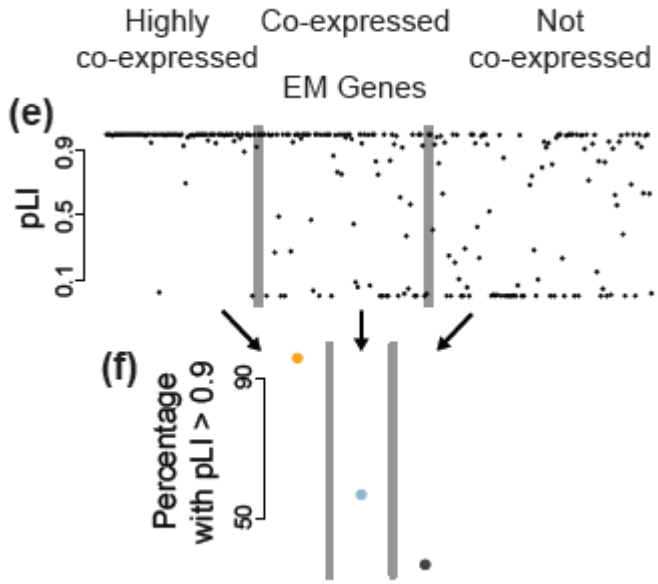


Boukas et al. Genome Res, 2019

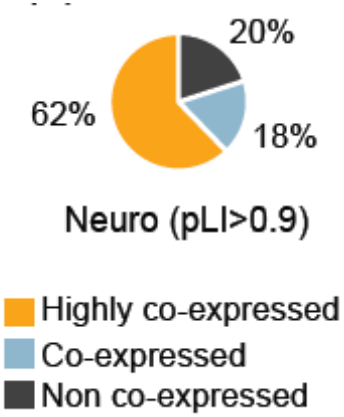
A subset of the 300 epigenetic machinery (EM) genes are highly co-expressed







Could disruption of co-expression play a role in disease pathogenesis?



Boukas et al. Genome Res, 2019

Summary (4):

- Enzymatic functions of EM factors are **mutually exclusive** (one enzyme function per factor);
- 74 of these factors form a large co-expression module, and those factors are very highly intolerant to variation and enriched for neurological disease phenotypes (perhaps network itself plays role in disease and dosage sensitivity) and perhaps should be **prioritized for future disease gene discovery**;
- A list of all known EM factors (and our data):
  - www.epigeneticmachinery.org

Have we found all the EM disease factors?

De novo changes in *CHD1* cause autism and speech apraxia

4/6 had speech apraxia!

We have now made a mouse with R618Q which is lethal in homozygosity!

Pilarowski-Bjornsson syndrome (MIM#617682)

Pilarowski et al. Journal of Med Gen, 2017

De novo changes in *CHD3* also cause speech apraxia

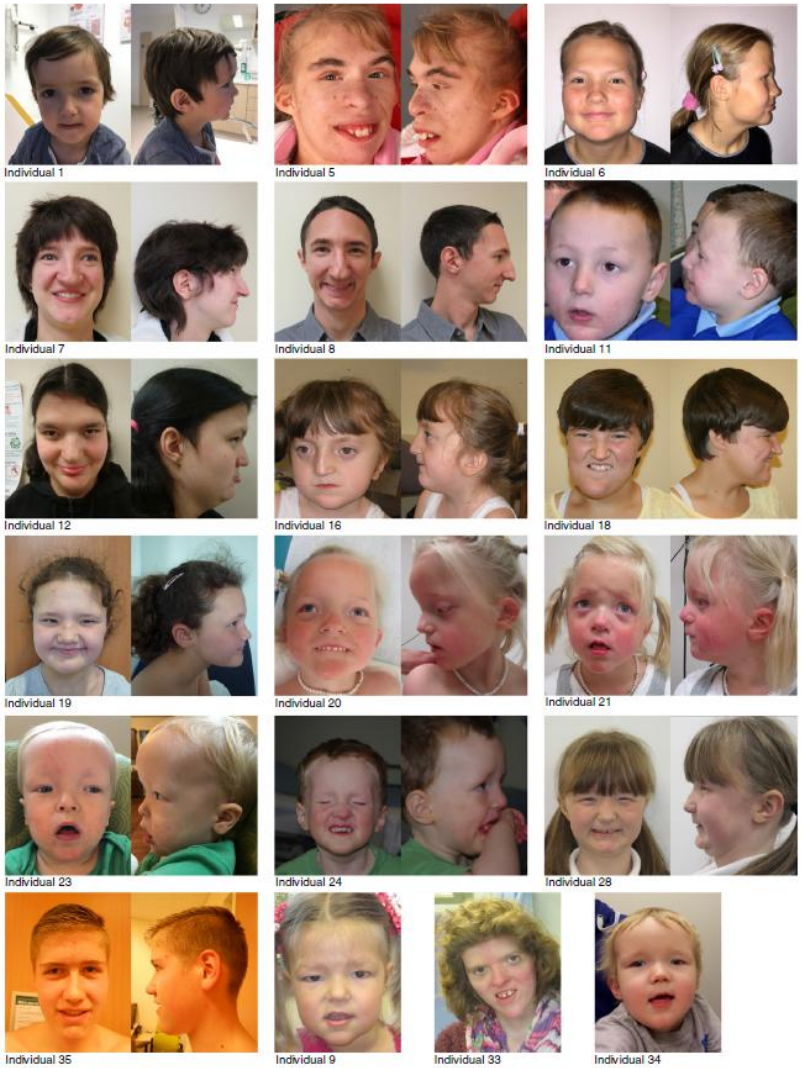


Table 1 Summary of phenotypes found in this cohort of probands with *CHD3* mutations

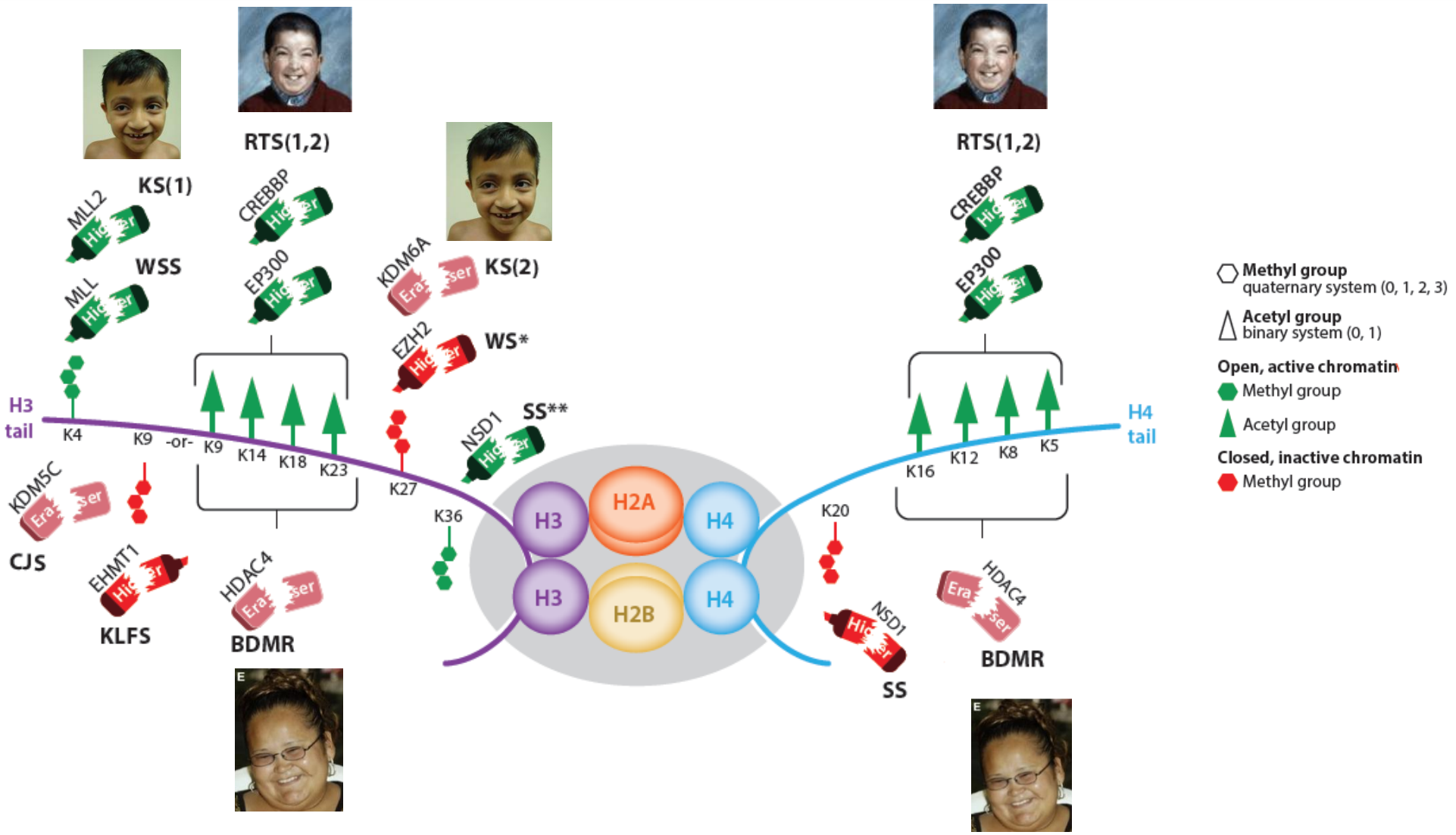
	Amount	Percentage
Development		
ID/DD	35/35	100%
Degree of ID/DD		
Borderline ID	3/35	9%
Mild or mild-moderate ID	9/35	26%
Moderate or moderate-severe ID	8/35	23%
Severe ID	7/35	20%
DD/level unknown	8/35	23%
Speech delay/disorder	33/33	100%
Autism or autism-like features	9/31	29%

Snijders Blok. Nature Commun, Nov, 2018

Summary (5):

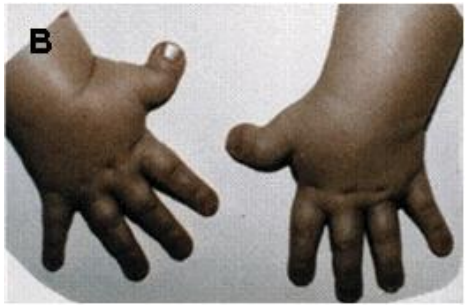
- **Pilarowski-Bjornsson syndrome (PILBOS)** is a novel Mendelian disorder of the epigenetic machinery caused by mutations in the chromatin remodeler CHD1;
- CHD3 also leads to a novel ID syndrome, which also has a strong connection to **speech problems**, indicating that these related factors and/or chromatin remodeling have something to do with speech.

Three illustrative writer/eraser disorders



Rubinstein-Taybi syndrome: a writer disorder

A disorder of histone acetylation
Autosomal dominant
Mutations in either *CREBBP* or *EP300*
Intellectual disability, dysmorphic features Immunodeficiency (1)
1/100000



Hennekam et al. EJHG 2006.

RTS, Type 1 and 2: ***CREBBP/EP300***

Ac Ac Ac

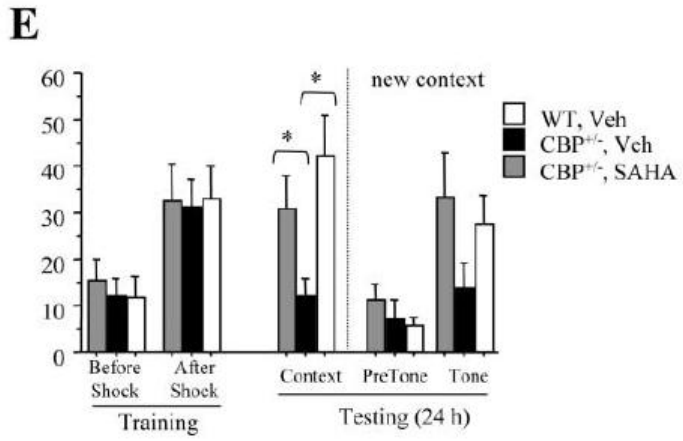
ARTKQ//KAPRKQLATK (H3) ⟶ ⟵ ARTKQ//KAPRKQLATK (H3)

14 18 23 14 18 23

Closed chromatin **Open chromatin**

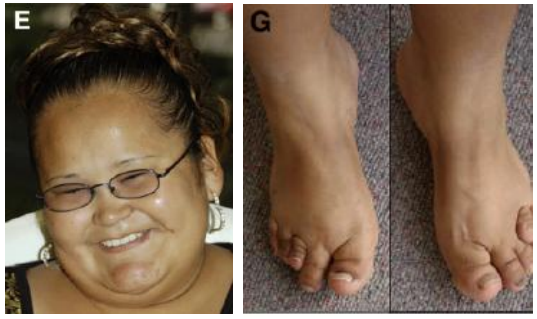
Postnatal treatment with histone deacetylase inhibitors can rescue memory defect in mouse models of RTS:

Alarcon et al. Neuron 2004
Wood et al. Neuron, 2004



Brachydactyly Mental Retardation syndrome

Disorder of histone acetylation
Autosomal dominant
Mutations in *HDAC4*
Brachycephaly, brachydactyly type E, intellectual disability
Rare (less than 100 cases)



Williams SR et al. AJHG 2010

BDMR

HDAC4

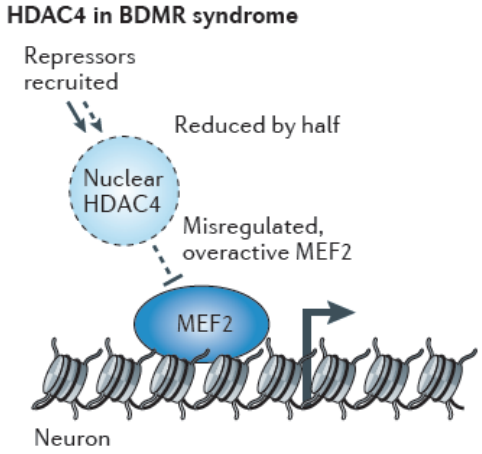
Ac Ac Ac

ARTKQ//KAPRKQLATK (H3)
14 18 23

Closed chromatin

ARTKQ//KAPRKQLATK (H3)
14 18 23

Open chromatin

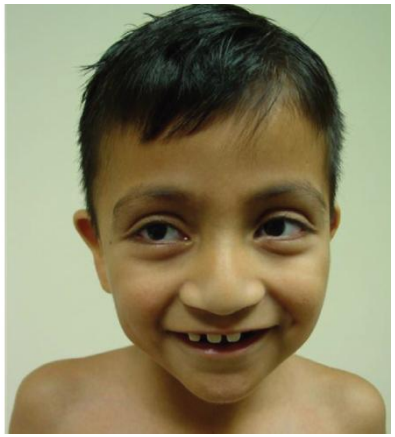


Ronan JL et al. Nature Reviews 2013

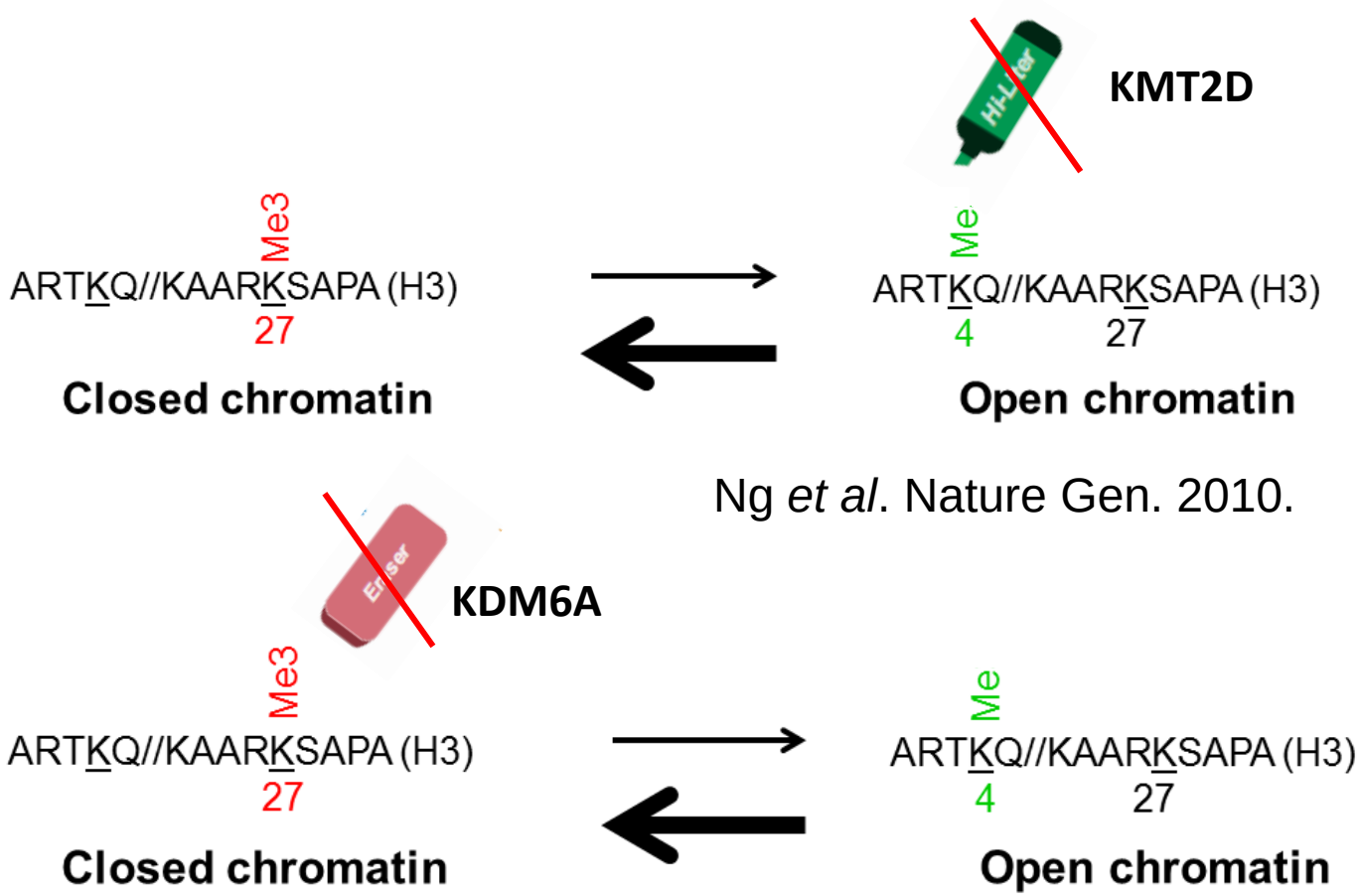
Either too little or too much acetylation can lead to intellectual disability.

Kabuki syndrome: An imbalance of open and closed chromatin?

Disorder of histone methylation
Autosomal dominant/ X linked (escapes)
Mutations in *KMT2D* or *KDM6A (UTX)*
Variable intellectual disability, postnatal growth retardation
1/30,000



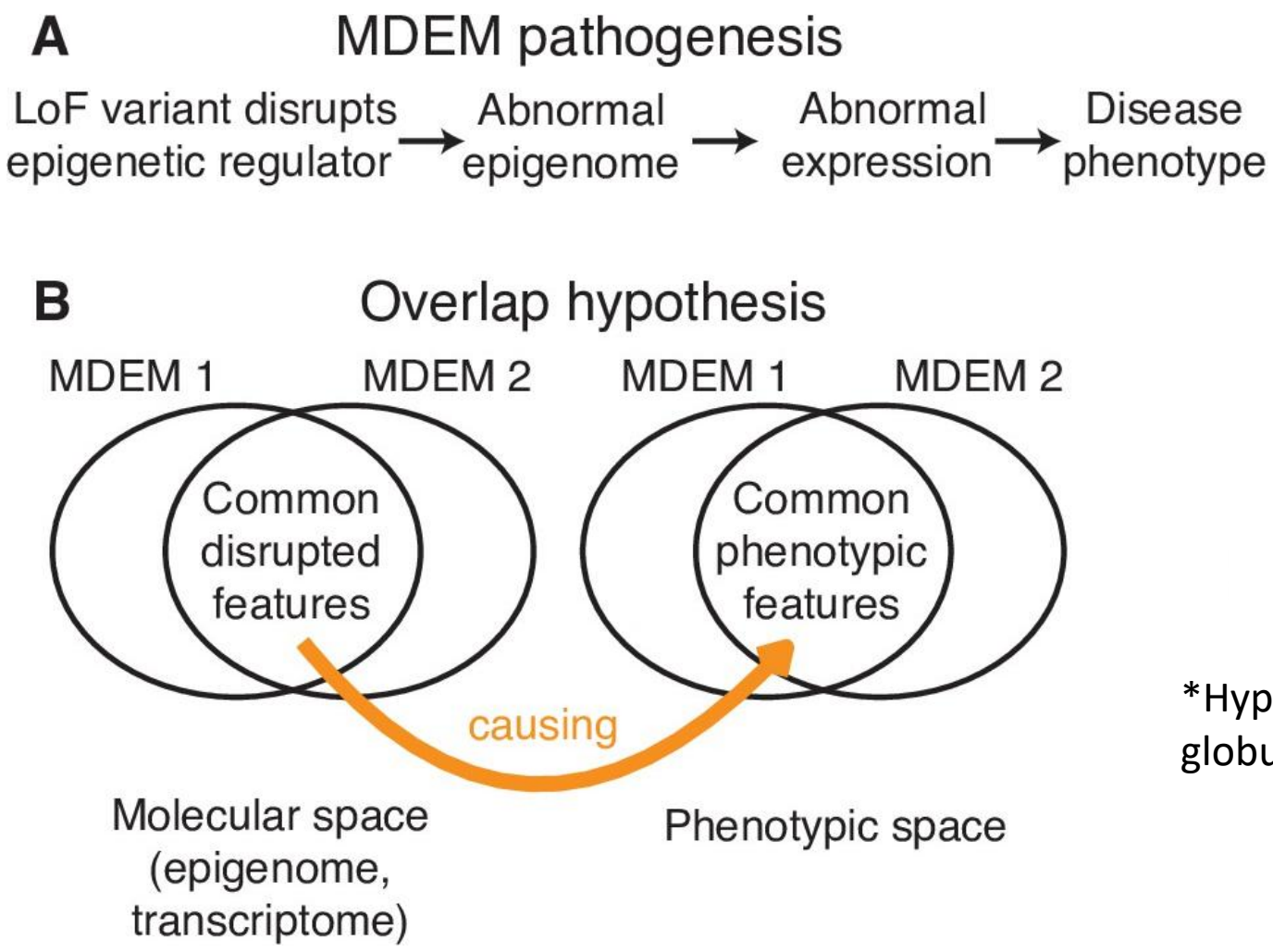
Adam MP *et al*. Clin. Genet. 2005

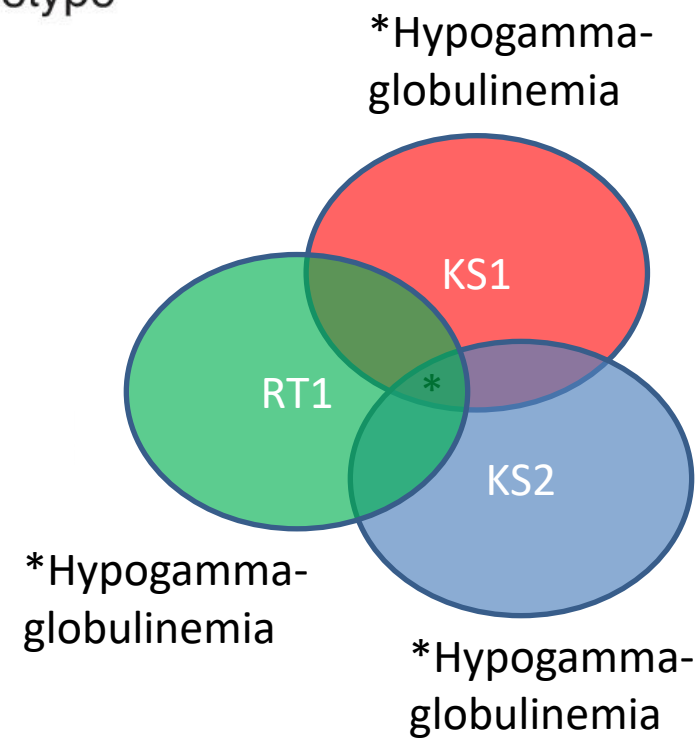


Ng *et al*. Nature Gen. 2010.

Lederer *et al*. Am. J. Hum Genet. 2012.

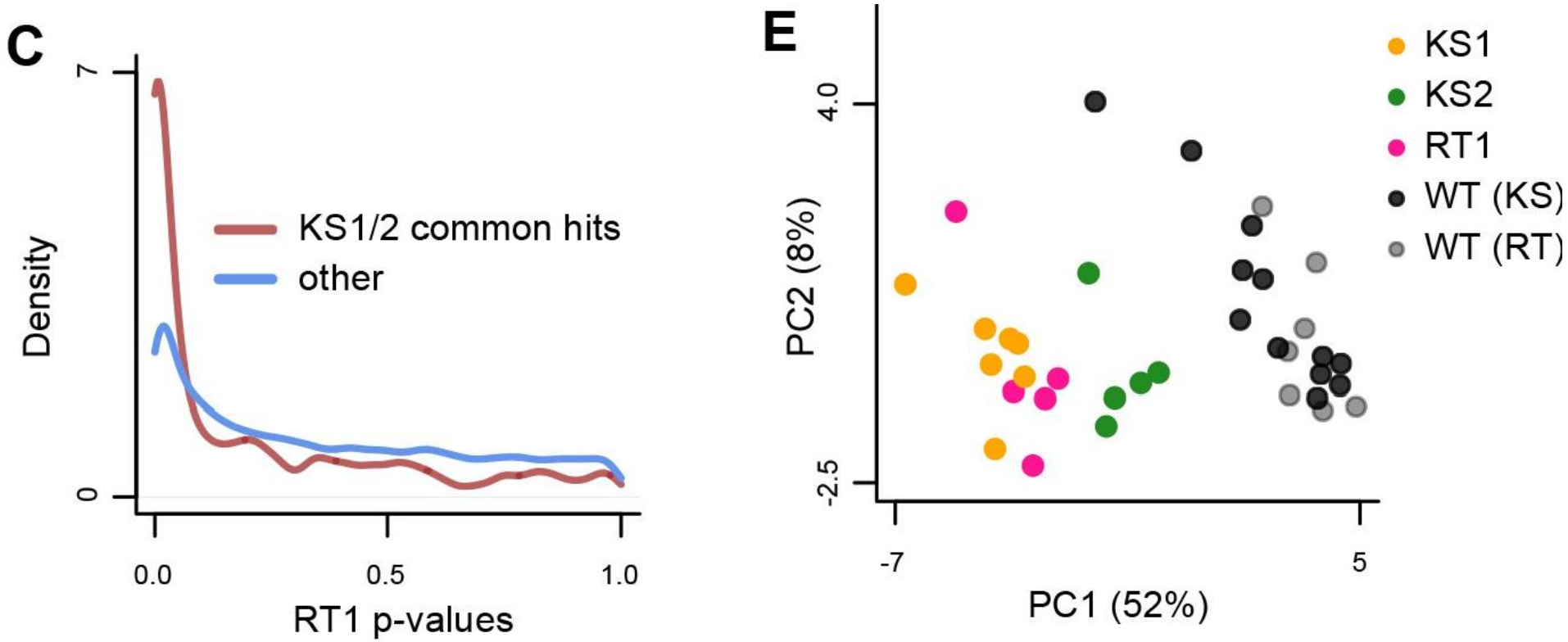
Are there shared chromatin abnormalities between KS1, KS2 and RT1?





Luperchio, Boukas et al. , eLIFE 2021

Sharing of chromatin promoter abnormalities



724 promoter peaks disrupted in both KS1 and KS2 (98% concordant direction)
67% are differential in RT1 as well (Figure 2C; p < 2.2e-16, 5 RT1 vs. 7 wild-type mice)

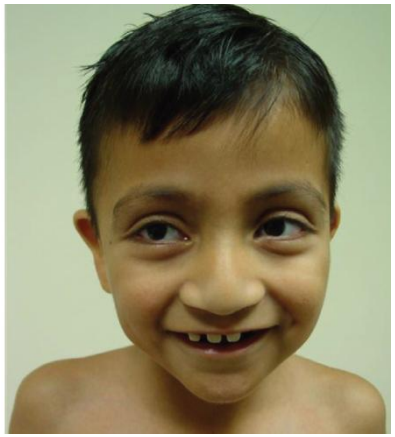
Luperchio, Boukas et al. , eLIFE 2021

Summary (6):

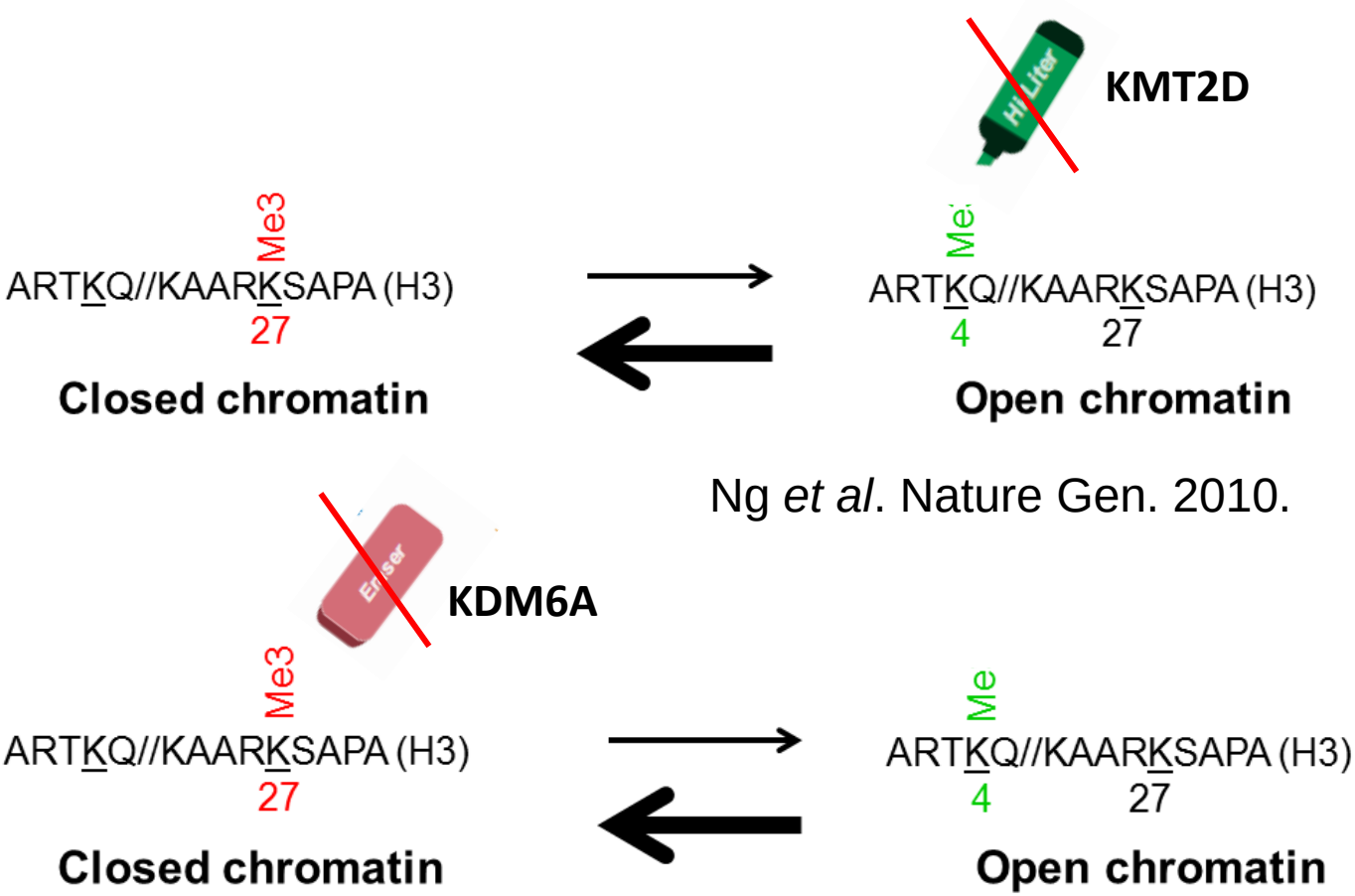
- The apparent dosage sensitivity and disease states when histone acetylation is either increased or decreased indicate **tight regulation of the levels** of these marks;
- The two types of Kabuki syndrome suggest that an **imbalance between open and closed chromatin** states may play a role;
- Some of these disorders (KS and RTS) appear to have **shared molecular abnormalities** which hints at shared pathogenesis which could be a target for therapeutic development if fully understood;

Kabuki syndrome: An imbalance of open and closed chromatin?

Disorder of histone methylation
Autosomal dominant/ X linked (escapes)
Mutations in *KMT2D* or *KDM6A (UTX)*
ID, immune problems, postnatal growth retardation
1/30,000



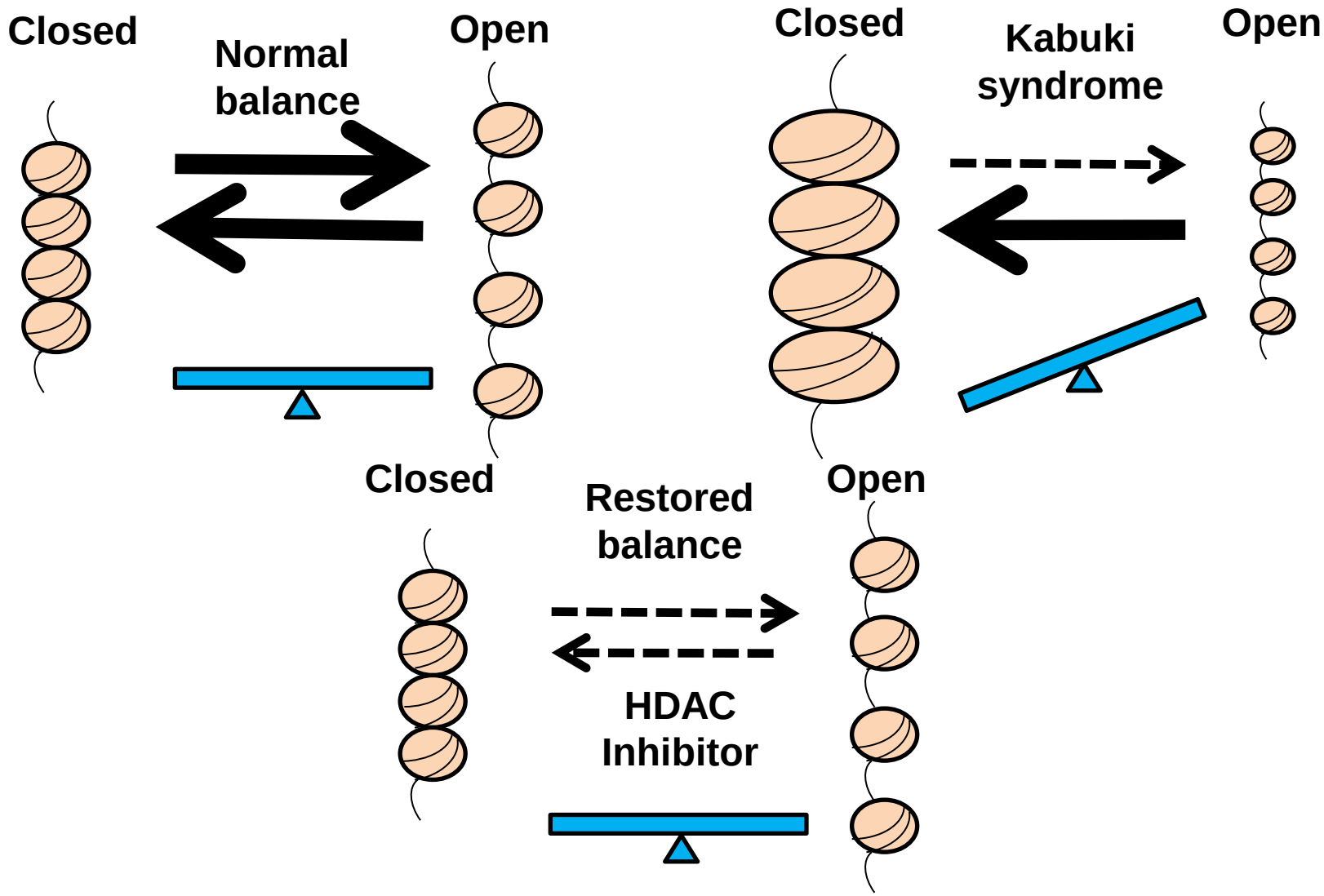
Adam MP et al. Clin. Genet. 2005



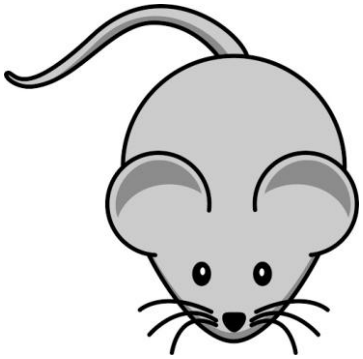
Ng *et al*. Nature Gen. 2010.

Lederer *et al*. Am. J. Hum Genet. 2012.

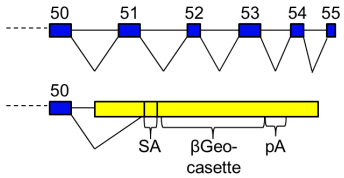
Kabuki syndrome (KS): A treatable cause of intellectual disability?

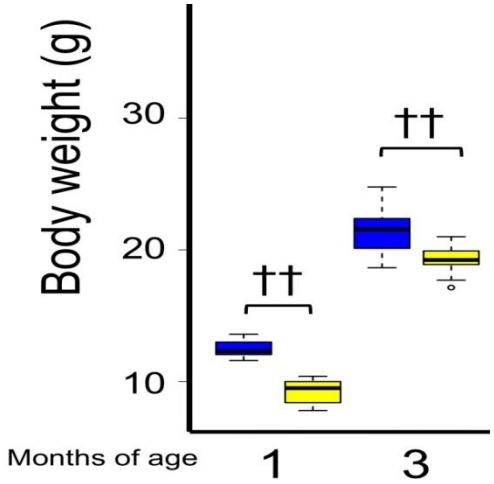


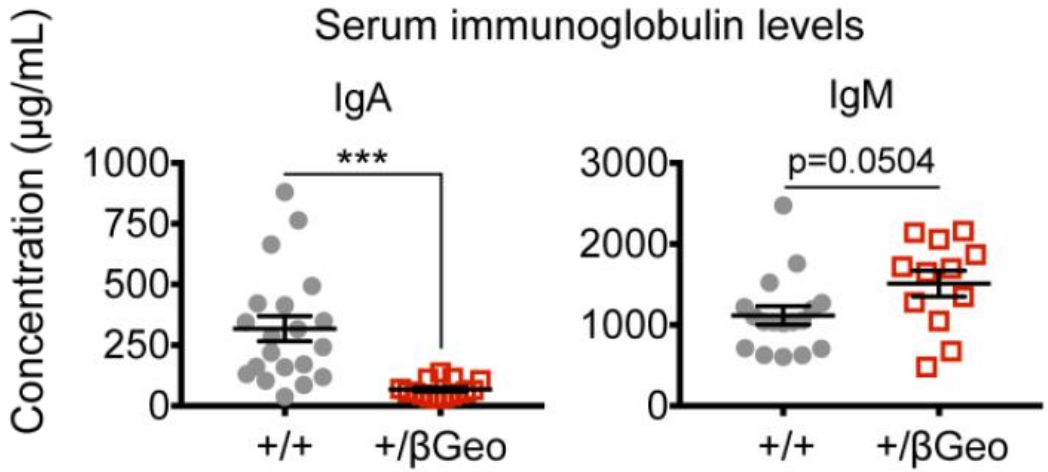
Kmt2d$^{+/\beta Geo}$ mice: Known and new phenotypes

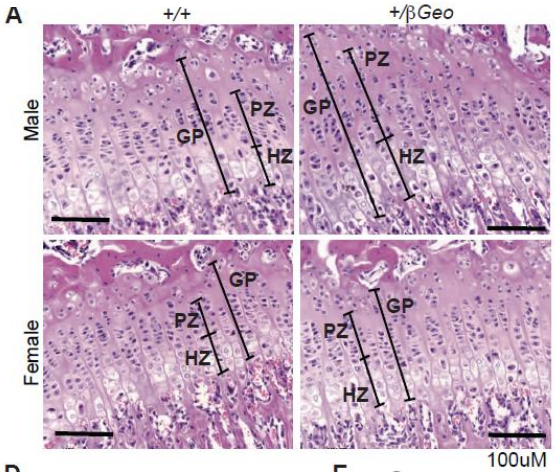


Kmt2d$^{+/\beta Geo}$

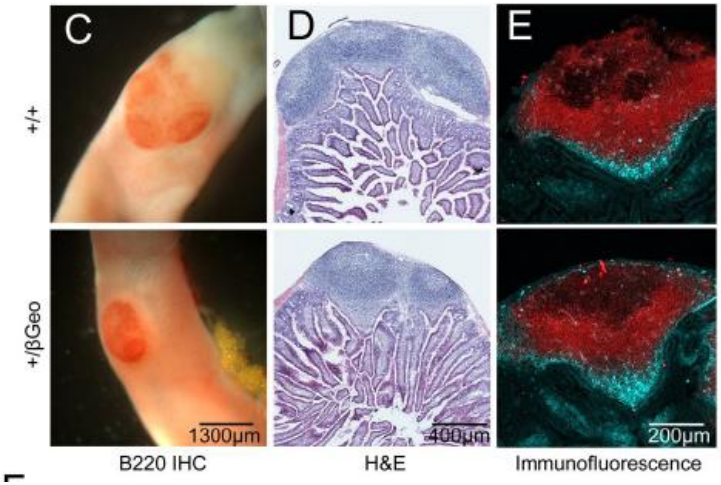








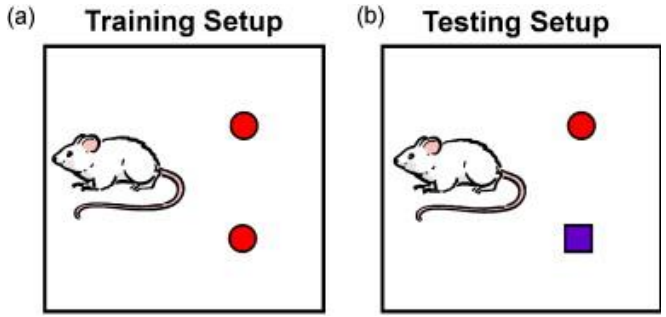
Expanded Growth plate



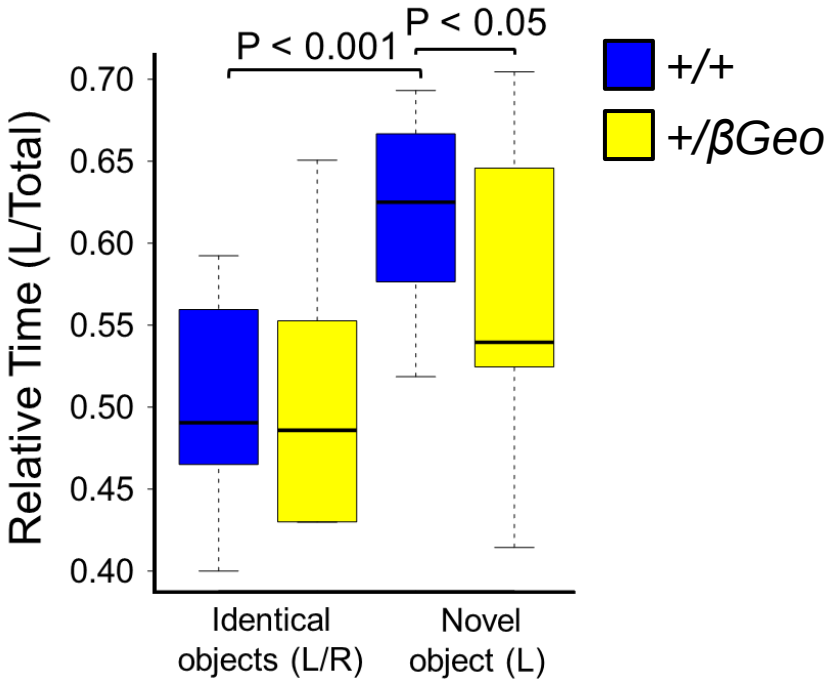
Smaller
Fewer
Peyer´s
patches

Bjornsson et al. Sci Transl Med. 2014, Pilarowski G et al. JACI, 2020; Farhner et al. JCI Insight, 2019

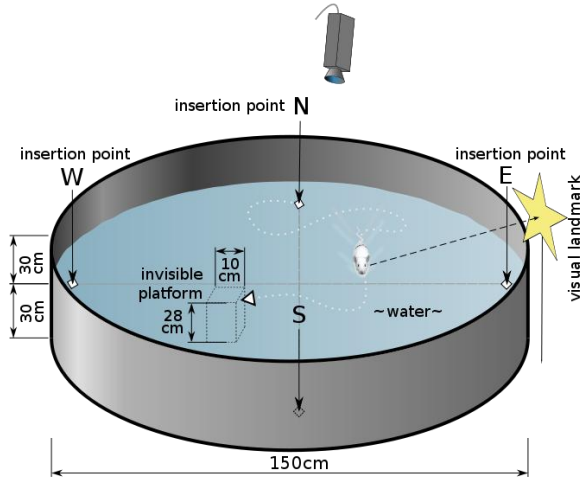
Kmt2d$^{+/\beta Geo}$ mice: hippocampal memory defects in NOR and MWM testing

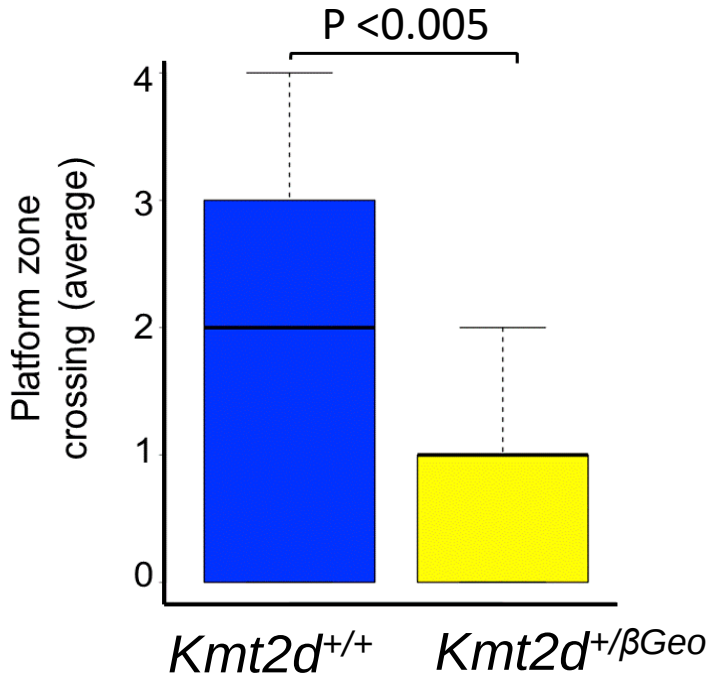


Taglialatela et al. Behav. Brain. Res. 2009



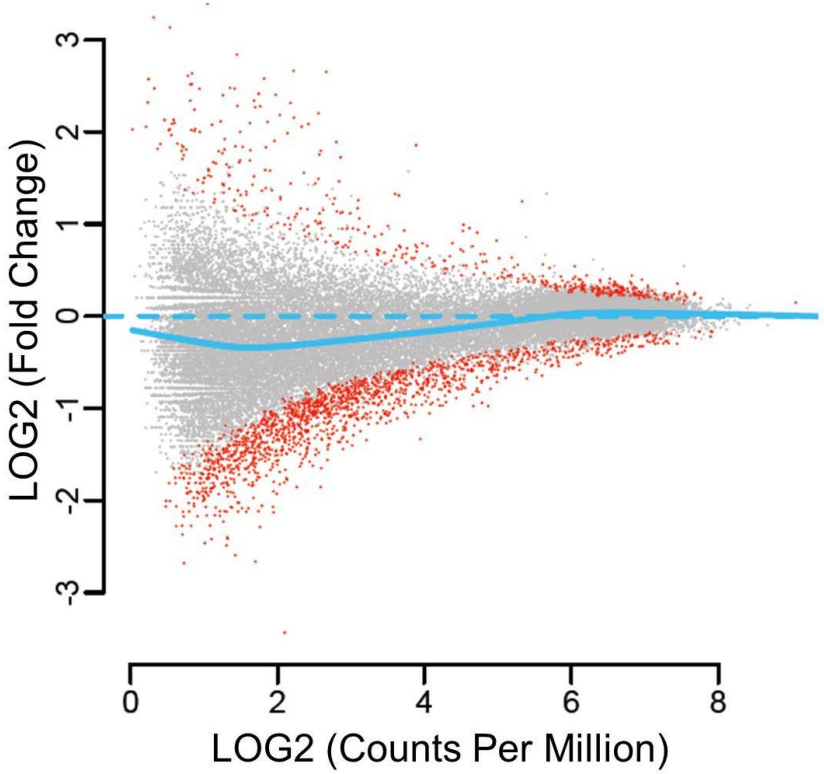
Bjornsson et al. Sci Transl Med. 2014





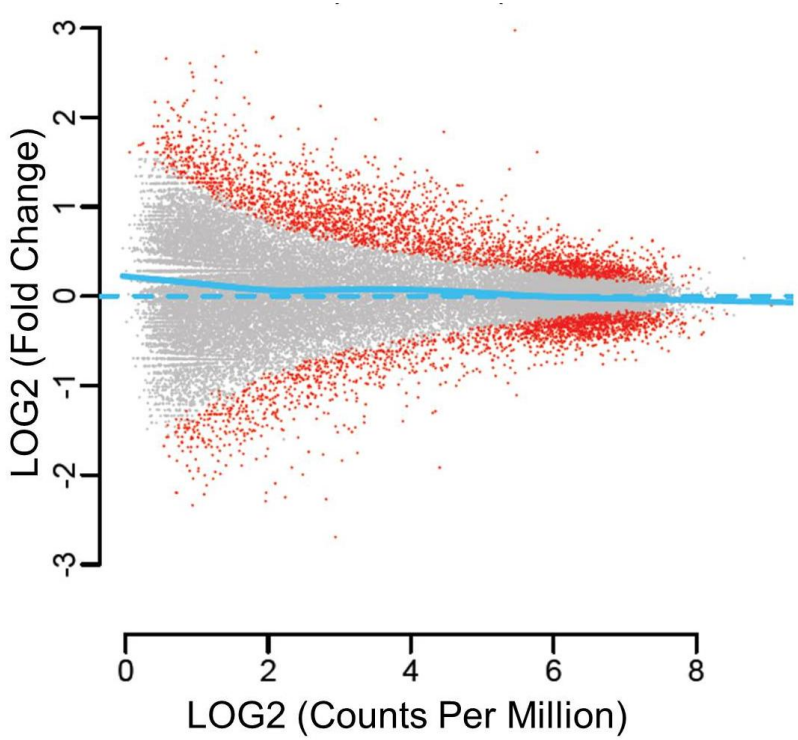
H3K4me3 deficiency is improved *in vivo* with HDACi AR-42

Kmt2d$^{+/\beta Geo}$ – *Kmt2d*$^{+/+}$



No drug

Kmt2d$^{+/\beta Geo}$ *(on AR-42)* – *Kmt2d*$^{+/+}$



AR-42

Bjornsson *et al.* Sci Transl Med. 2014

Summary (7):

- 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.
- *Kmt2d*$^{+/\beta Geo}$ mice have a deficiency of genome-wide **H3K4me3 levels** and this can be manipulated with histone deacetylase inhibition;

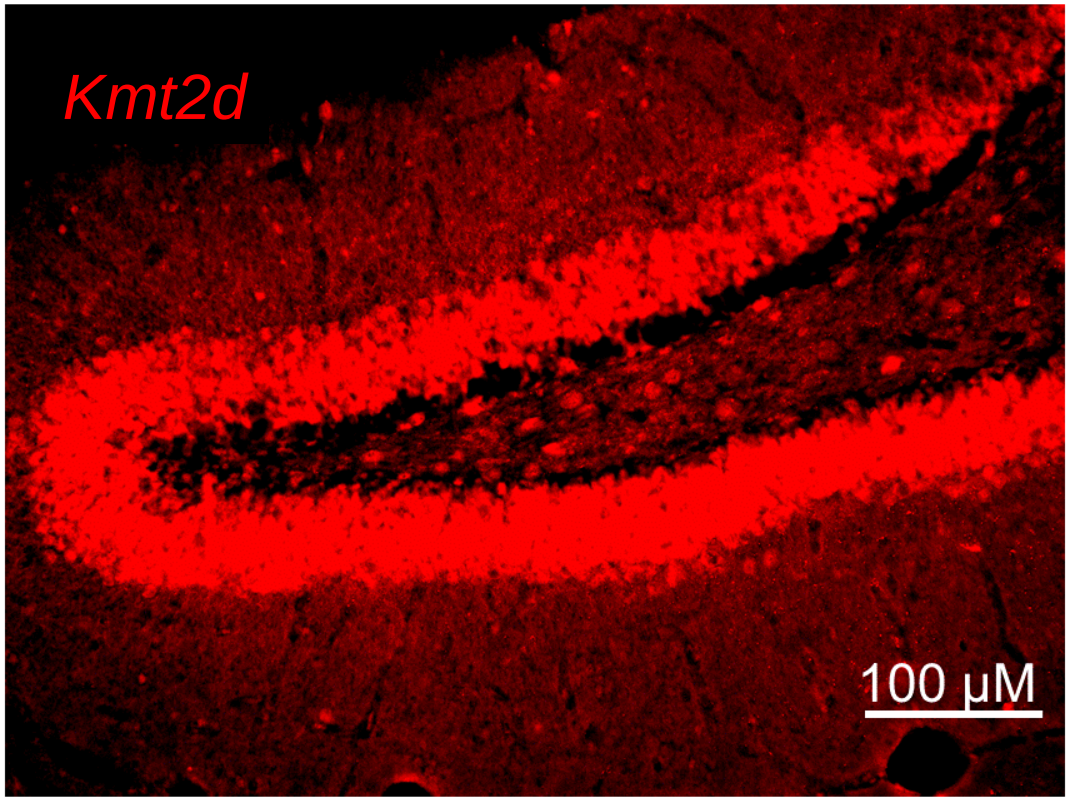
Kmt2d is highly expressed in Granule Cell Layer (GCL) of the dentate gyrus

Strongest expression in Granule Cell Layer (GCL);

GCL is within dentate gyrus which is part of the hippocampus;

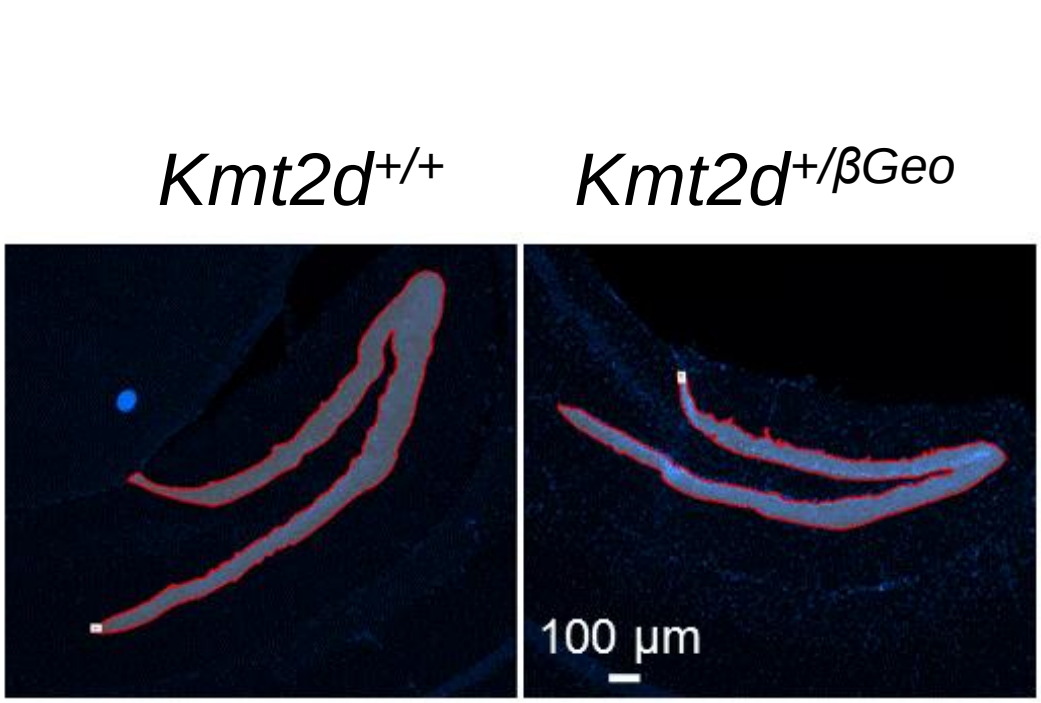
Adult neurogenesis occurs in subgranular zone (SGZ);

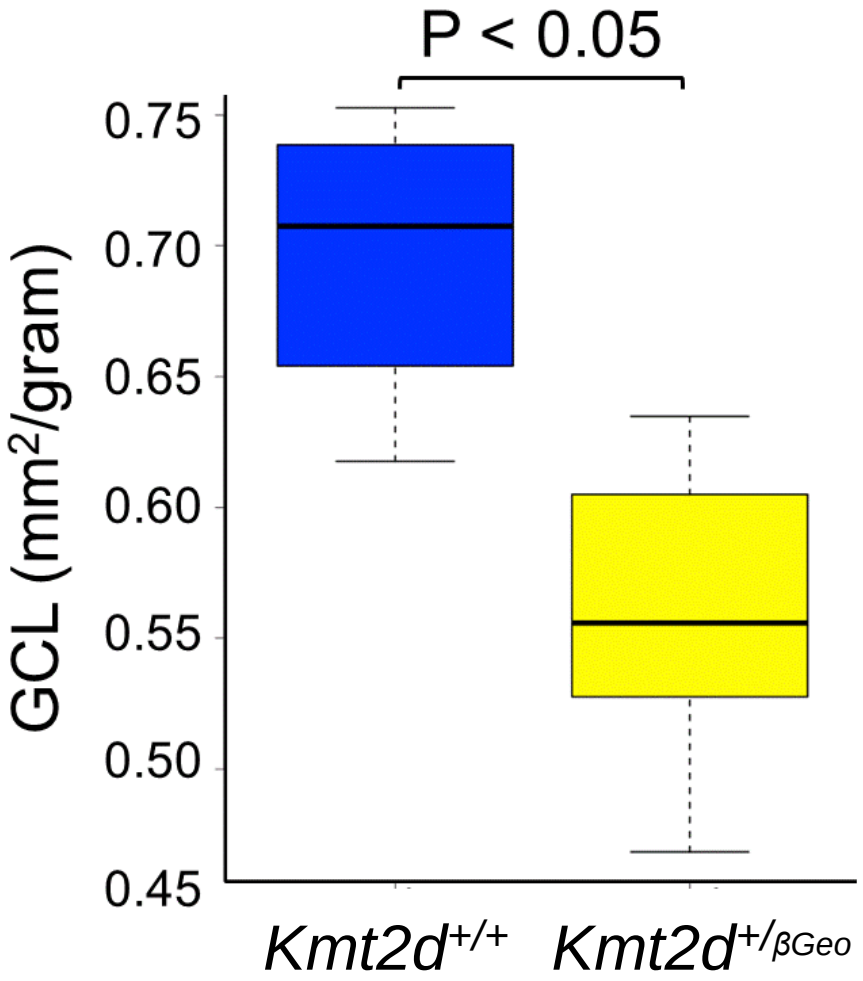
Defects of adult neurogenesis lead to hippocampal memory defects.



Immunofluorescence against *Kmt2d* (WT)

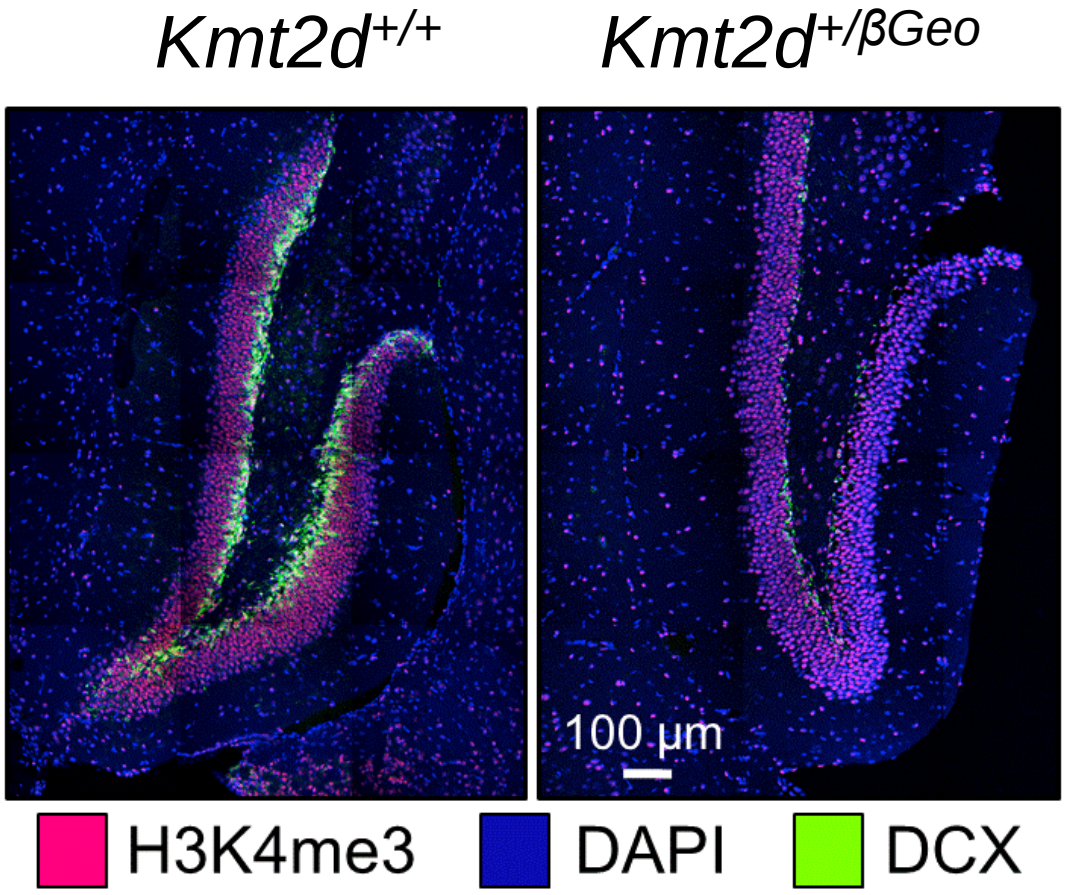
Kmt2d$^{+/\beta Geo}$ mice have a thinner GCL layer

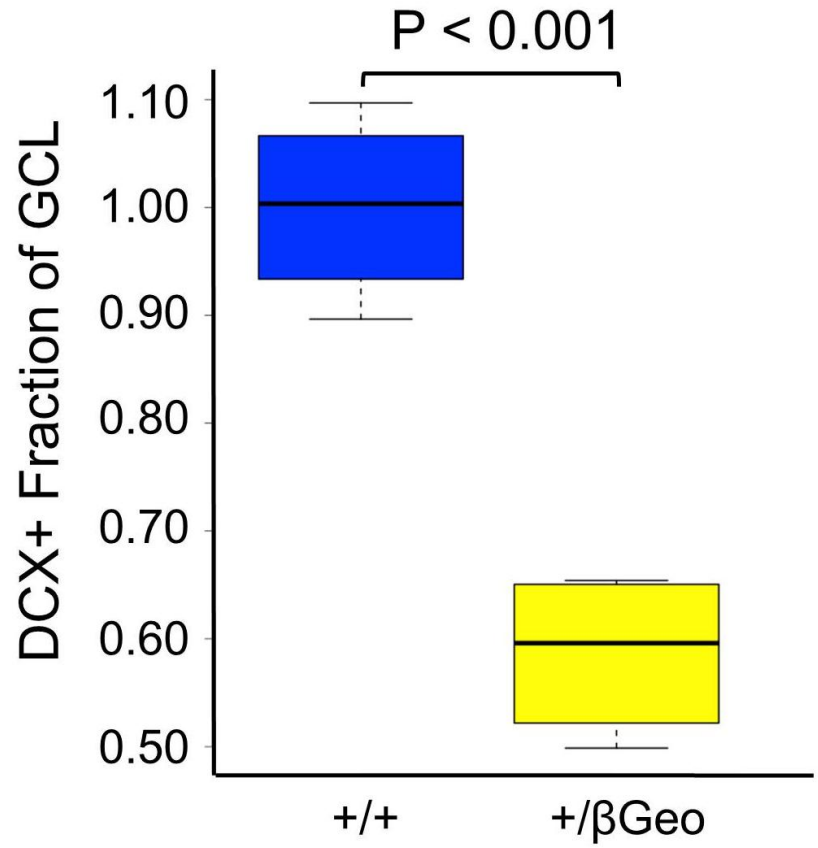




Bjornsson et al. Sci Transl Med. 2014

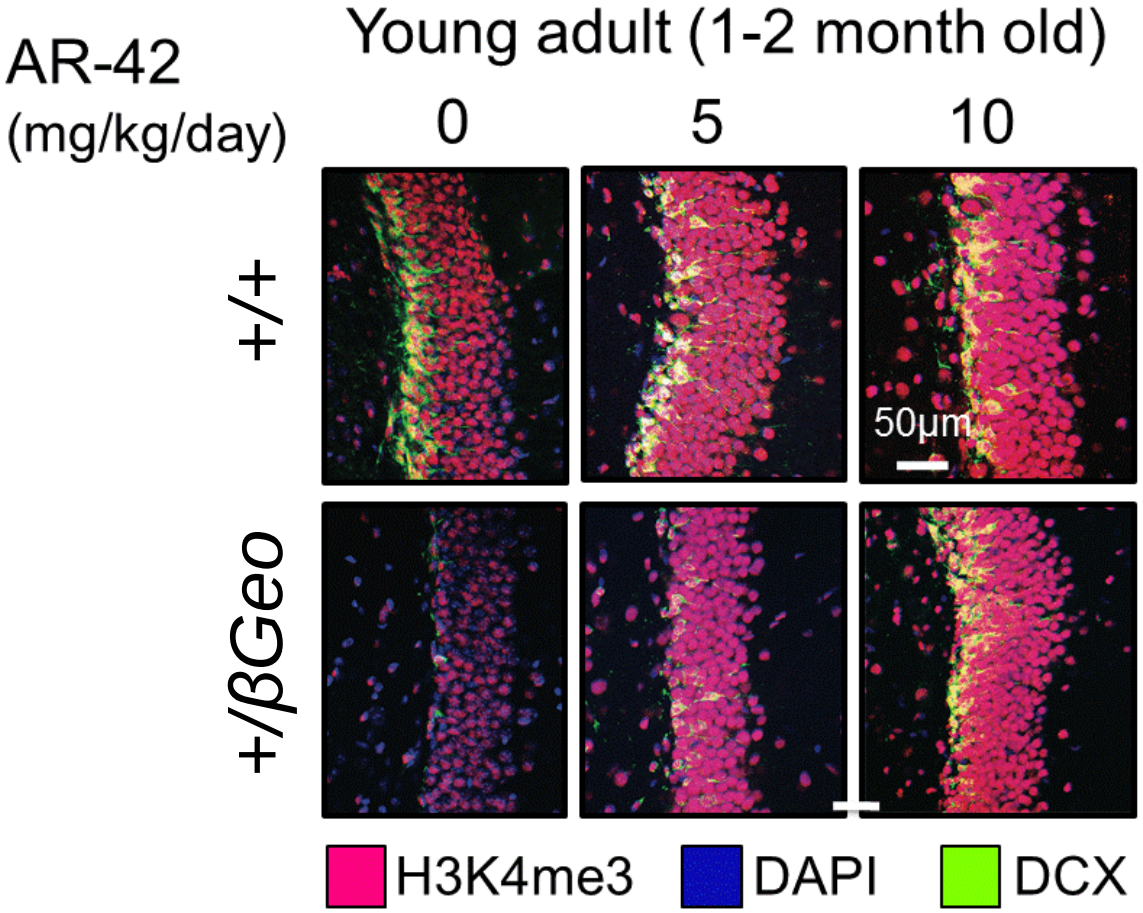
Kmt2d$^{+/\beta Geo}$ mice have a deficiency of neurogenesis in the GCL layer of the DG

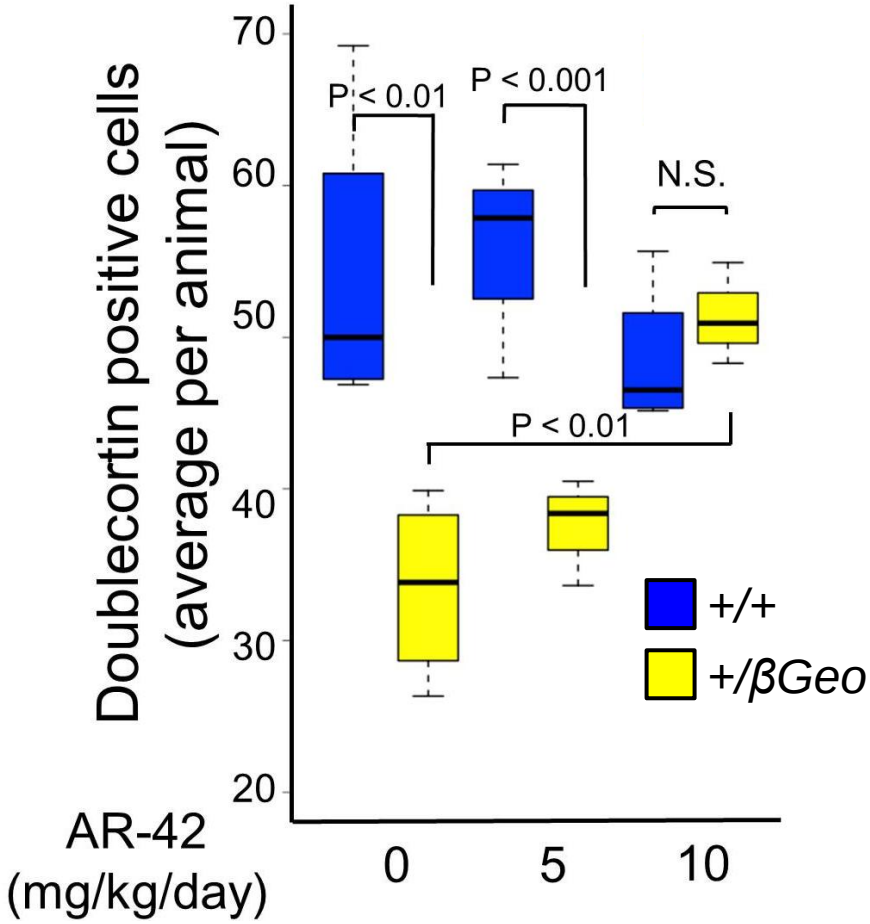




Bjornsson et al. Sci Transl Med. 2014

Impaired neurogenesis in *Kmt2d*$^{+/\beta Geo}$ mice is improved with 2 weeks of HDAC inhibitor AR-42

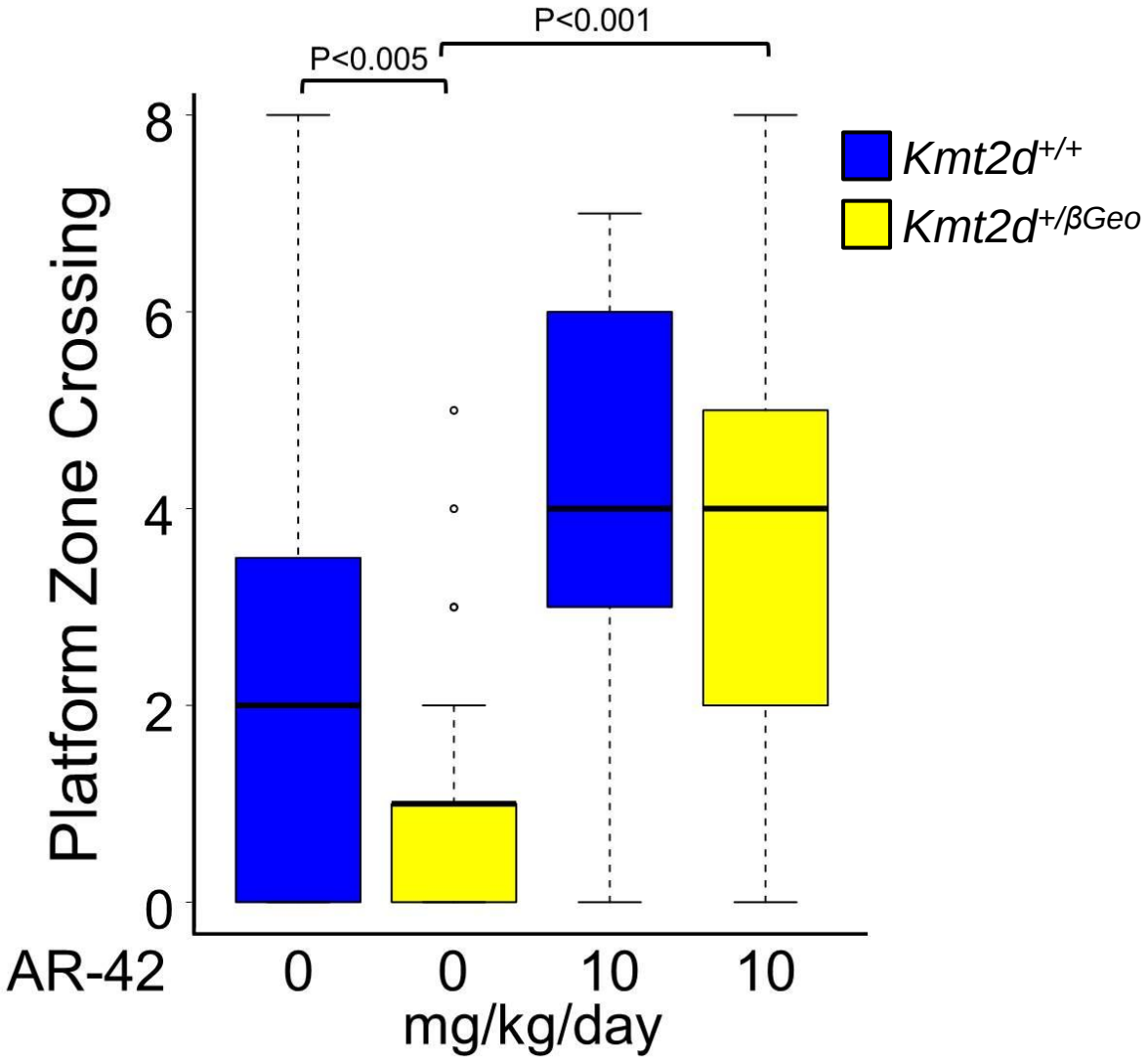




Five month old cohort

Bjornsson *et al.* Sci Transl Med. 2014

Hippocampal memory defect in *Kmt2d*$^{+/\beta Geo}$ mice improves after 2 weeks of HDAC inhibitor AR-42



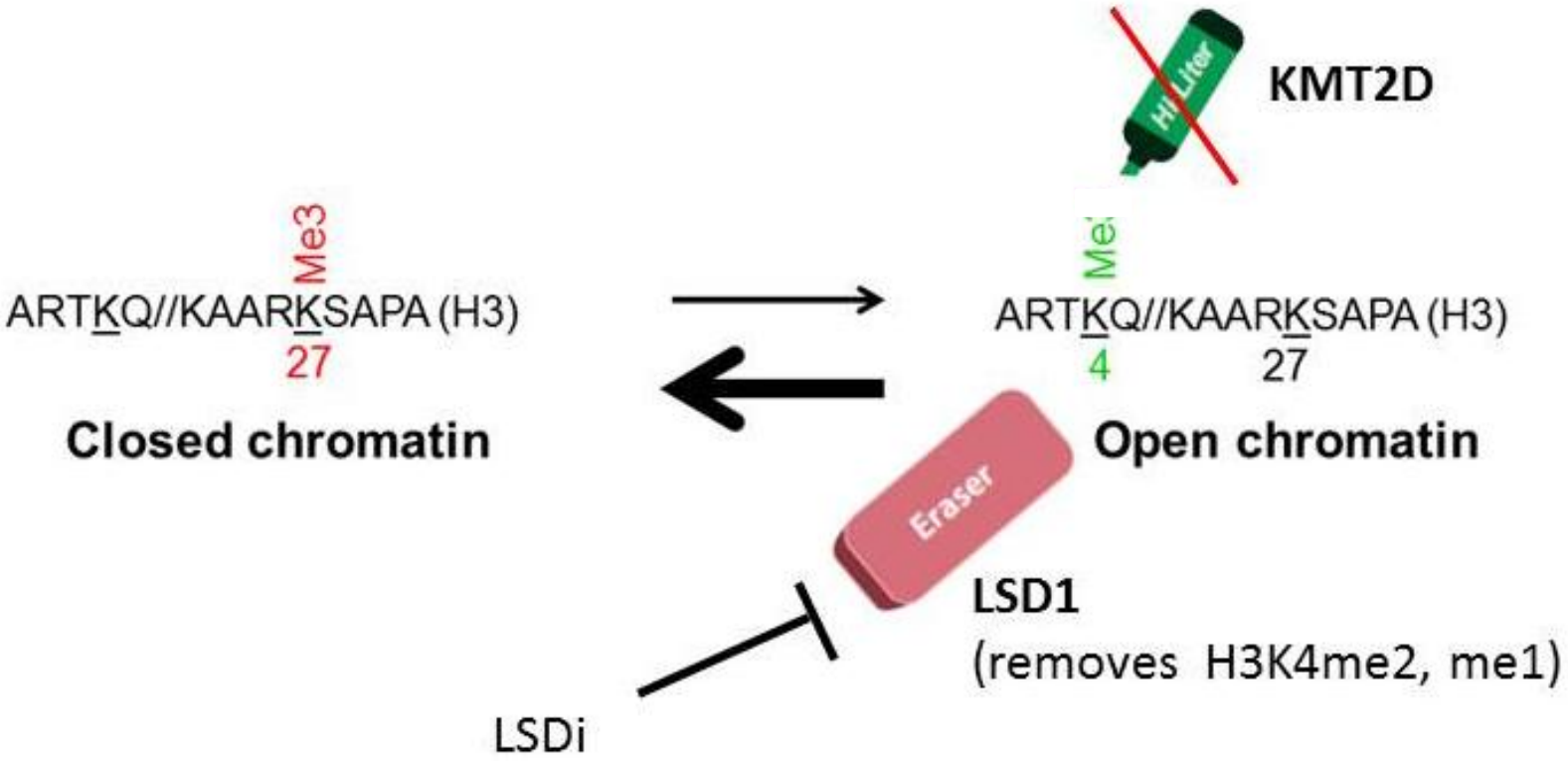
Bjornsson et al. Sci Transl Med. 2014

Summary (8):

- A mouse model of KS reveals **thinning of the GCL layer of dentate gyrus** 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**;

AR-42 is a cancer agent, are there more specific strategies?

LSD1 inhibition is theoretically a very specific way of increasing H3K4 methylation

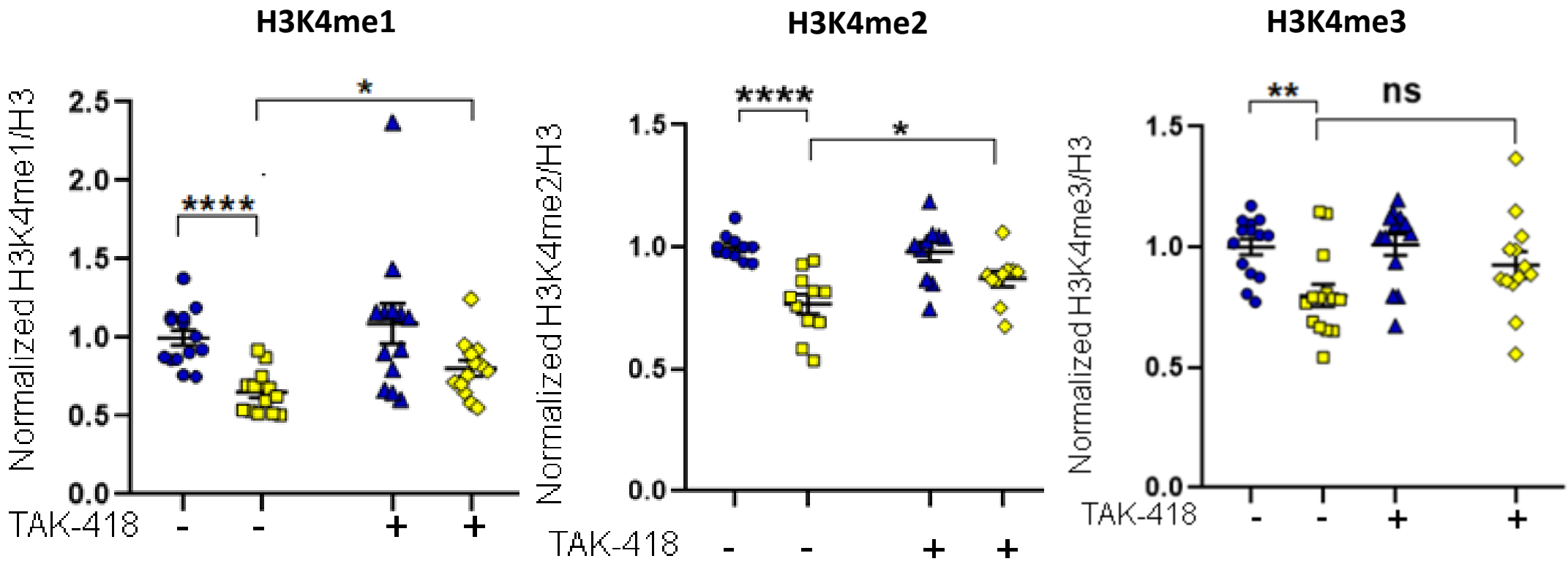


Zhang et al. Molecular Therapy: Methods and Clinical Development, 2021

Can LSD1 inhibition modify histone methylation levels in mouse hippocampus?

Used TAK-418, a LSD1 inhibitor developed by Takeda Pharmaceuticals.

Western blot (mouse hippocampus)



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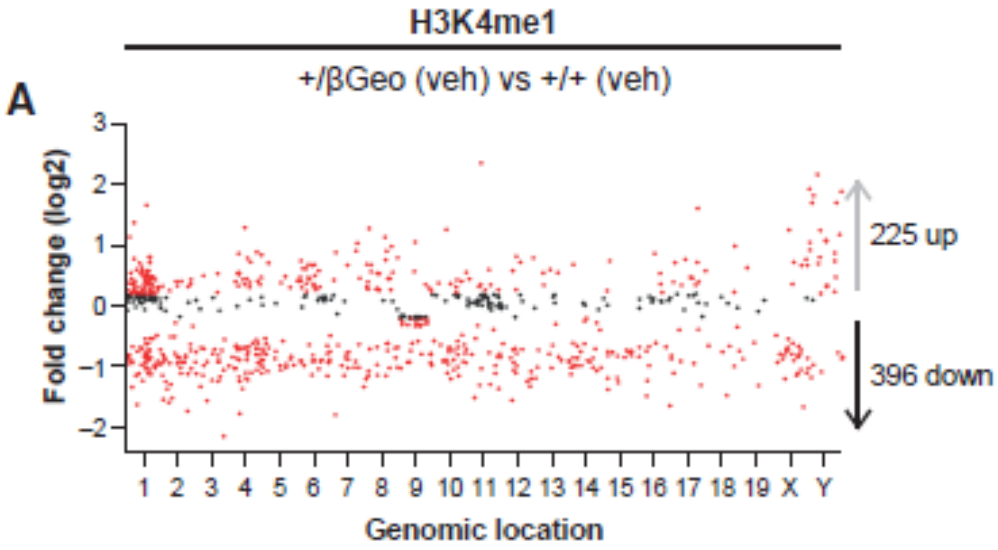
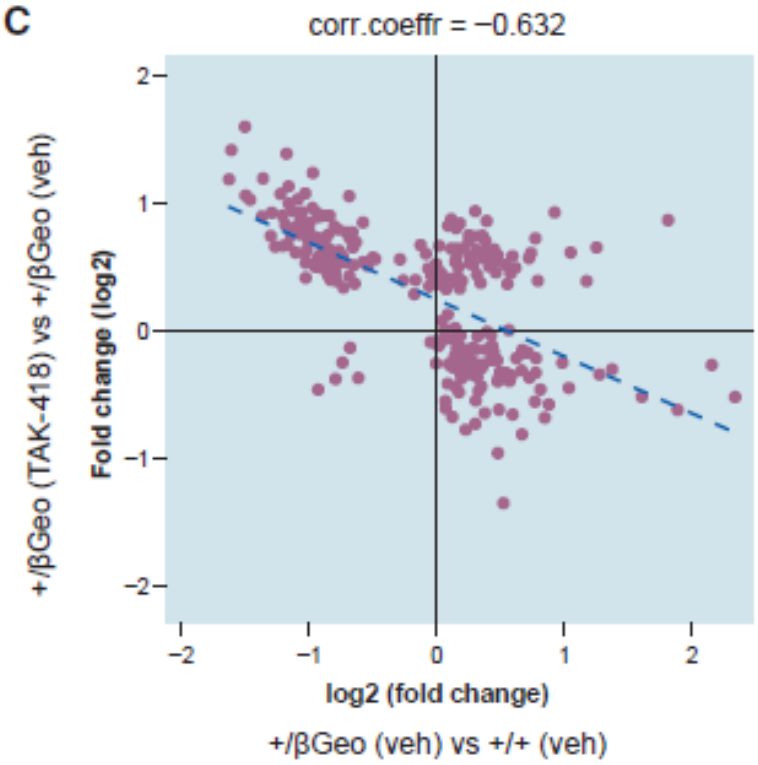
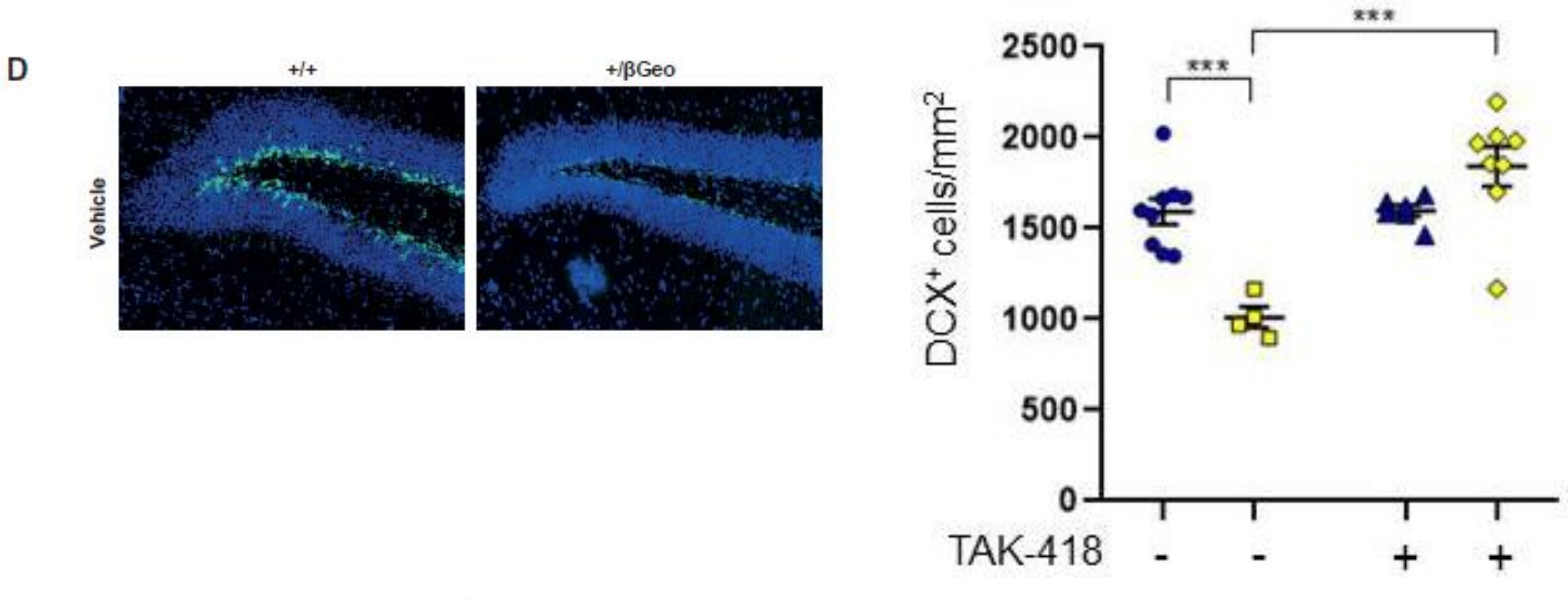




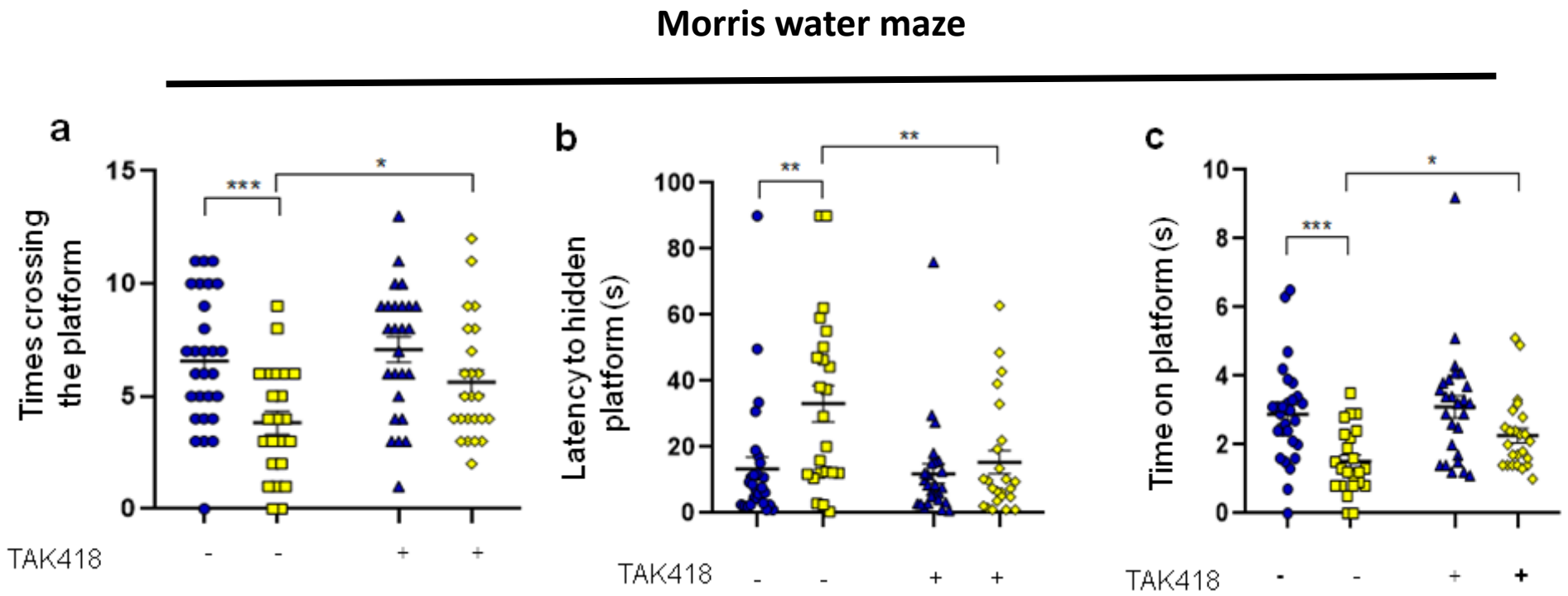
Zhang et al. Molecular Therapy: Methods and Clinical Development, 2021

Does LSD1 inhibition rescue defect of adult neurogenesis in dentate gyrus?



Zhang et al. Molecular Therapy: Methods and Clinical Development, 2021

Does LSD1 inhibition rescue hippocampal memory defects?



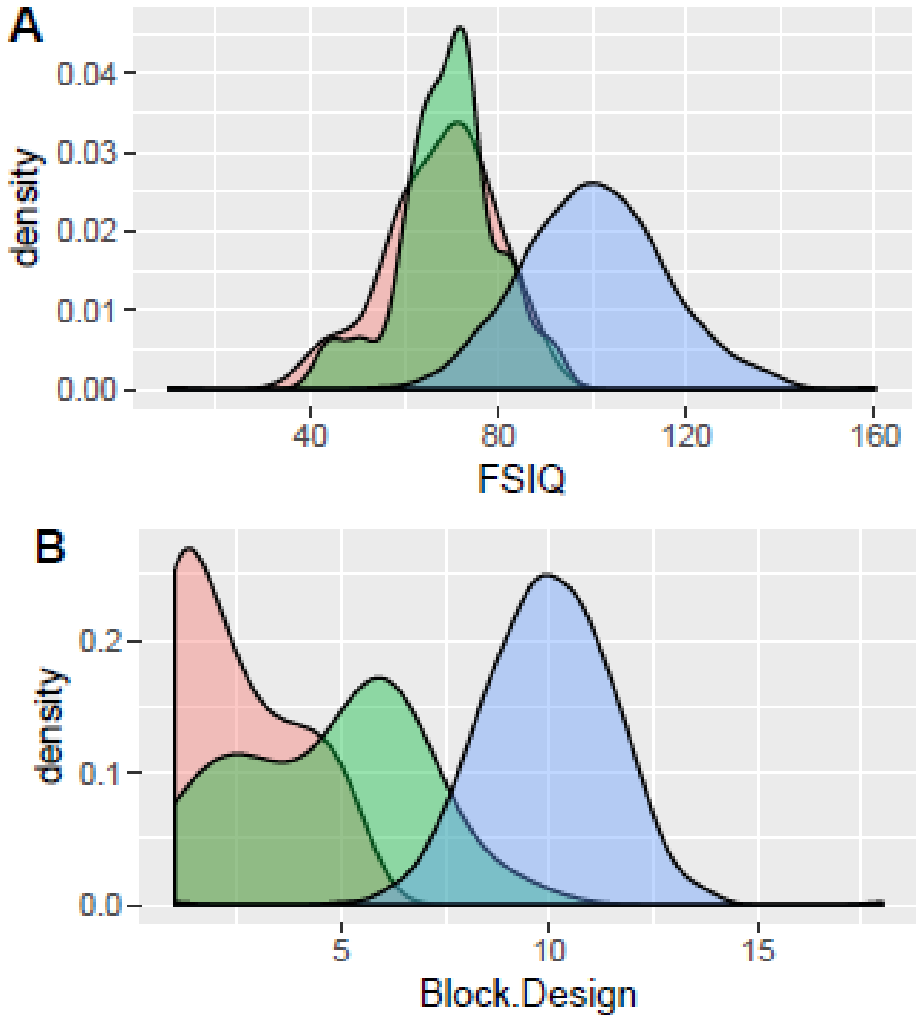
Zhang et al. Molecular Therapy: Methods and Clinical Development, 2021

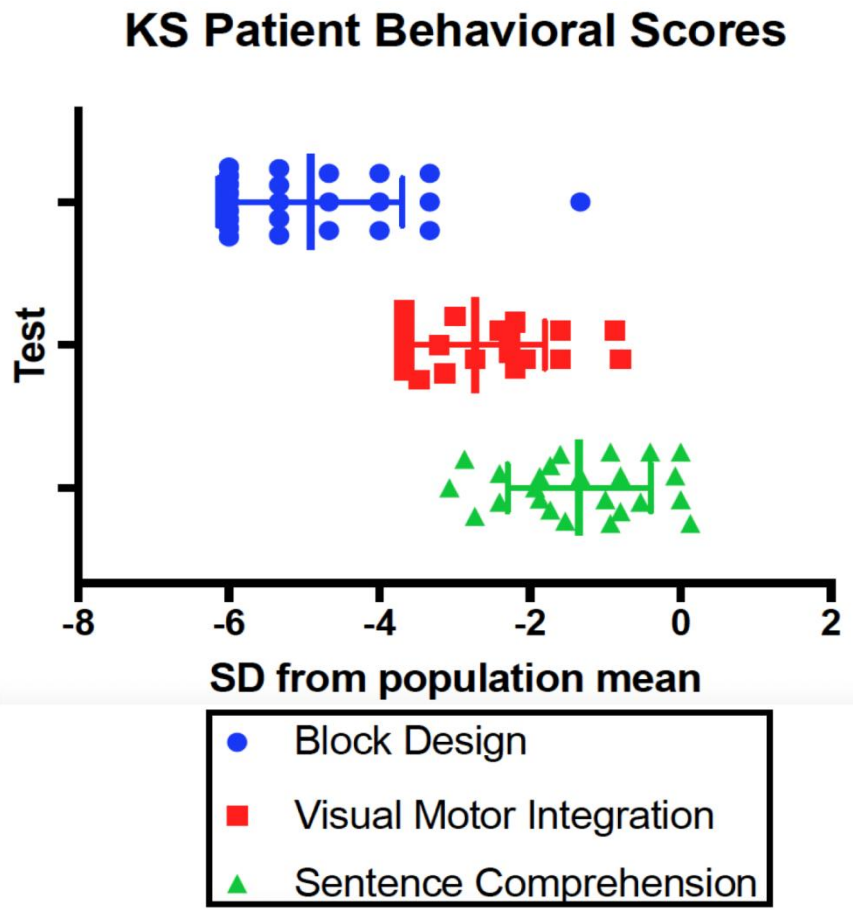
Summary (9):

- Defects in the dentate gyrus can be reversed using a drug that inhibits the **opposing epigenetic machinery (LSD1)**;
- This validates the prior non-specific strategy and suggests that aspects of the intellectual disability phenotype **in Kabuki syndrome may be treatable**;

Can we relate these mouse findings to humans?

Patients with KS have visuospatial defects





Harris J, Mahone M, Bjornsson HT, JIDR, 2019

Patients with KS have a significantly smaller dentate gyrus and hippocampus

Anatomical and functional abnormalities on MRI in kabuki syndrome

Jennifer Boisgontier[a,*], Jean Marc Tacchella[a], Hervé Lemaître[a,b], Natacha Lehman[c], Ana Saitovitch[a], Vincent Gatinois[c], Guilaine Boursier[c], Elodie Sanchez[c], Elza Rechtman[a], Ludovic Fillon[a], Stanislas Lyonnet[d], Kim-Hanh Le Quang Sang[d], Genevieve Baujat[d], Marlene Rio[d], Odile Boute[e], Laurence Faivre[f], Elise Schaefer[g], Damien Sanlaville[h], Monica Zilbovicius[a], David Grévent[a], David Geneviève[c], Nathalie Boddaert[a]

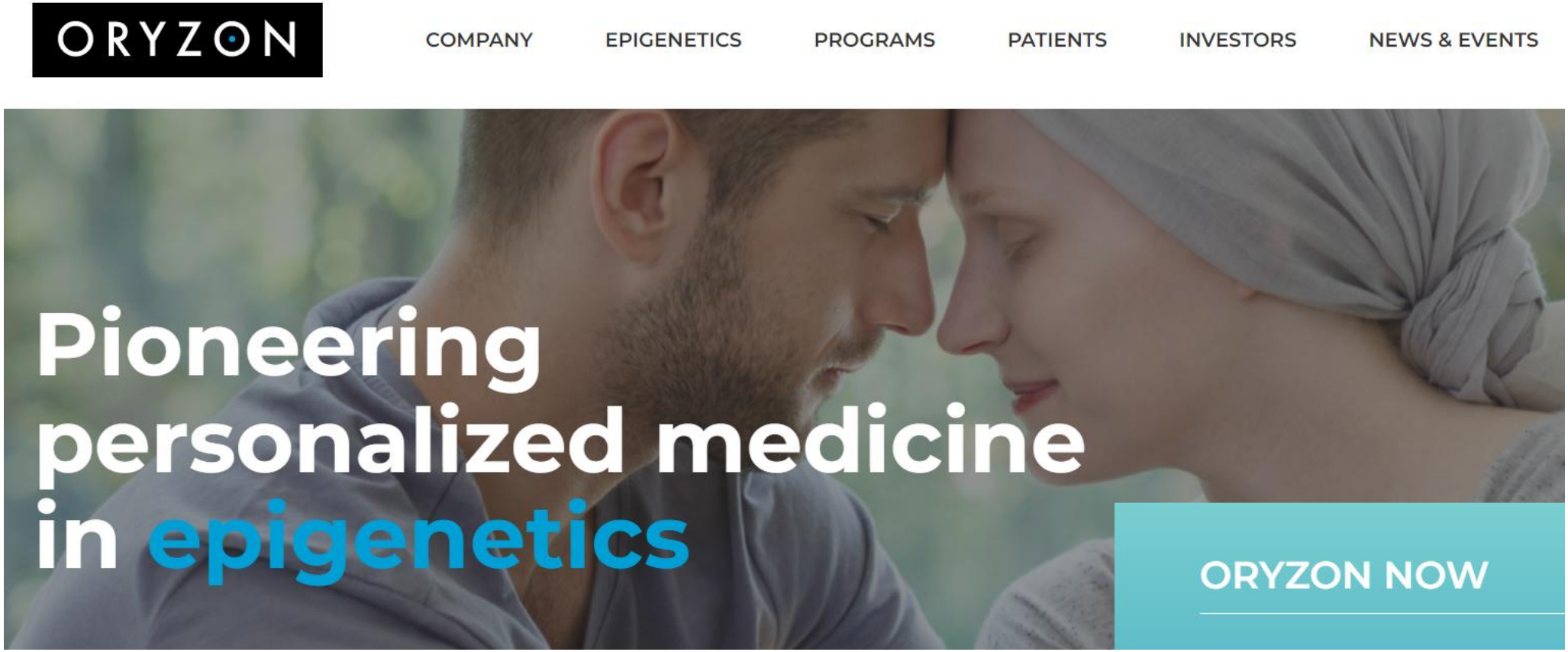
Table 2
Volume of hippocampus and dentate gyrus in patients with KS and healthy controls.

Volume of:	KS participants (n = 6)	Healthy controls (n = 26)	Statistics	*df*	*p*
Left hippocampus (in mm^3), mean [SD]	3283.06 [510.45]	3814.43 [336.94]	$t = 3.93$	28	0.0005*
Right hippocampus (in mm^3), mean [SD]	3427.31 [624.17]	3855.47 [373.06]	$t = 2.83$	28	0.008*
Left dentate gyrus (in mm^3), mean [SD]	230.65 [38.24]	268.53 [29.41]	$t = 3.11$	28	0.004*
Right dentate gyrus (in mm^3), mean [SD]	247.47 [38.69]	278.83 [32.90]	$t = 2.73$	28	0.01*

Abbreviations: SD: Standard Deviation; df: degrees of freedom.
* $p < .05$

Boisgontier et al. Neuroimage Clinical, 2018

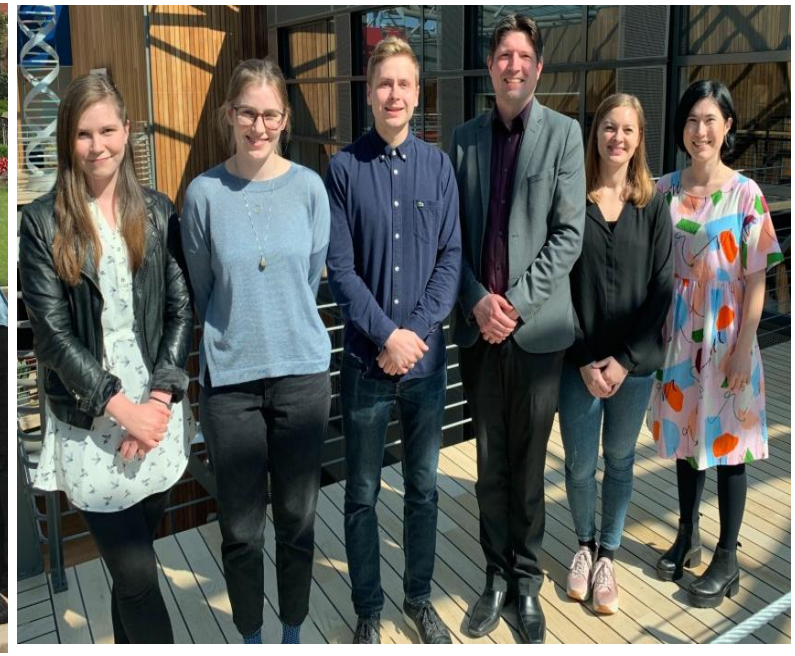
HOPE trial with Vafidemstat (planned 2022)



Summary (10):

- Patients with molecularly confirmed Kabuki syndrome type 1 (*KMT2D*) have **visuospatial defects**, symptoms that localize to the dentate gyrus of the hippocampus and **smaller hippocampi and dentate gyri**;
- HOPE trial planned (Oryzon) 2022!

Acknowledgements



Funding:
NIH (Early Independence)
Louma G. Foundation
Takeda Pharmaceuticals
Icelandic Research Fund (#195835, #206806, #217988)
Icelandic Technology Fund (#2010588)
Icelandic Cancer Society
Göngum Saman
Landspítali Research Fund
University Research Fund
Kabuki families
InfraStructure Fund

Video summary of our Kabuki work:
https://www.youtube.com/watch?v=AVK-tWIhXBk&feature=youtu.be

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