DONDERS INSTITUTE

IPSC-derived neural cells and strategies for upregulating the wildtype allele in patient cells

Hans van Bokhoven,
Molecular Neurogenetics

Kleefstra Syndrome
Scientific Conference 2023

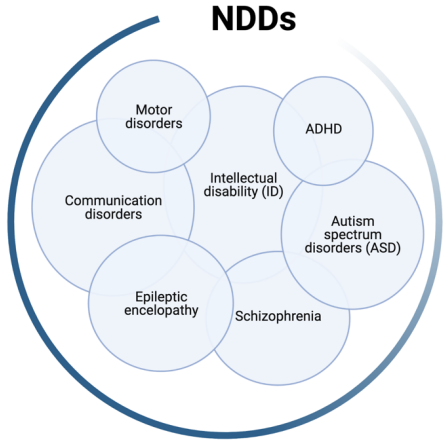
Ljubljana, 1-2 June

Human Genetics Nijmegen

Radboudumc
university medical center

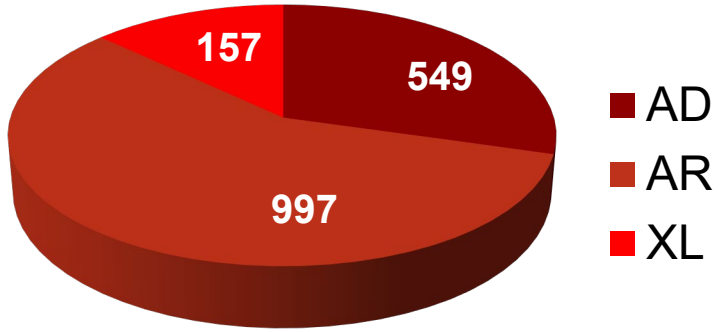
Radboud University Radboudumc

The molecular basis of Neurodevelopmental Disorders (NDDs)



ID genes January 2023

- 1566 total proven
- 157 X-Linked (XL)
- 549 Aut. Dominant (AD)
- 997 Aut. Recessive (AR)
- 9 mitochondrial
- (133 AD +AR)



- The vast majority of all AD disorders involve hapoloinsufficiency
- Opportunity for therapy: upregulation of the remaining wt allele

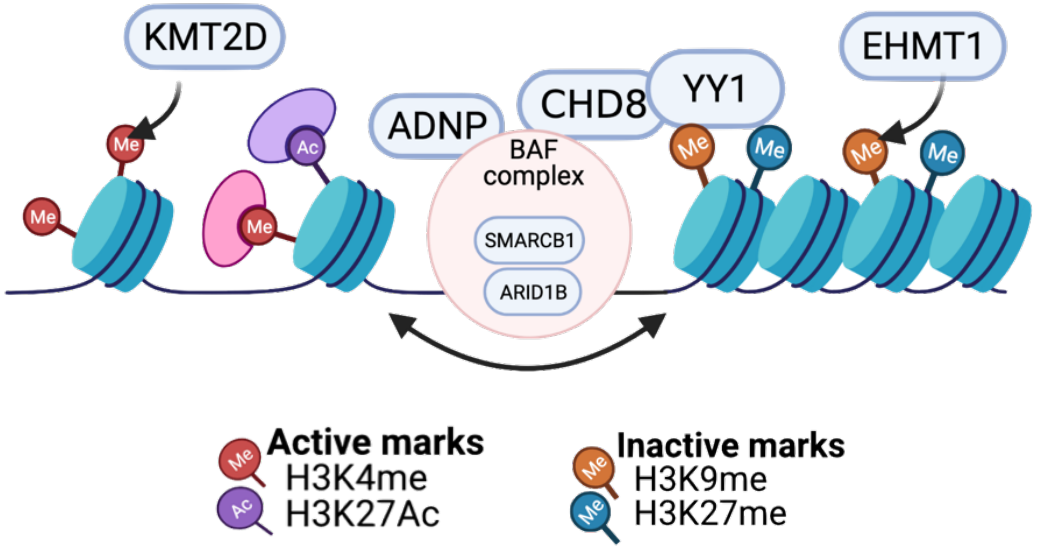
Kleefstra Syndrome

- Haploinsufficiency of the *EHMT1* gene
- EHMT1: Euchromatine histone methyltransferase
- Developmental delay
- intellectual disability
- ASD

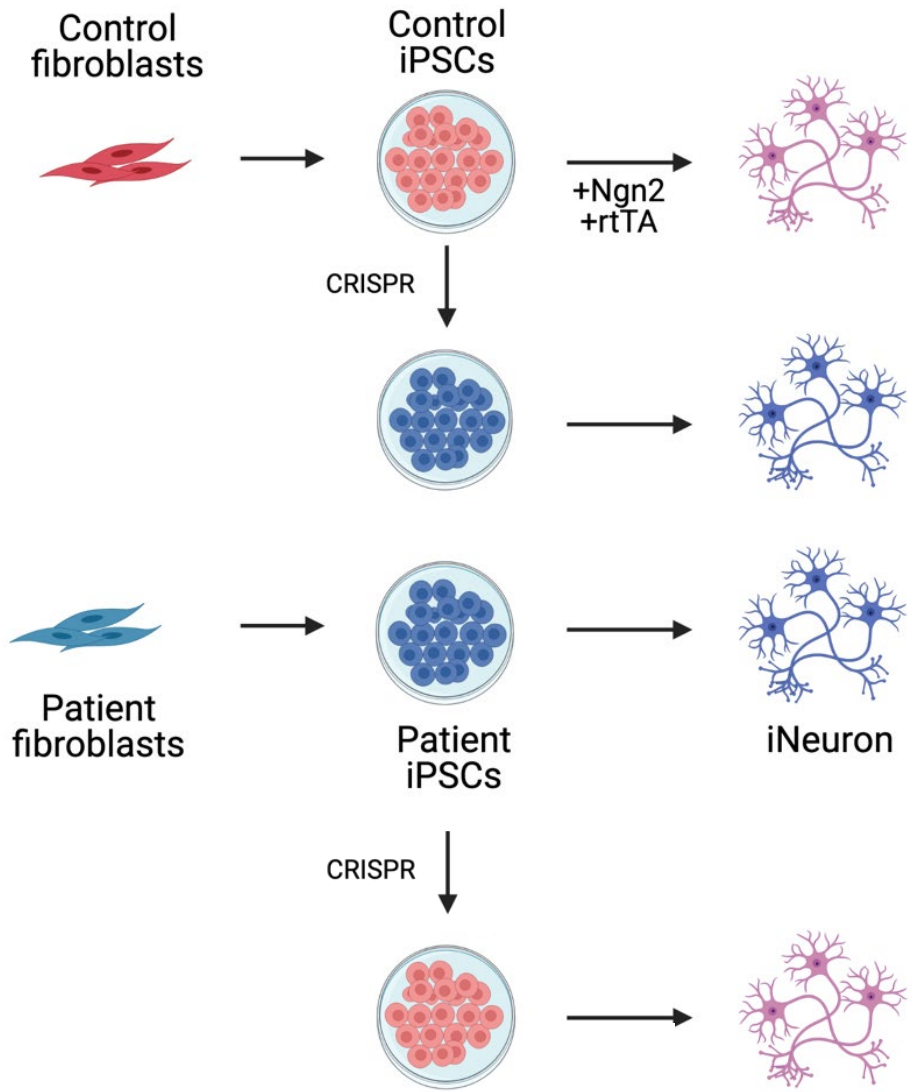
	Kleefstra
ASD (ADOS)	96%
Major depressive disorder	42%
Anxiety disorder	46%
(Hypo)mania	30%
OCD	33%
Psychosis	30%
Unspecified disorder	38%
Regression	50%
Sleep disorder	42%

Vermeulen et al. Am J Med Genet 2016

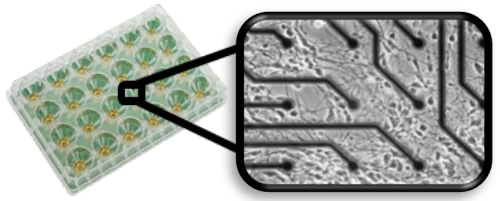
Protein	Disorders
KMT2D	Kabuki syndrome
YY1	Gabriele-de-Vries syndrome
ADNP	Helsmoortel-Van der Aa syndrome
CHD8	Syndromic ASD (AUTS18)
EHMT1	Kleefstra syndrome
SMARCB1	Coffin-syris syndrome; Kleefstra syndrome spectrum
ARID1B	Coffin-syris syndrome; Syndromic ID

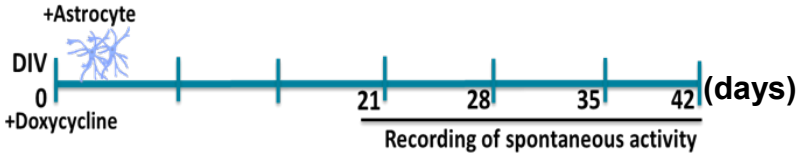


General strategy



Neuronal network phenotyping on MEAs

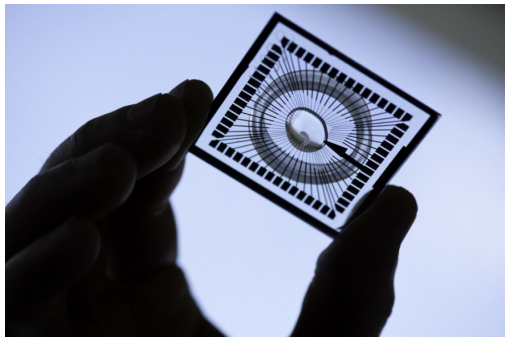


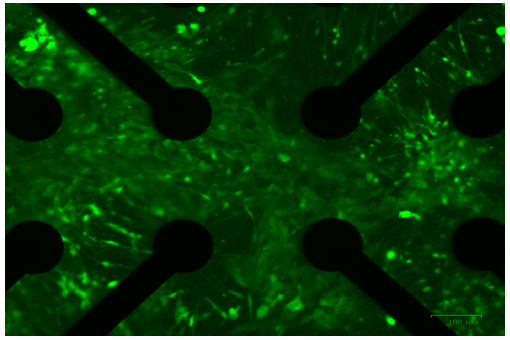


- glutamatergic neurons
- Inhibitory PV⁺ neurons
- Astrocytes
- Combinatorial cultures

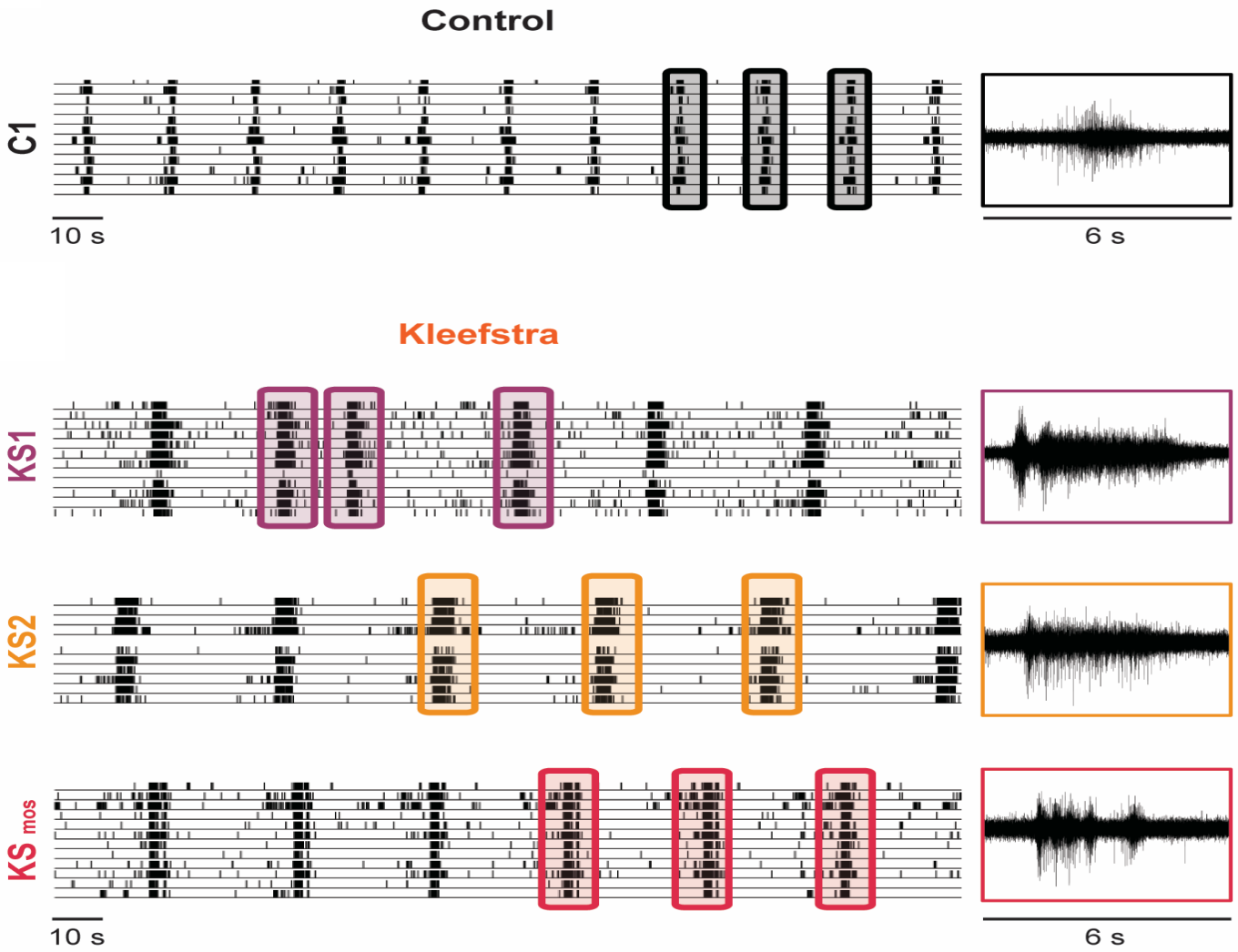
Measuring neural network activity of iPSC-derived networks

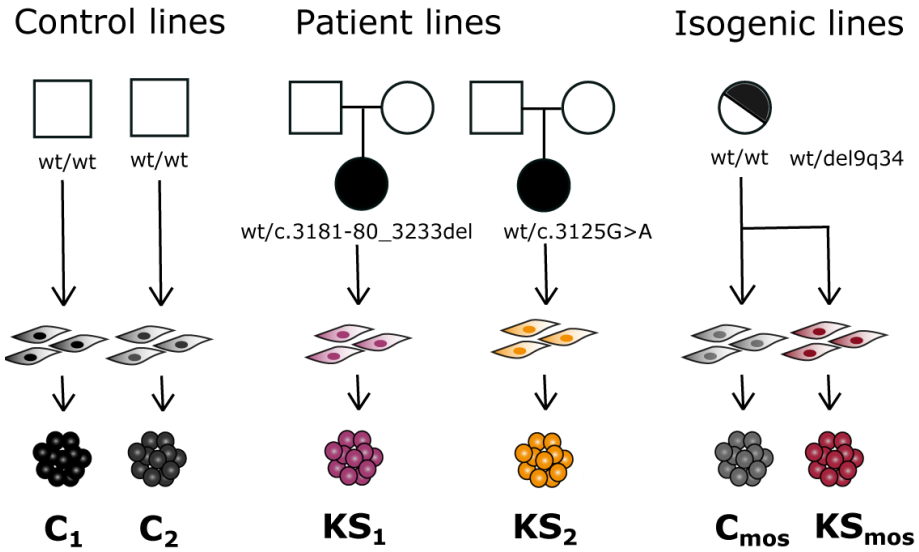
Micro-electrode arrays





Altered network activity in Kleefstra syndrome patient derived networks





Bursts of long duration

Reduced network burst frequency

Reduced network organization

Frega et. al., Nat Comm 2019

Network dysfunction in Kleefstra syndrome is mediated by enhanced NMDAR signaling

Neuronal Network

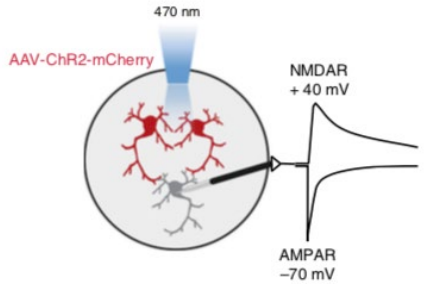
Bursts of long duration

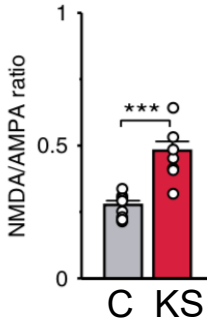
Reduced network burst frequency

Reduced network organization

Single cell- Synaptic

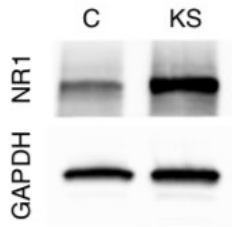
Increased NMDAR function

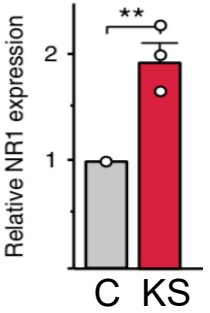




Molecular

Increased *GRIN1* expression and decreased H3K9me2 at *GRIN1* loci





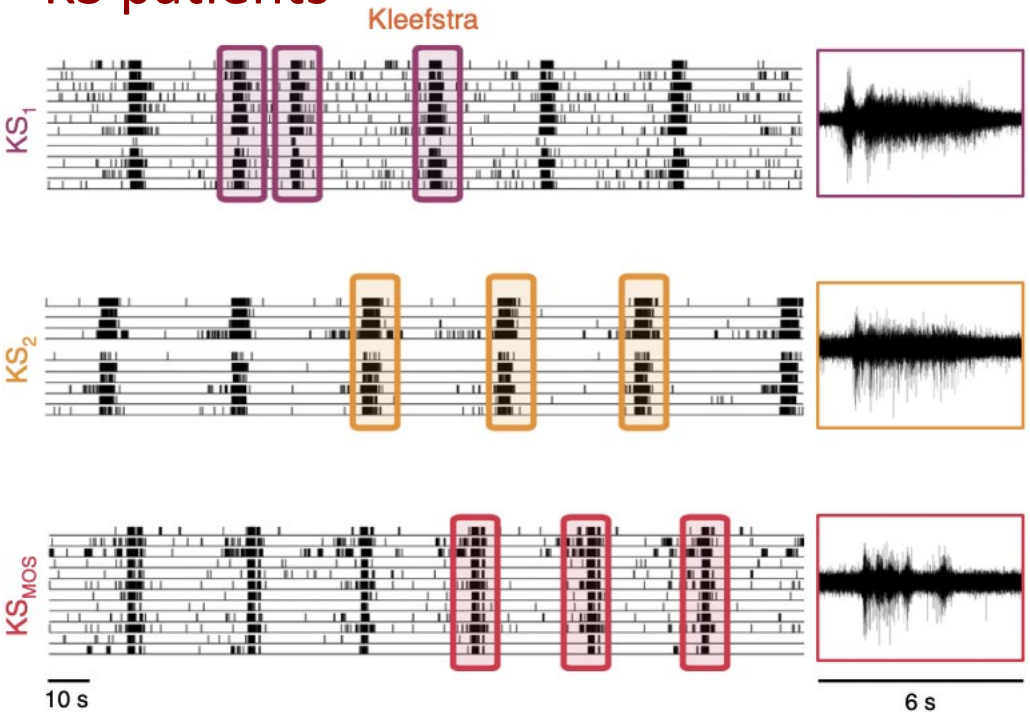
Rescue with NMDAR antagonists (MK-801)

Frega et. al., Nat Comm 2019

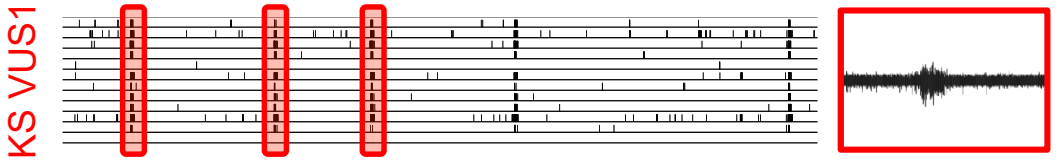
Can we use Kleefstra MEA fingerprint to test variants of unknown significance?

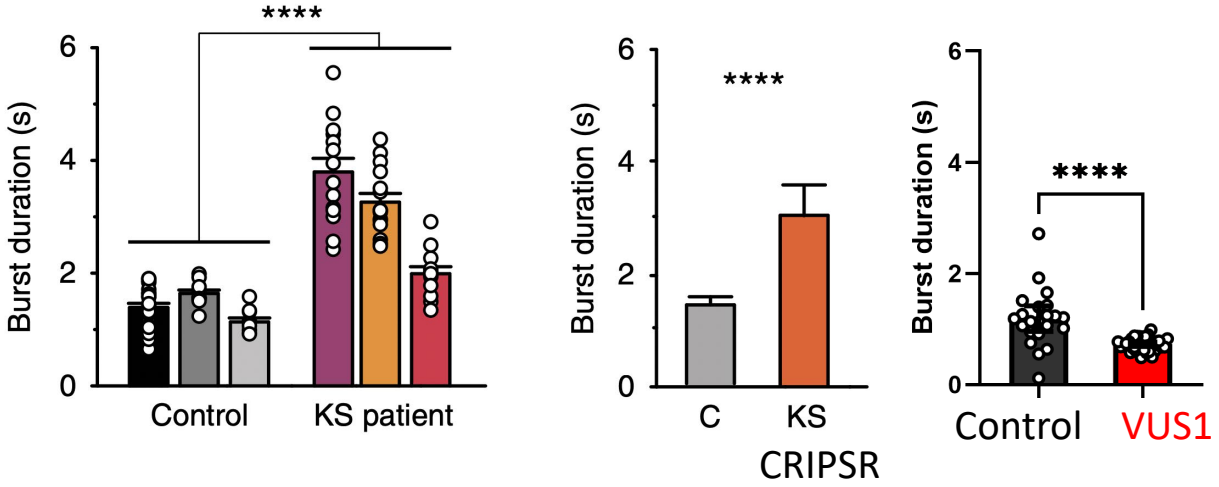
Control

KS patients



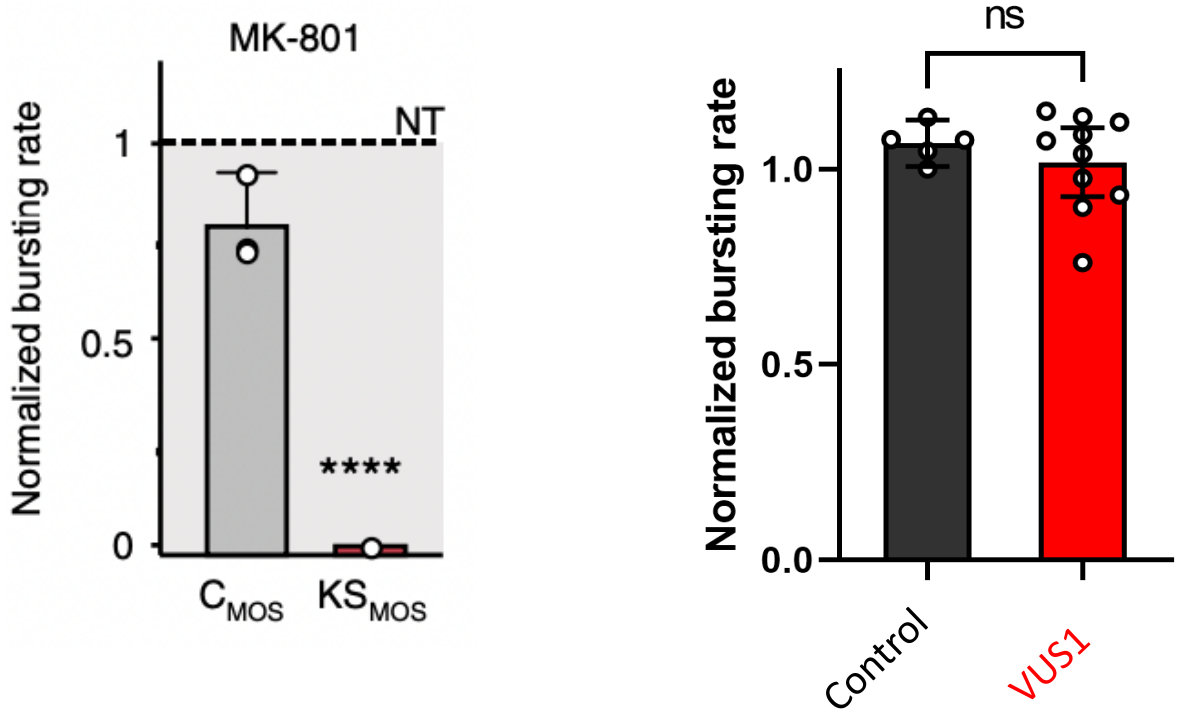
VUS 1: synonymous-splicing p.(Ala597=) which in fact is p.550_597del





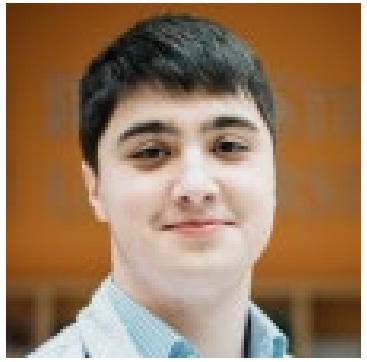
Neurons behave differently compared to previous KS neurons!

Altered response to NMDAr antagonist (MK801) in VUS1 neurons



Applications of iPSC-derived neurons

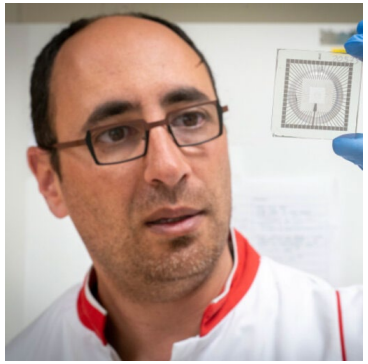
Dmitrijs Rots
Friday, 10.20 a.m.



Diagnostic:
Interpretation of VUS

Studying mechanisms of disease

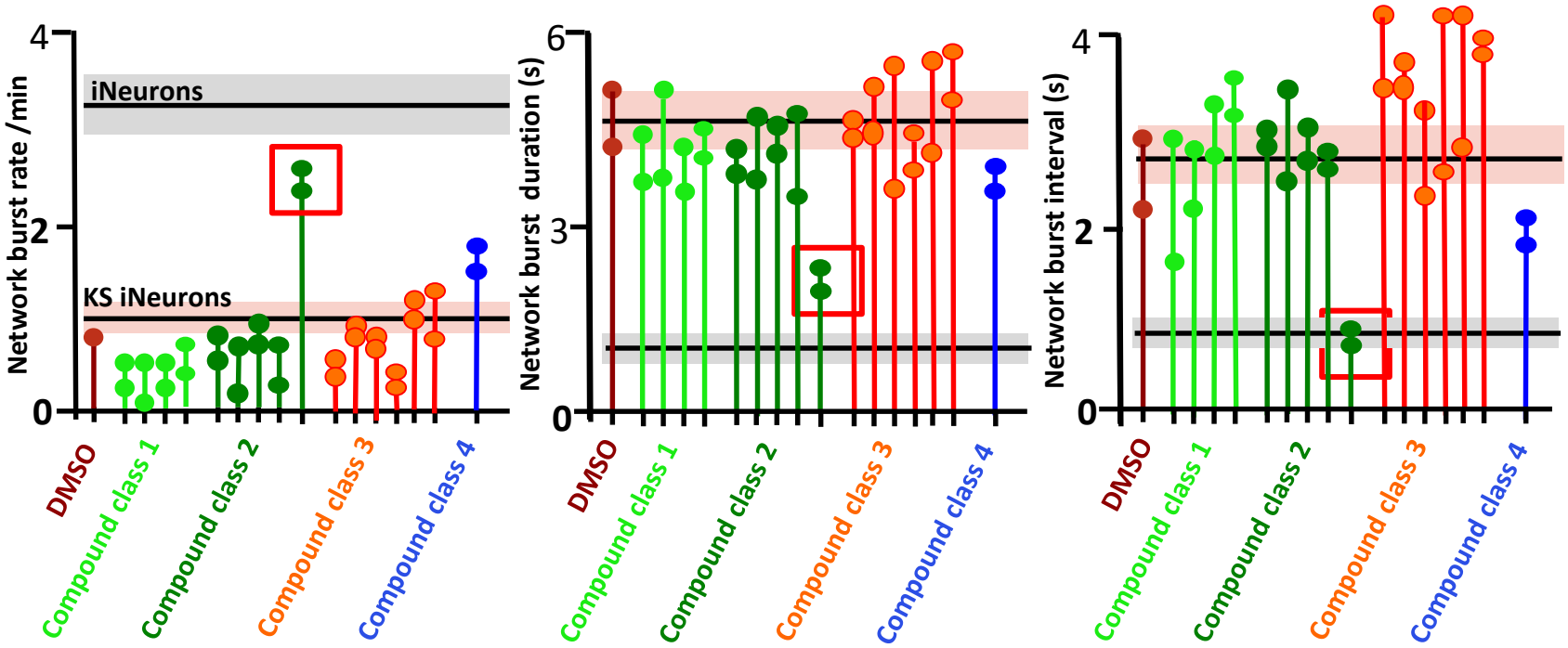
Finding therapeutic targets
& preclinical testing

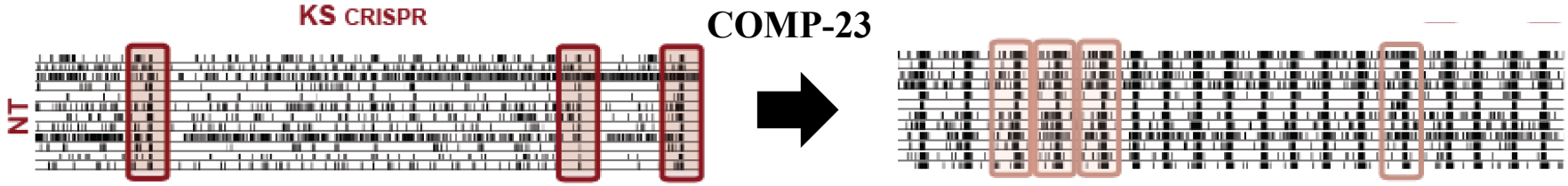


Nael Nadif Kasri
Friday, 10.00 a.m.

Personalized medicine: Patient specific drug testing

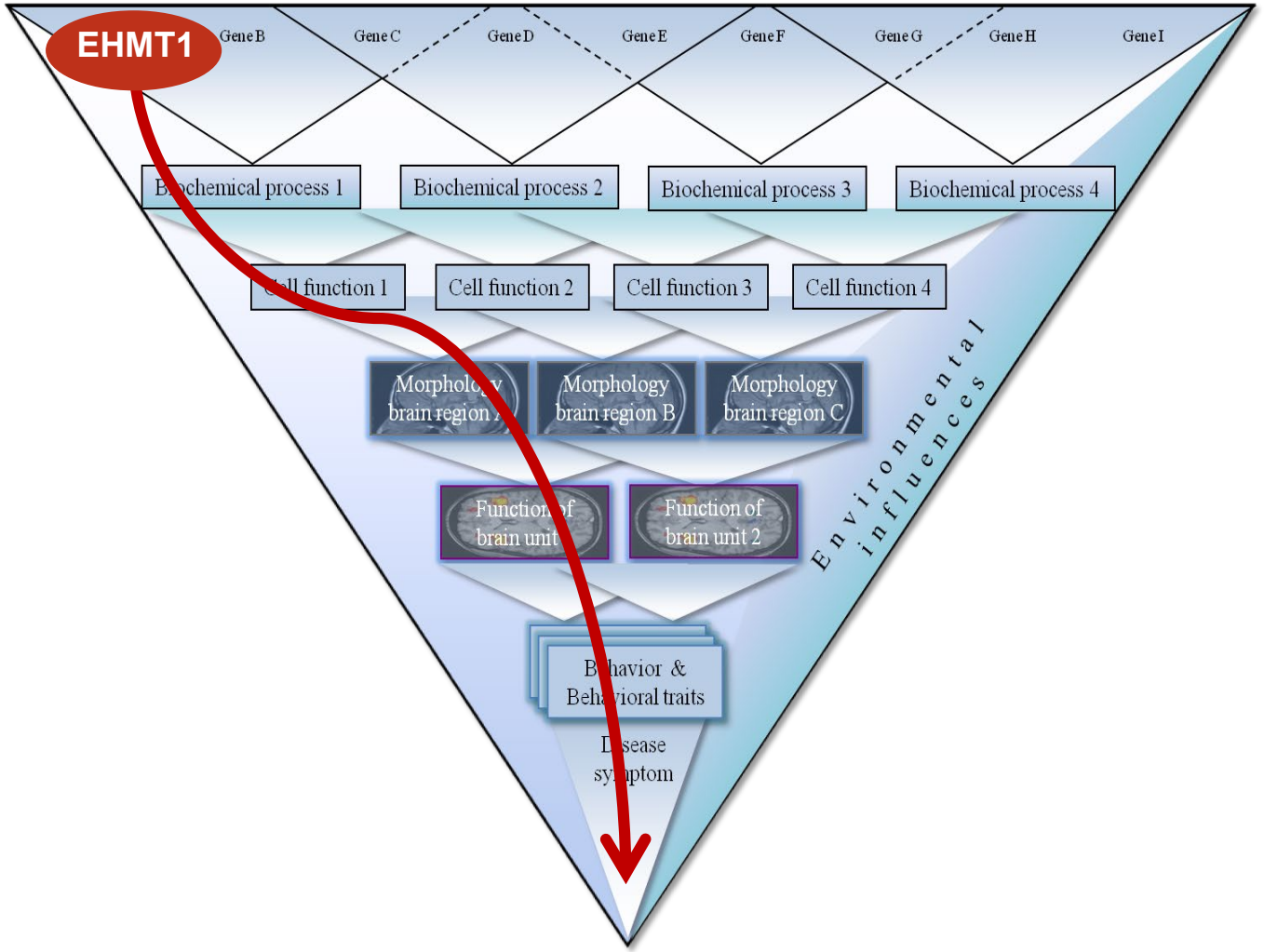
- Testing >200 compounds: different classes of compounds that ”modify” the epigenome
- 1 dose/compound tested on 1 patient line and CRISPR line





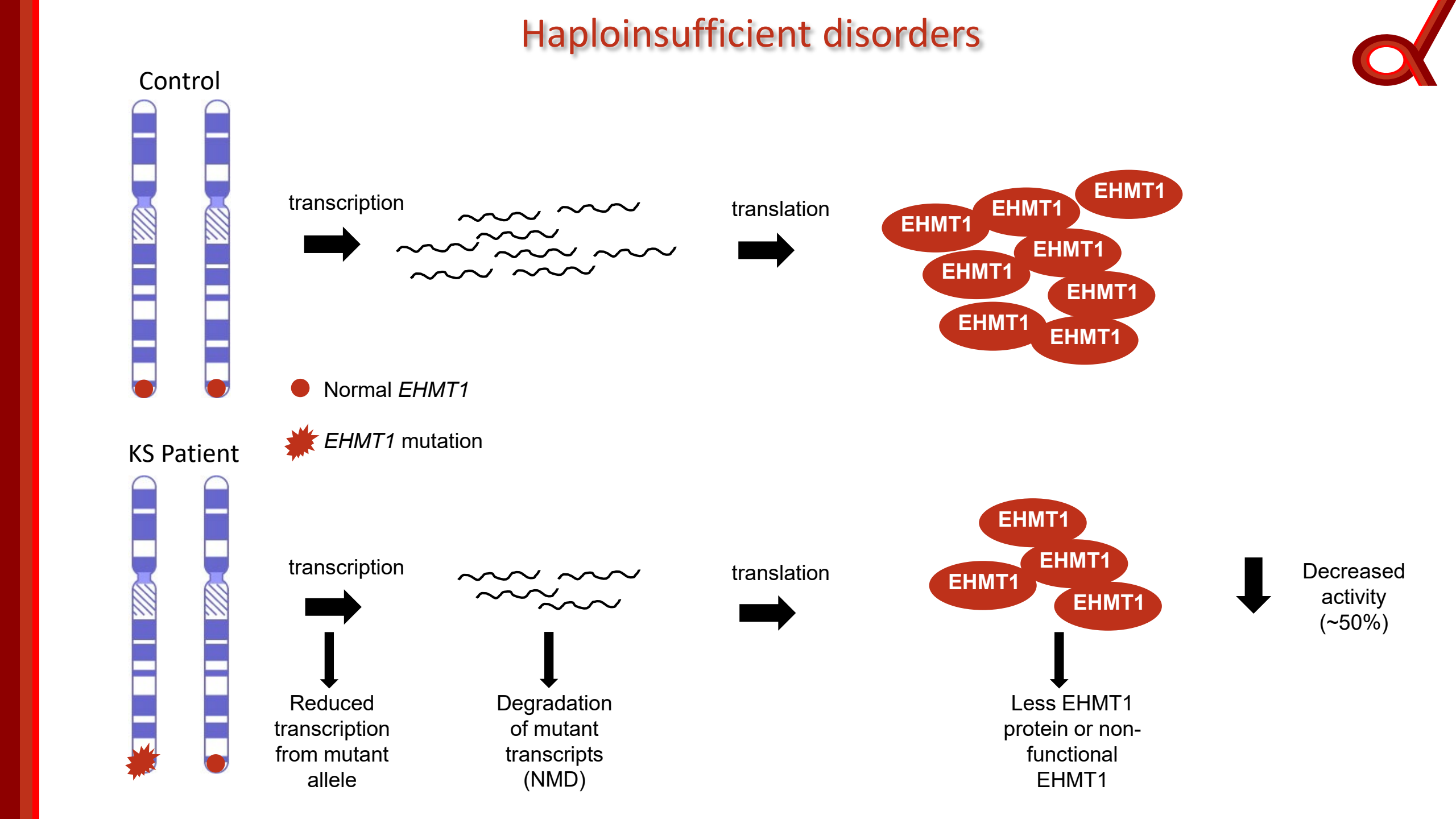
NDDs: Convergence from genes to phenotypes

Upregulation of haploinsufficientNDD genes may provide a **direct personalized** treatment for postnatal symptoms



Convergence of NDD genes onto common biological processes may facilitate the developnment of generic **symptomatic** treatments

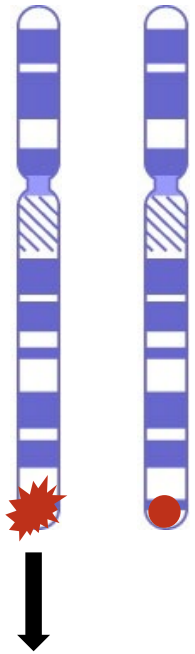
Haploinsufficient disorders



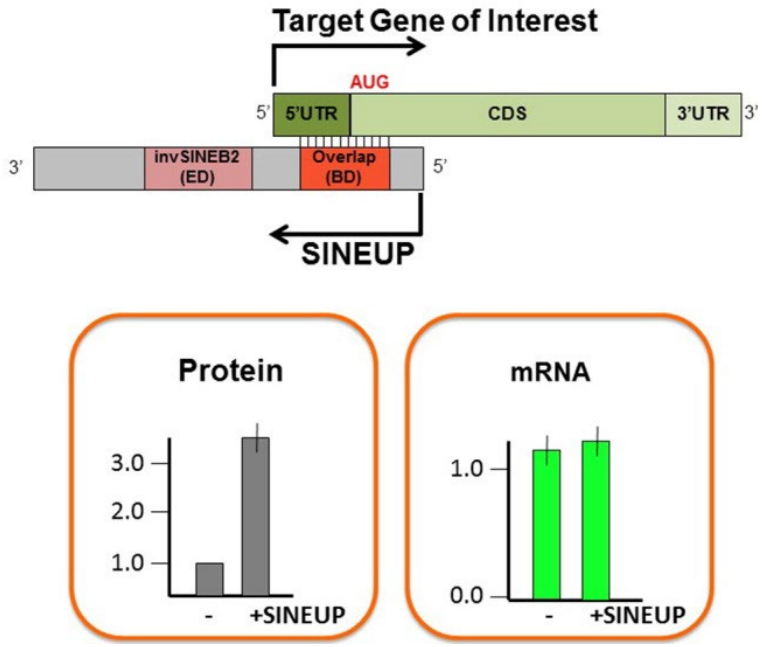
Upregulate translation of the wildtype *EHMT1* transcript

- SINEUPs: recruitment of translation factors to enhance translation

KS Patient



Reduced transcription from mutant allele

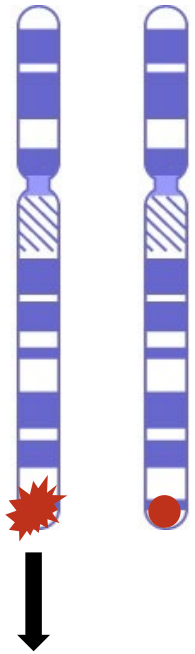


Stefano Gustincich,
Transine therapeutics

+ Easy delivery (viral, RNA)
+ Accurate dosage (physiological level)
- Selection of effective binding site

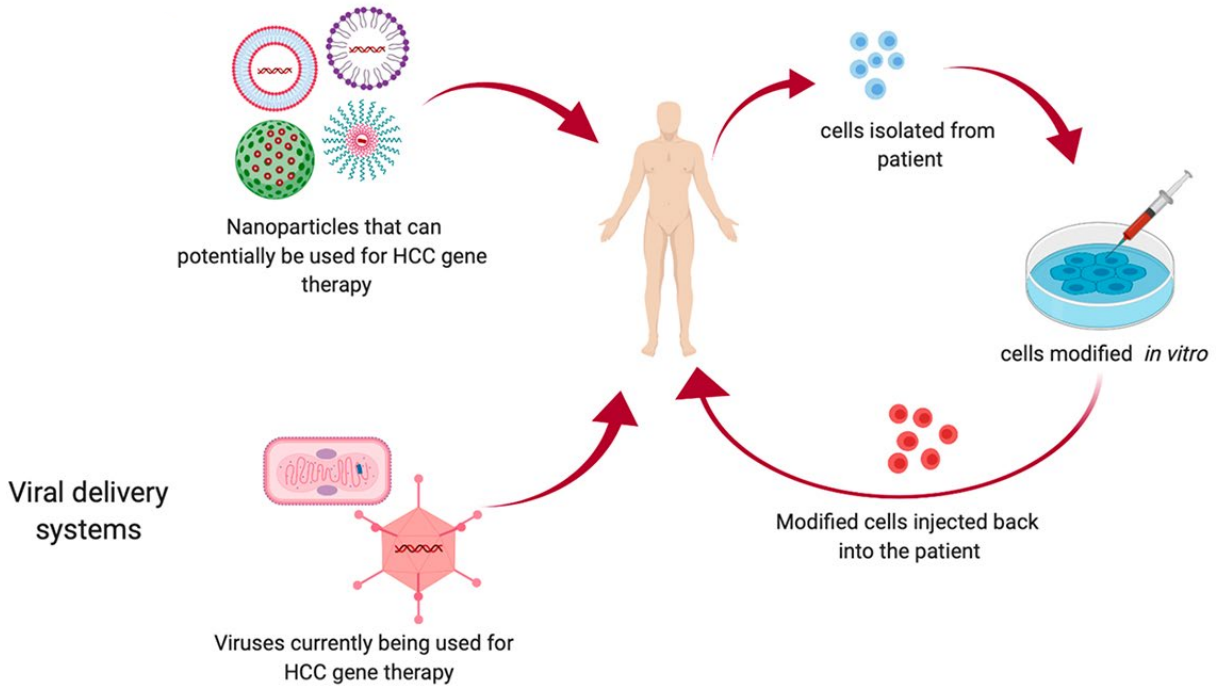
Upregulate transcription of the wildtype EHMT1 copy

KS Patient



Reduced transcription from mutant allele

- Introduction of an exogenous EHMT1 copy



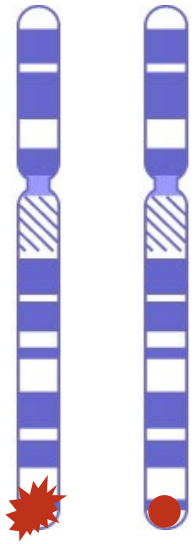
+ Choices for delivery (viral, RNA-nanoparticles, ex vivo)

- Accurate dosage (calibration) is difficult

- Possible off-target effects

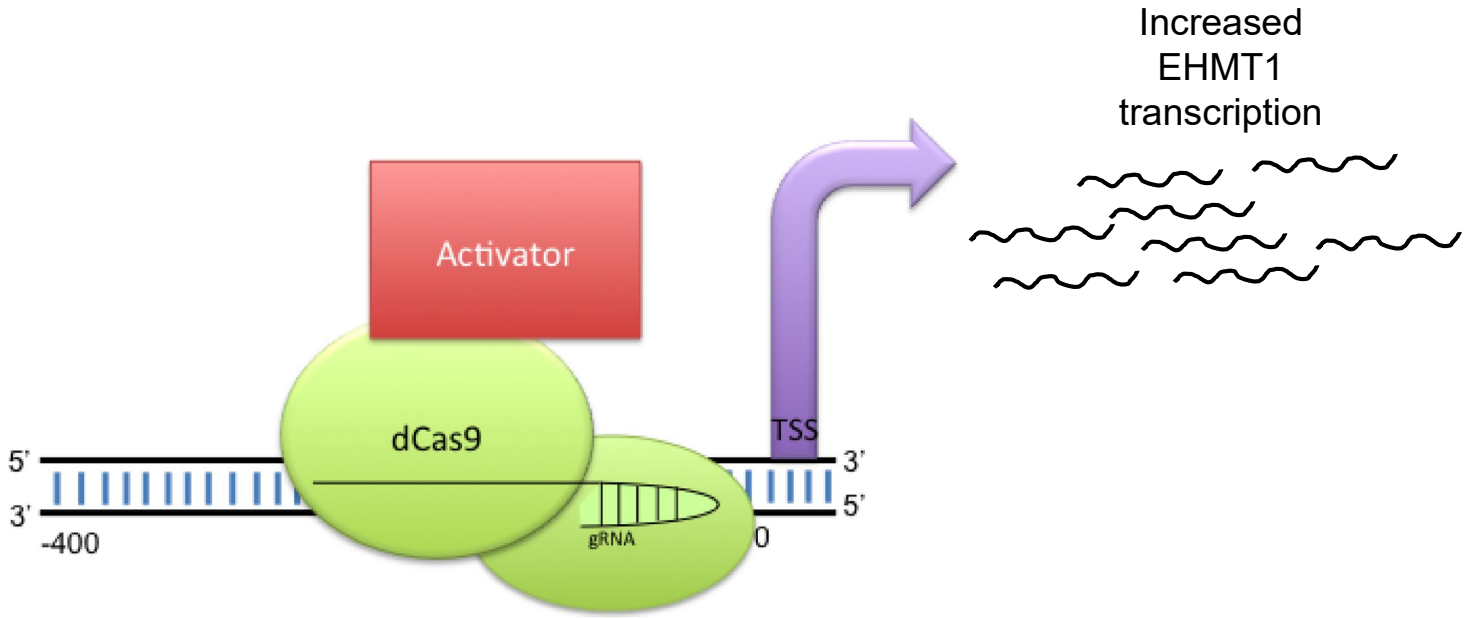
Upregulate transcription of the wildtype EHMT1 copy

KS Patient



Reduced transcription from mutant allele

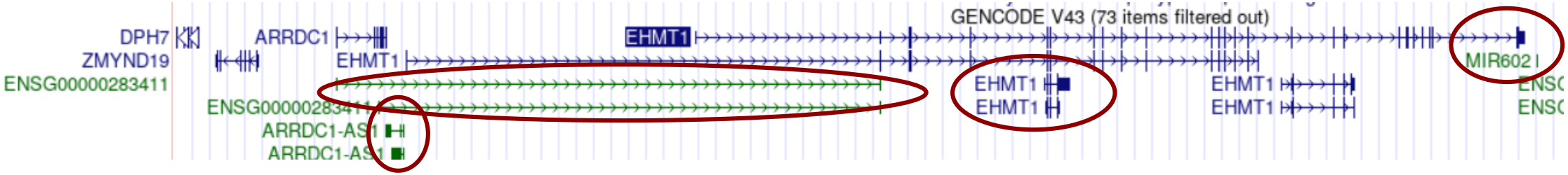
- dCRISPR-mediated gene enhancement



+ **Delivery vehicles (viral, RNA-nanoparticles)**
- **Accurate dosage (calibration) is difficult**
- **Possible off-target effects**

upregulation of the remaining wt allele

- Dampen the expression of negative regulators



- Modulate antisense RNAs and lncRNAs
- microRNAs
- upstream open reading frames: overlapping
- upstream open reading frames: non-overlapping
- Reduce non-productive splicing: Targeted Augmentation of Nuclear Gene Output (TANGO)

\+ **Easy delivery (viral, RNA)**
\+ **Accurate dosage (physiological level)**
\- **Dependent on the presence of negative regulators**

Acknowledgement

PI's: Hans van Bokhoven, Nael Nadif Kasri, Dirk Schubert, Tjitske Kleefstra

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Brooke Latour
Elly Lewerissa
Xiuming Yuan
Eline van Hugte
Ka Man Wu
Katrin Linda
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Ilse van der Werf
Moritz Negwer
Britt Mossink
Emma Dyke
Rachel Mijdam
Imke Schuurman
Yi Lu
Naoki Kogo

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European Institute of Oncology & Experimental Oncology
Giuseppe Testa

SickKids Hospital Toronto
James Ellis

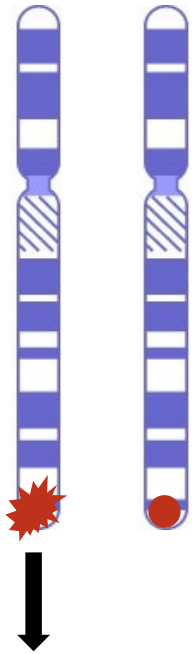
Institute of Science and Technology Austria
Gaia Novarino

CNRS and University of Montpellier
Giacomo Cavalli

University of Antwerp
Frank Kooy

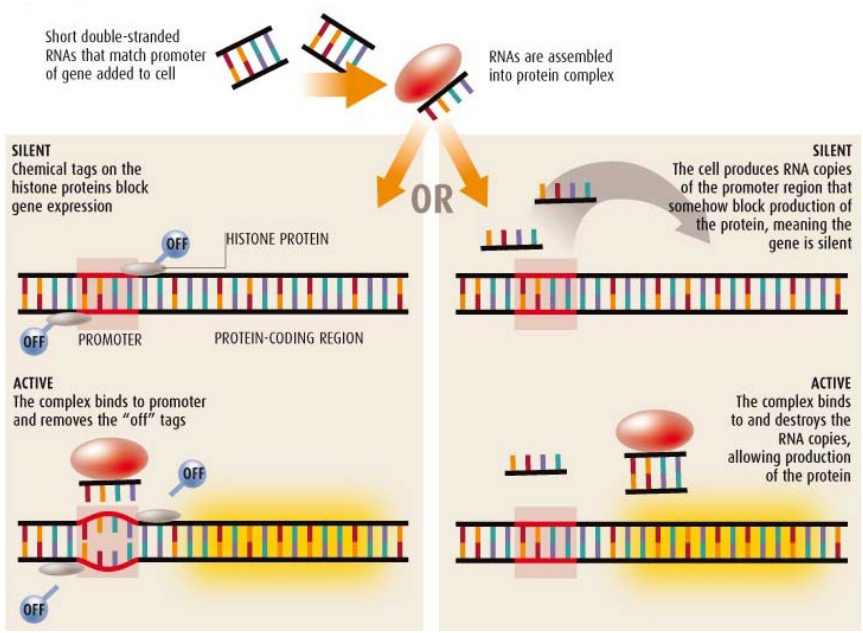
Upregulate transcription of the wildtype EHMT1 copy

KS Patient



Reduced transcription from mutant allele

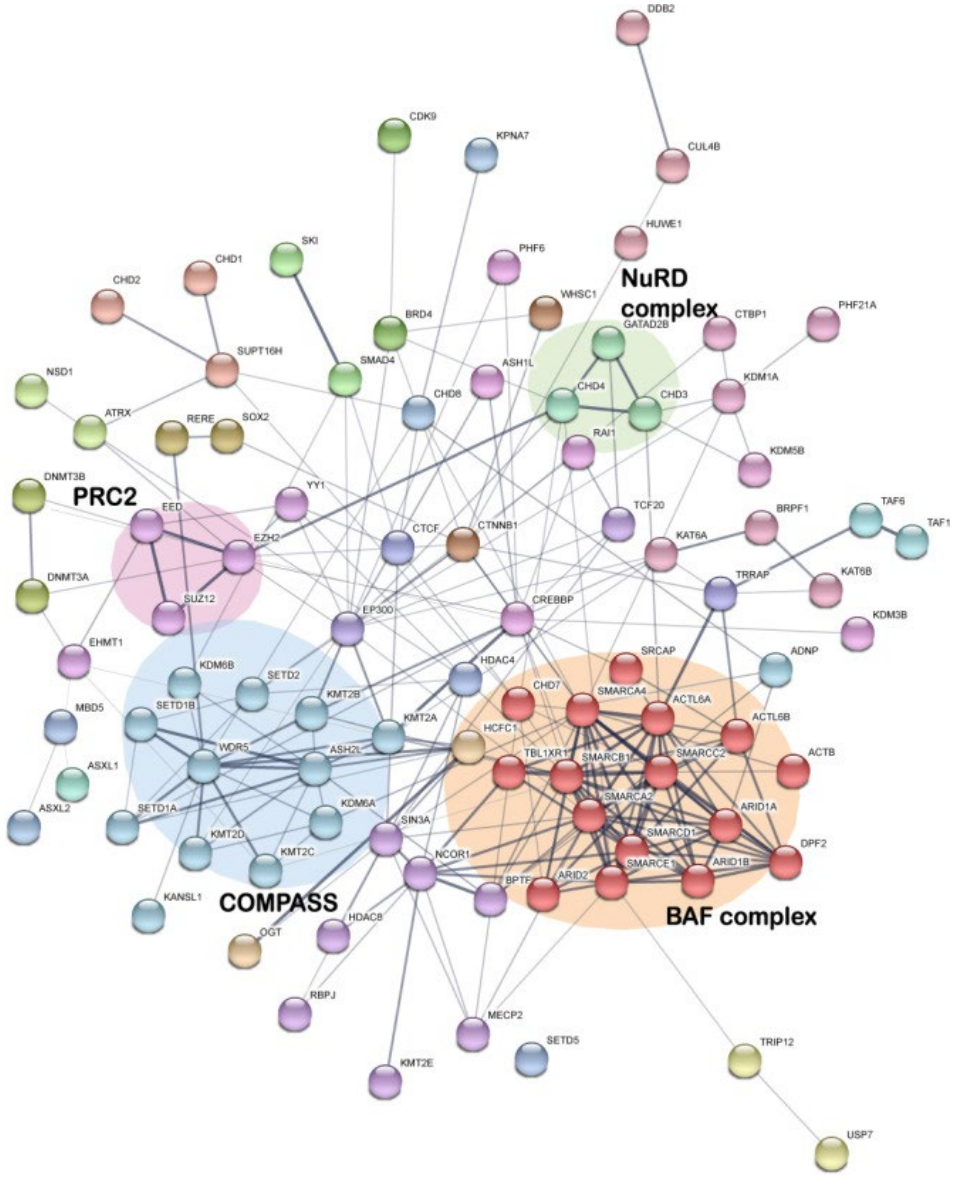
- Activating RNAs (aRNAs) acting at silencing promoter elements



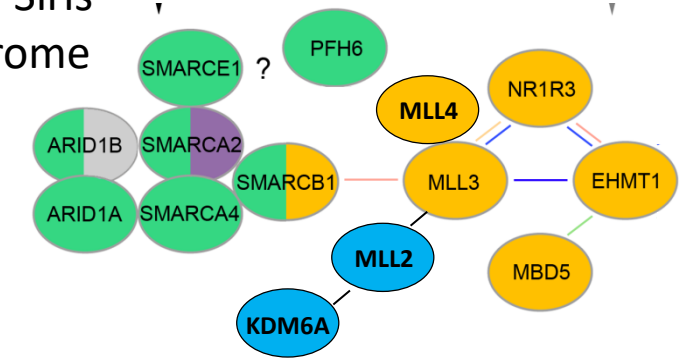
small activating RNA (gene-quantification.de)

\+ Easy delivery of RNA molecules
\- Requires a prescreen of RNA libraries
\- Accurate dosage (calibration) is difficult

Epigenetic networks in ID: “Chromatinopathies” >200 genes



Coffin-Siris syndrome

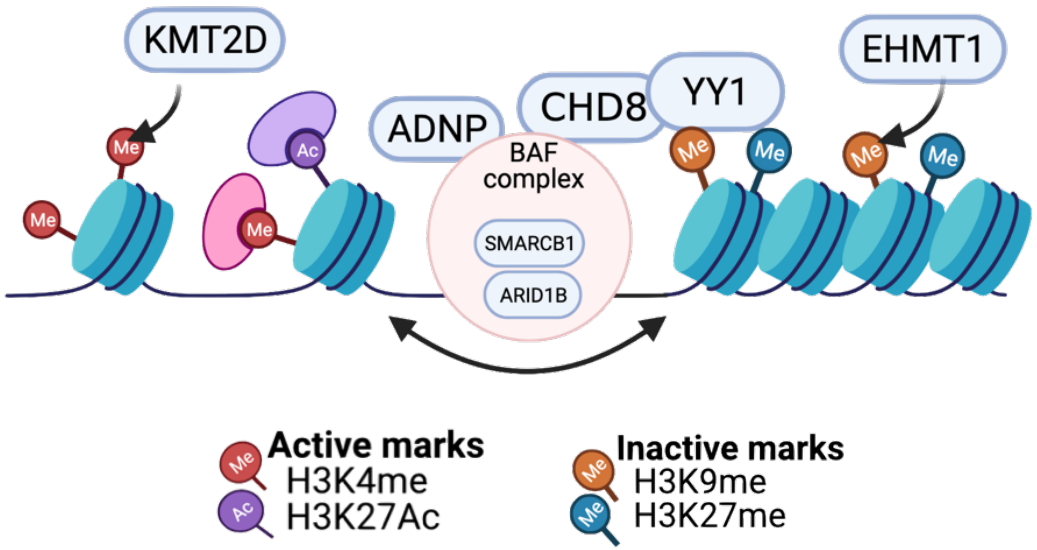


Kleefstra syndrome

Kabuki syndrome

Common downstream Pathways?

IMPACT: Identification of Converging Molecular Pathways Across Chromatinopathies as Targets for Therapy



Protein	Disorders
KMT2D	Kabuki syndrome
YY1	Gabriele-de-Vries syndrome
ADNP	Helsmoortel-Van der Aa syndrome
CHD8	Syndromic ASD (AUTS18)
EHMT1	Kleefstra syndrome
SMARCB1	Coffin-syris syndrome; Kleefstra syndrome spectrum
ARID1B	Coffin-syris syndrome; Syndromic ID

IMPACT: Identification of Converging Molecular Pathways Across Chromatinopathies as Targets for Therapy

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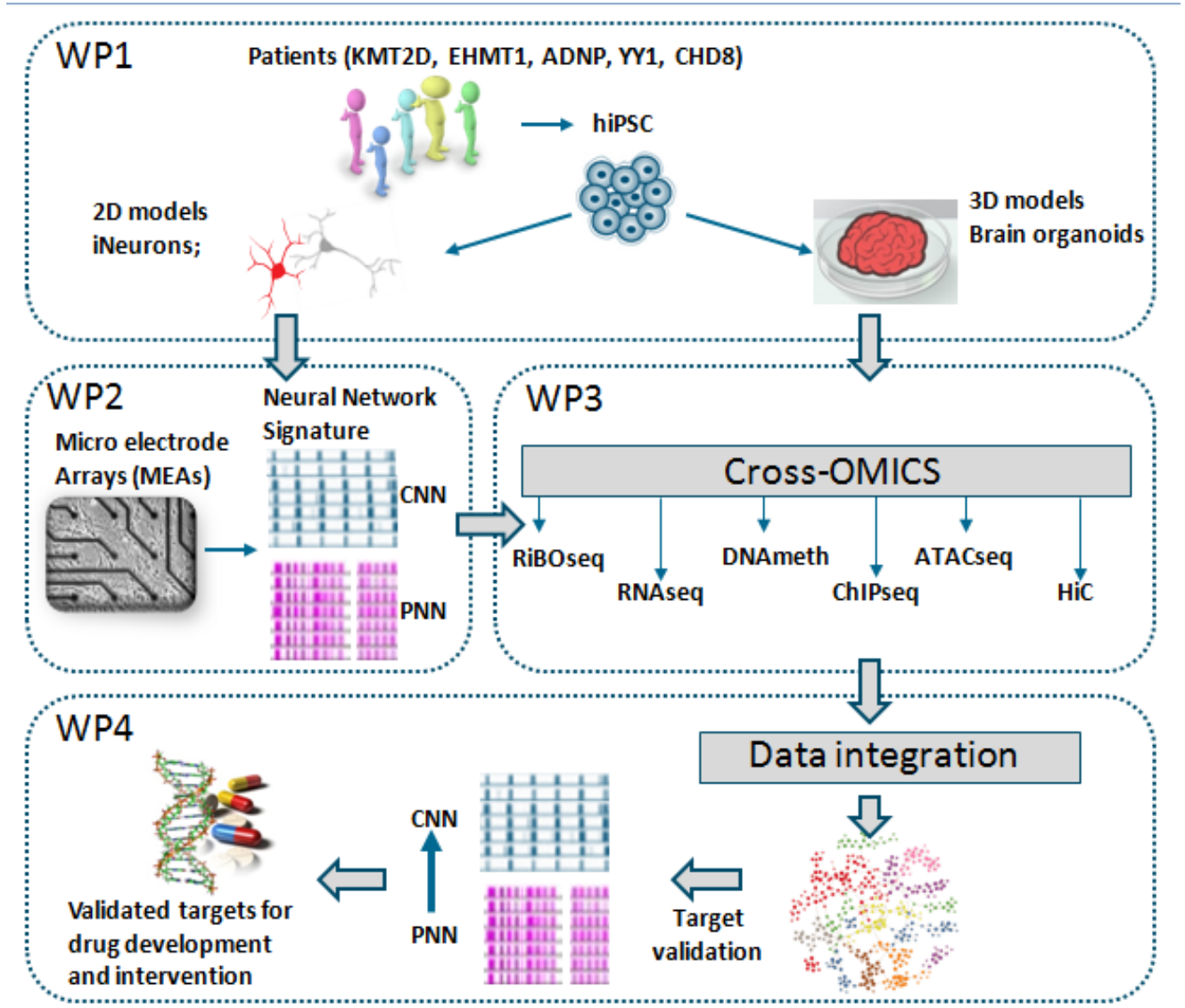
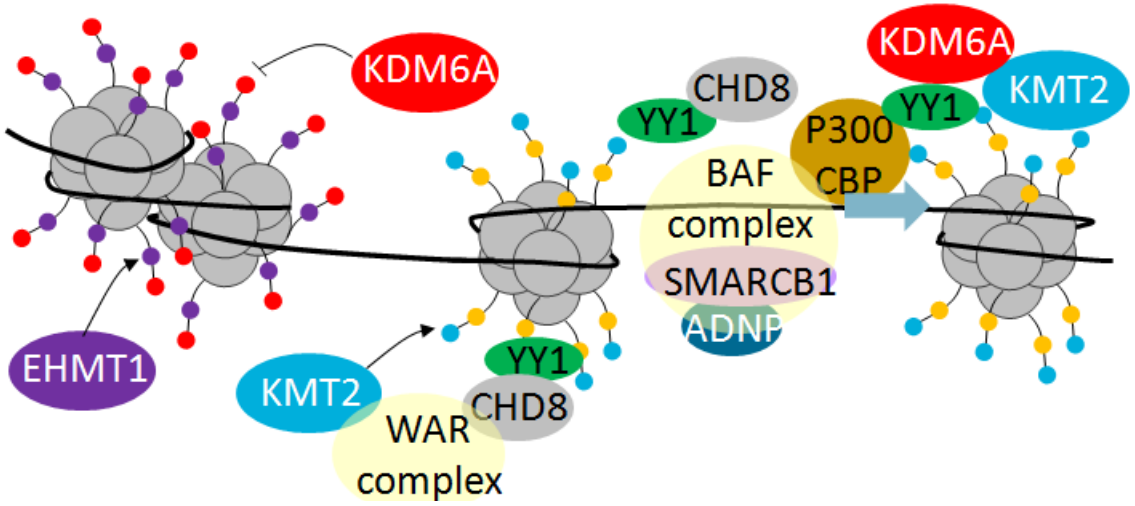


IMPACT: Identification of Molecular Pathways Across Chromatinopathies as Targets for Therapy

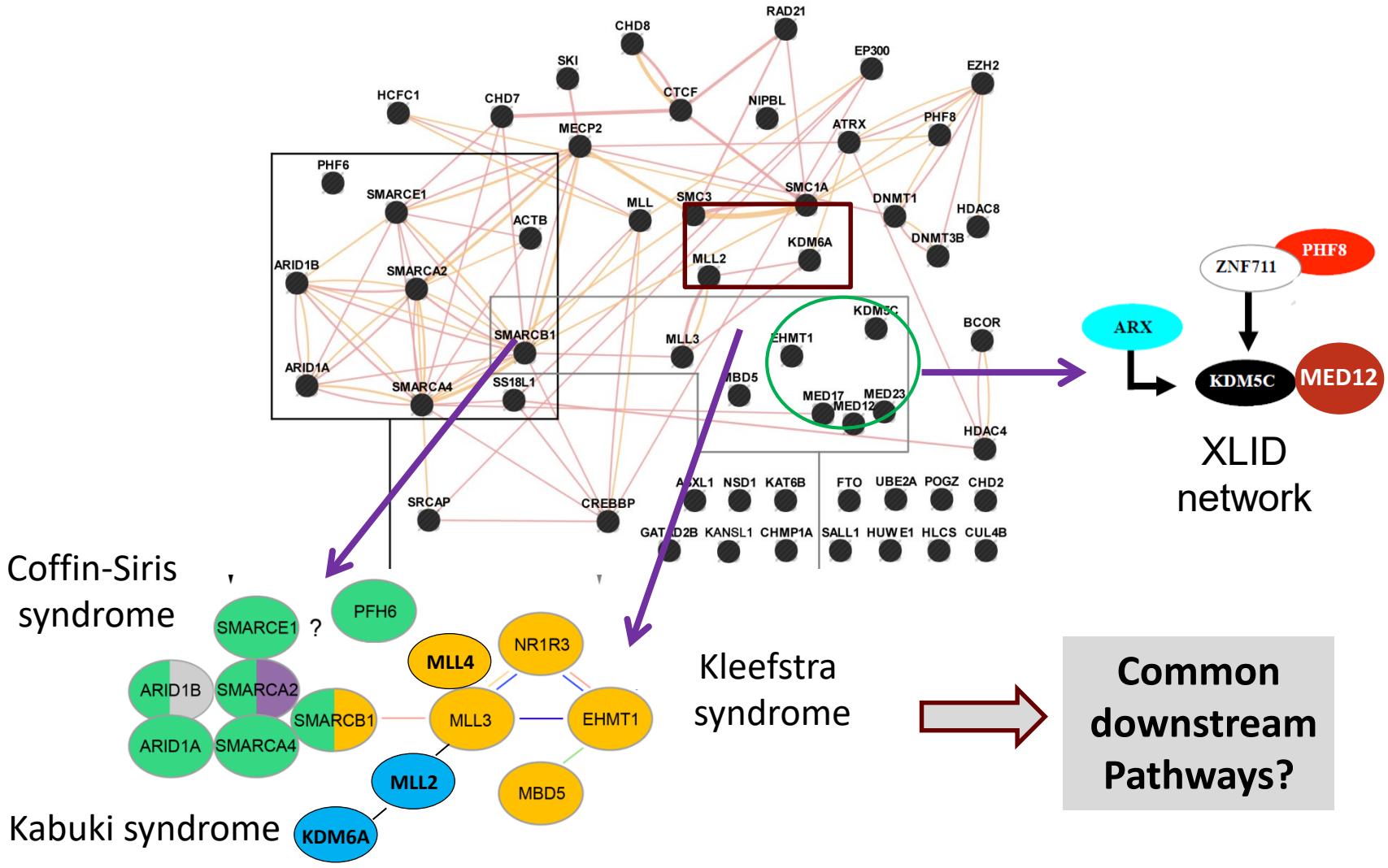


Inactive marks
- H3K9me
- H3K27me

Active marks
- H3K4me
- H3K27Ac

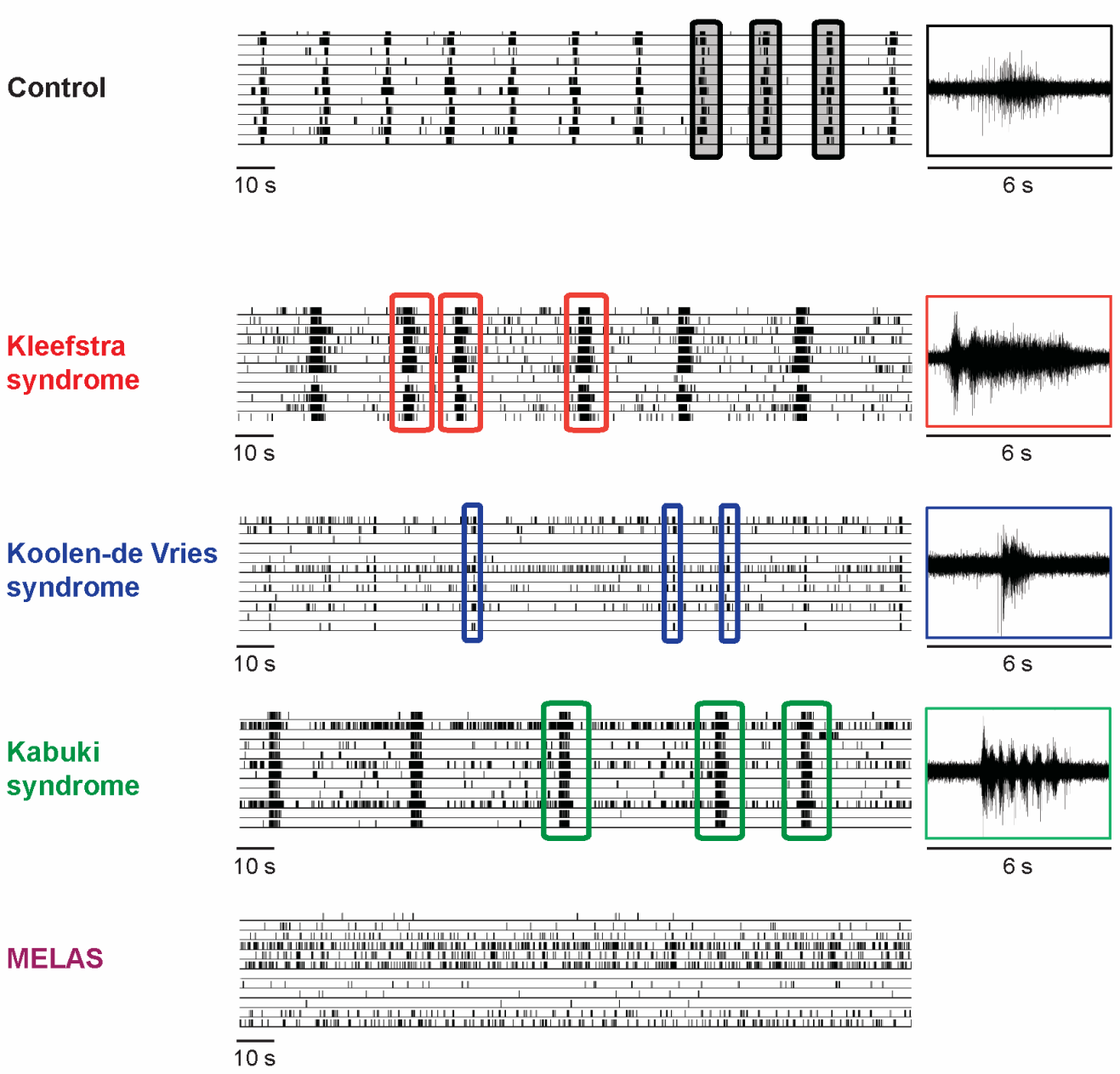
Protein	Functional interaction	activity	Disorder
KMT2D	WAR complex	H3K4me	Kabuki syndrome
EHMT1	KMT2C, SMARCB1 (BAF complex)	H3K9me1/2	Kleefstra syndrome
YY1	P300/CBP, KMT2D, UTX, CTCF, BAF complex	Chromtain remodeler	Gabriele-de Vries syndrome
ADNP	BAF complex	Chromatin remodeler	Helsmoortel- van der Aa syndrome
CHD8	WAR complex (+ similar to BAF complex)	Chromatin remodeler	Syndromic autism; AUTS18

Epigenetic networks in ID: "Chromatinopathies" >200 genes

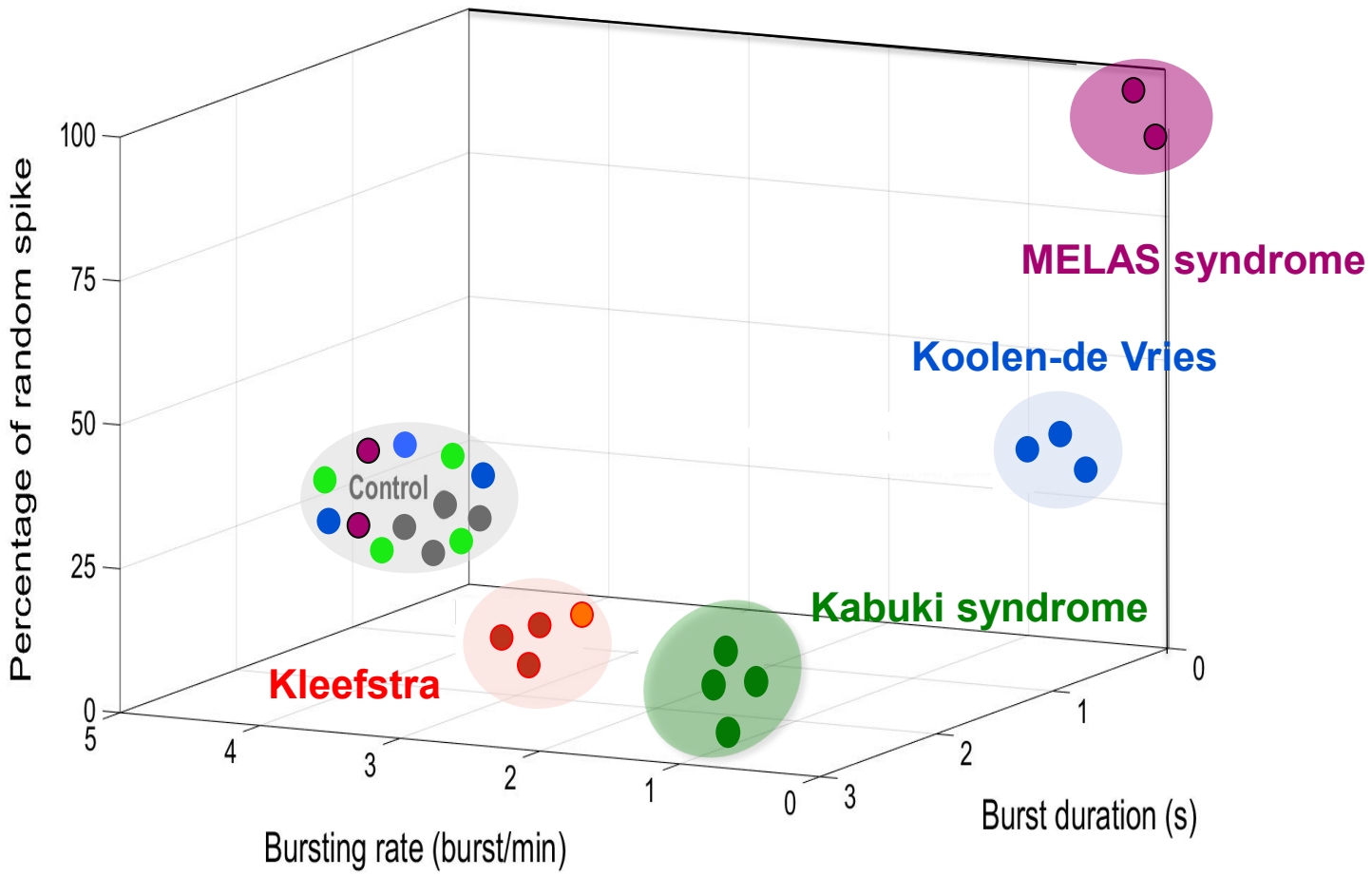


Adapted from Kleefstra et al. 2014, Neuropharmacology

Disease-specific neuronal network phenotypes



Disease-specific neuronal network phenotypes

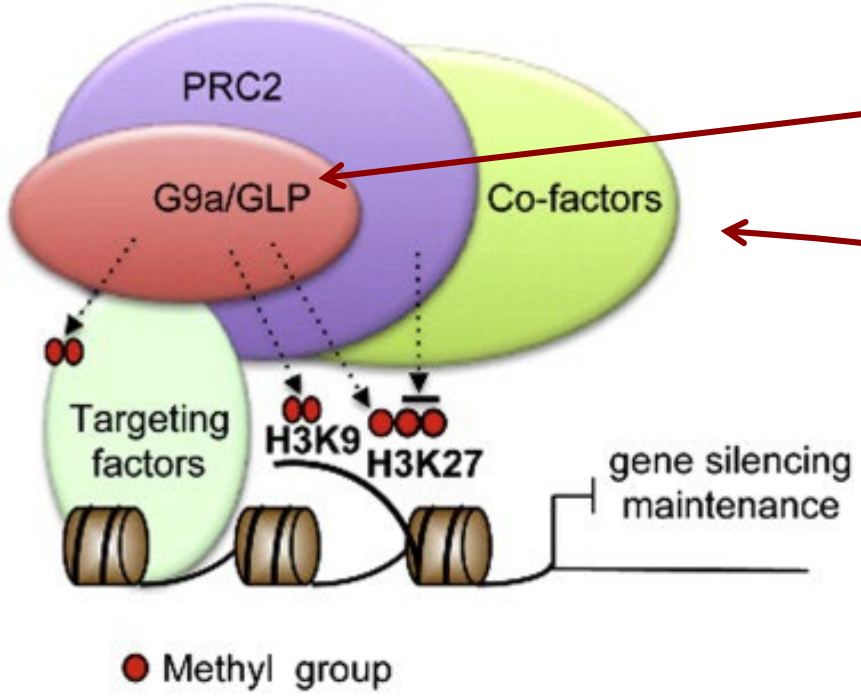


- **Genotype-phenotype for NDD?**
- **Validation of VUS**

KS-like phenotypes without *EHMT1* mutation

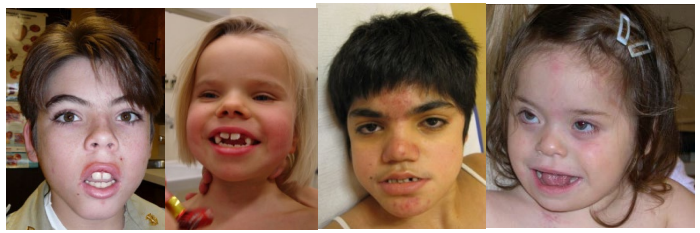
25%

Kleefstra syndrome



?

75%



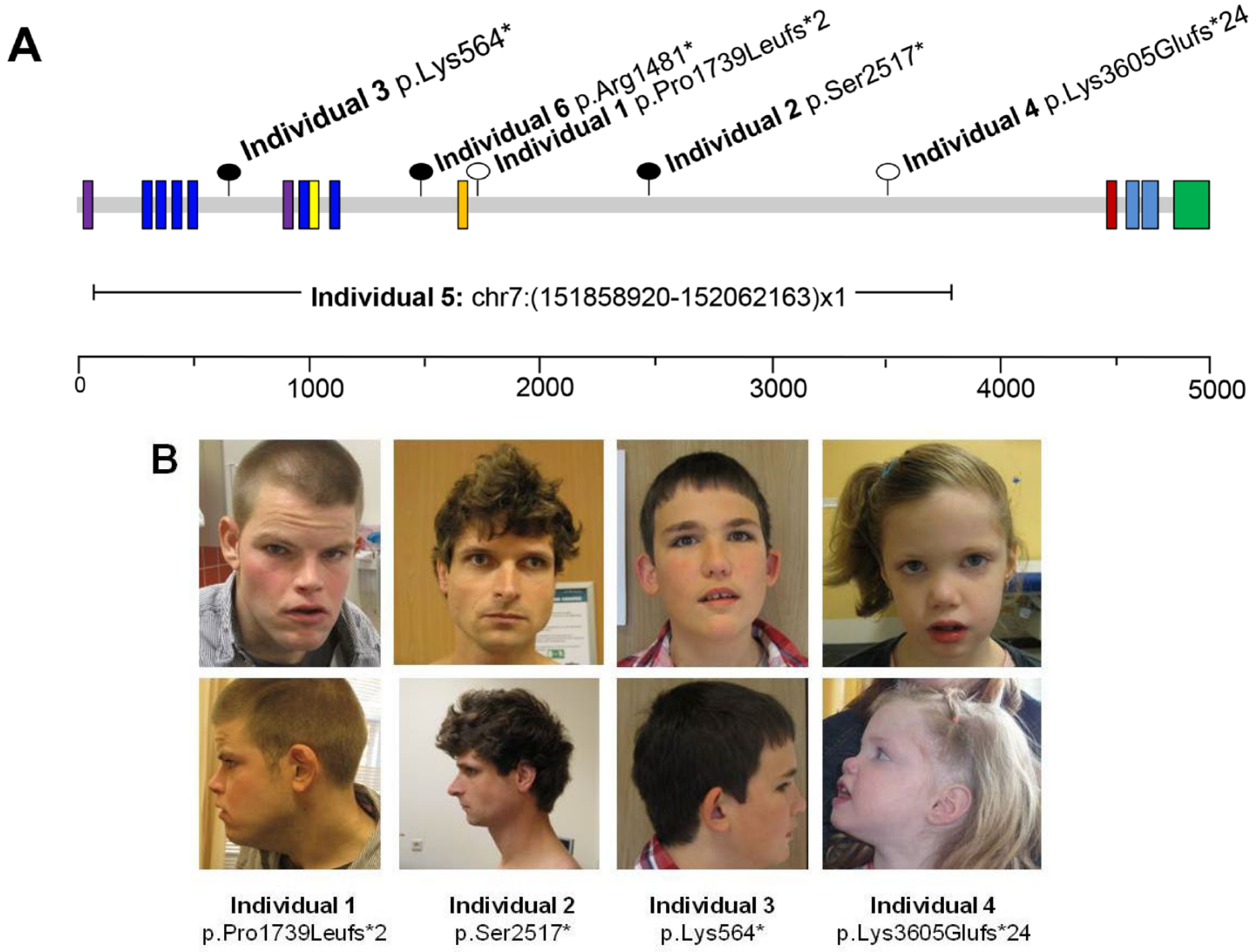
Kleefstra Syndrome Spectrum

Mozzetta et al. Mol Cell 2014

Other KS-like variants affect epigenetic regulators

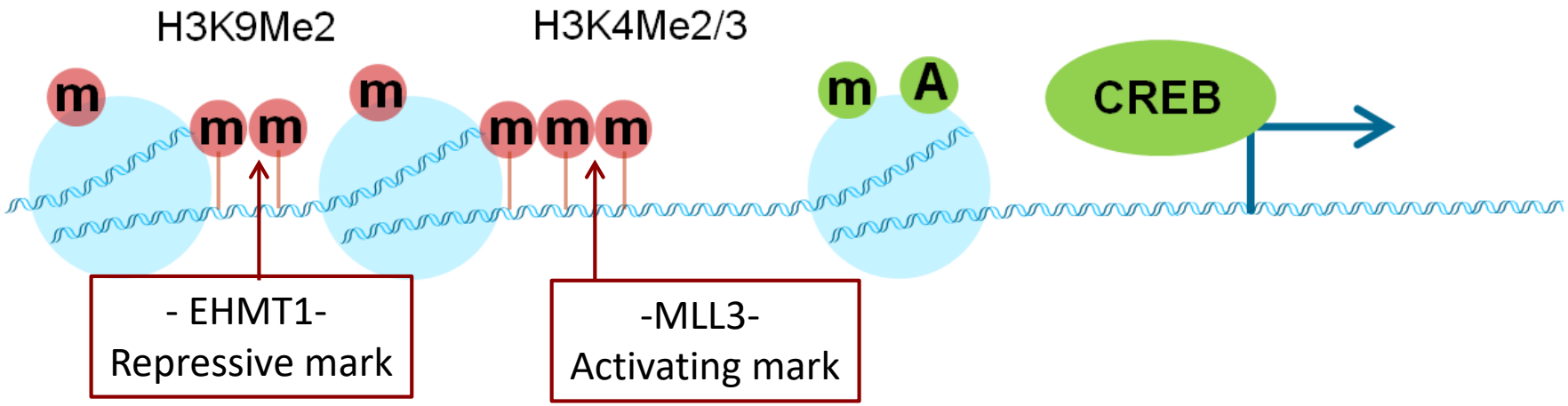
Gene	Variant	Inheritence	Function	Mutations
MBD5	Thr52Hisfs	de novo	Methyl CpG Binding	Smith-Magenis-like Autism
NR1I3	Phe247Ser	de novo	Nuclear receptor	-
SMARCB1	Arg37His	de novo	Swi/Snf helicase	Coffin-Siris syndrome Medulloblastoma
MLL3	Arg1481X	de novo	H3K4me3	Medulloblastoma Autism
MLL4	Pro819Leu	AR	H3K4me3	AD Dystonia Medulloblastoma

De novo *MLL3/KMT2C* mutations cause a KS-like phenotype

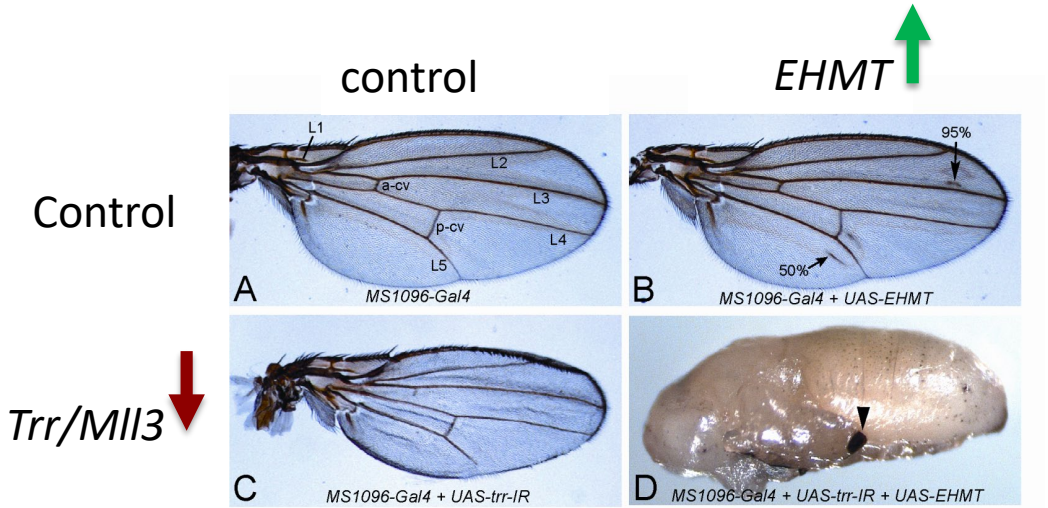


Koemans et al. PLoS Genetics 2017

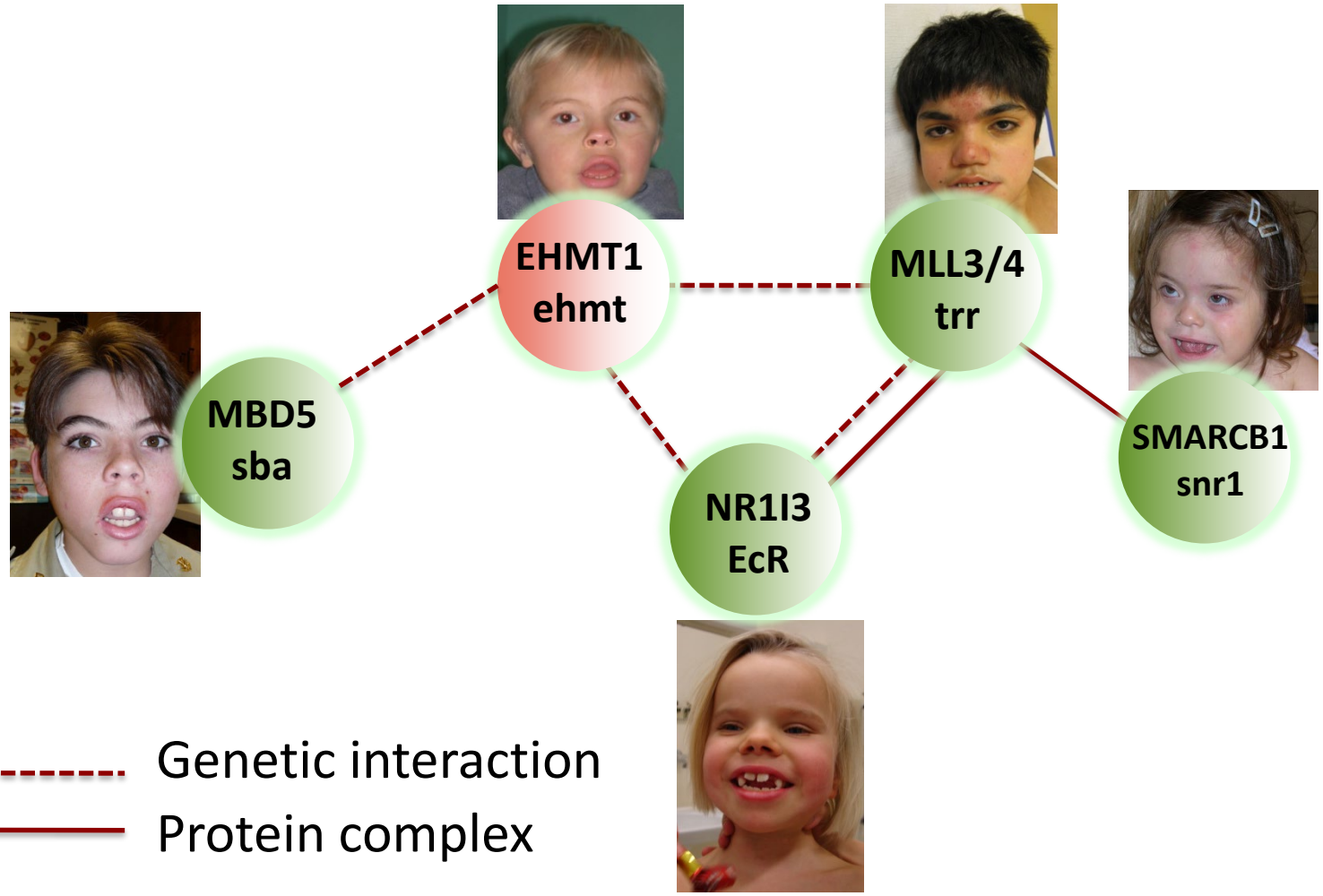
Antagonistic activity of EHMT1 and MLL3



Antagonistic effects reflected in mutant fly phenotype



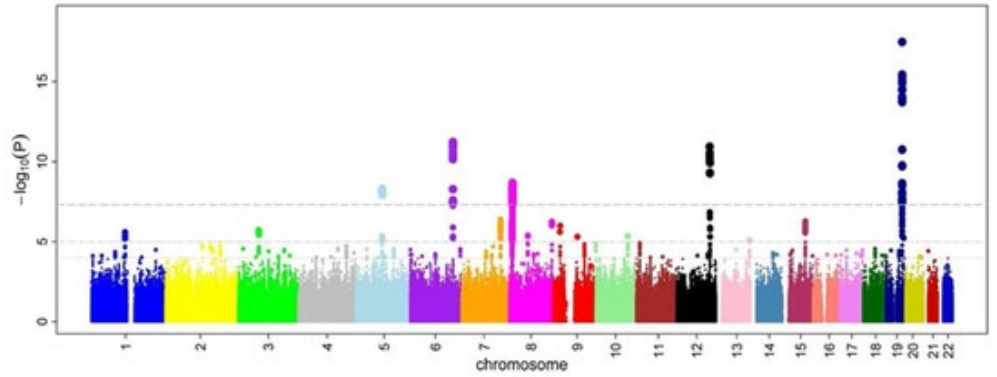
Establishment of an epigenetic module underlying ID



Kleefstra et al., Am J Hum Genet 2012

Genome-wide analyses

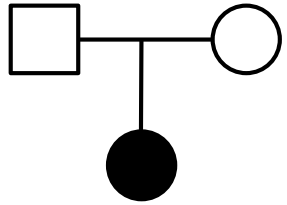
Microarrays

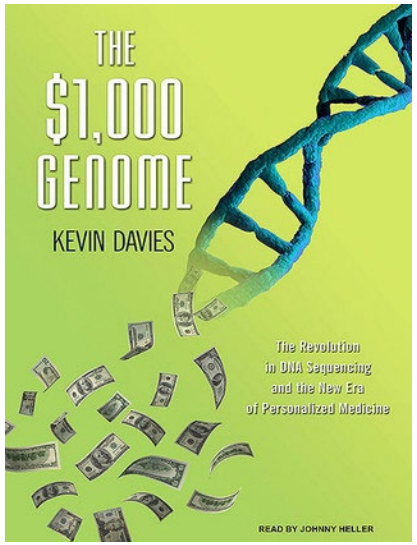


- Copy Number Variation (CNV) analysis
- Genome-wide association studies (GWAS)

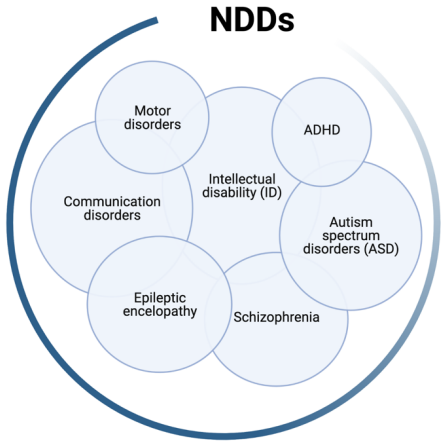
Next generation sequencing (exome/genome)

Trio sequencing
de novo mutations





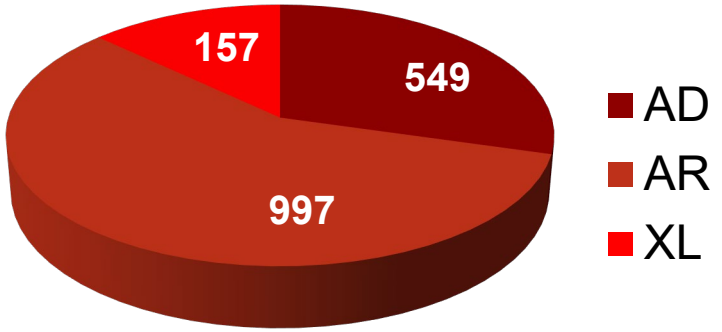
Elucidation of the molecular basis of Neurodevelopmental Disorders (NDDs) comprising Intellectual Disability (ID)



- Deficits in intellectual AND adaptive functioning
- Evident in developmental phase
- Clinically and genetically highly heterogenous
- High co-morbidity of other (cognitive) defects
- >1500 ID genes identified; >1800 distinct ID syndromes
- Some 1000 more to be expected

ID genes January 2023

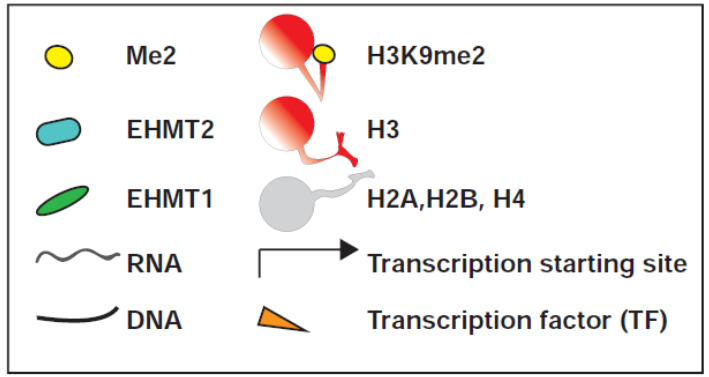
- 1566 total proven
- 157 X-Linked (XL)
- 549 Aut. Dominant (AD)
- 997 Aut. Recessive (AR)
- 9 mitochondrial
- (133 AD +AR)

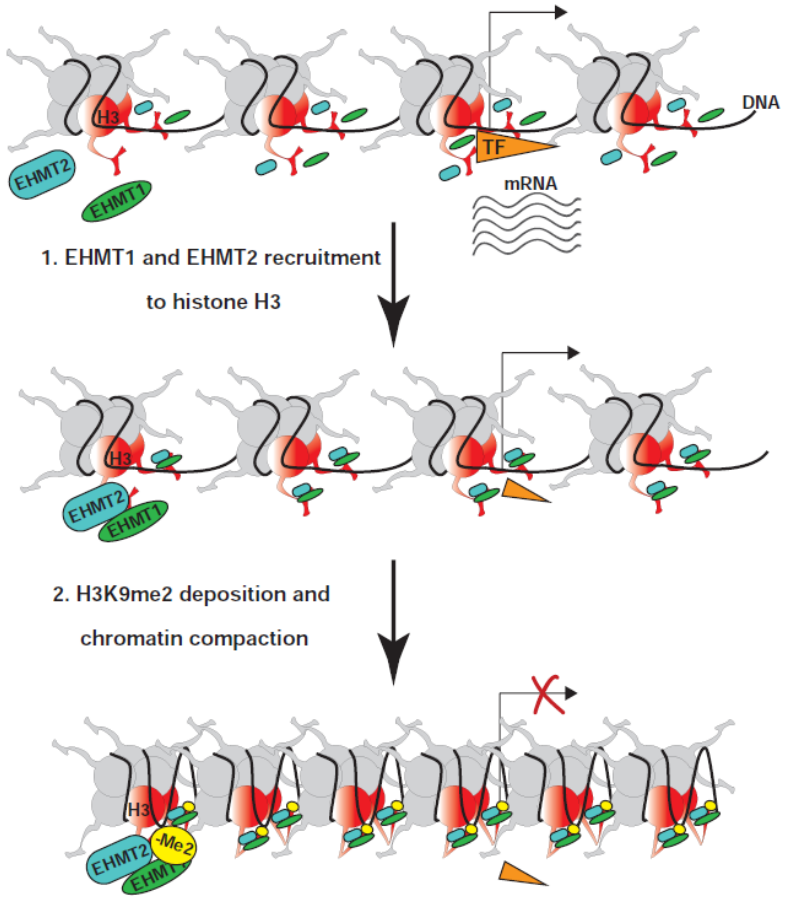


EHMT1: eu-chromatic histone methyltransferase 1

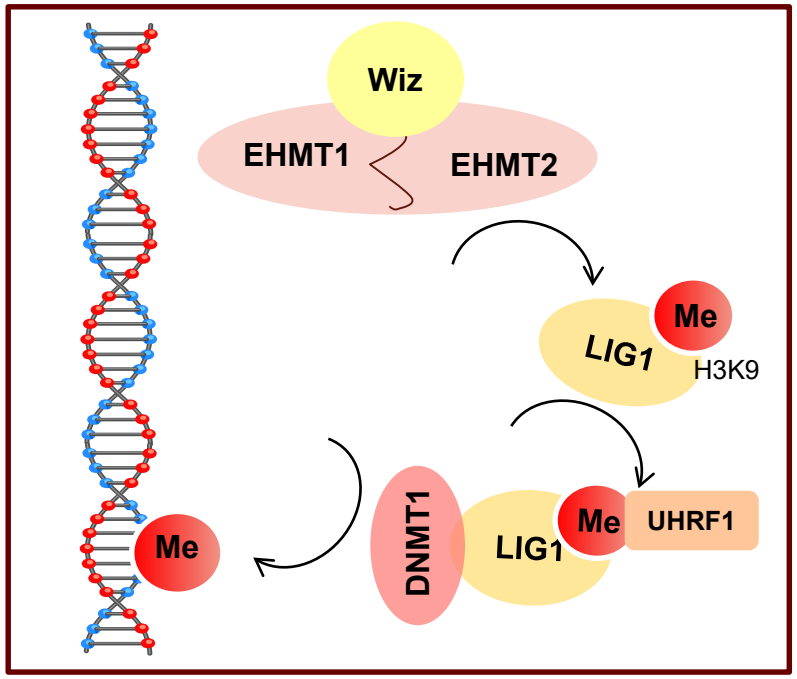
- De novo 9q subtelomeric deletions and L.o.F. mutations in *EHMT1*
- Mostly nonsense and frameshift variants
- Some missense variants in conserved domains
- EHMT1 acts in complexes with EHMT2 (G9a) H3K9me/me2 and DNA methylation → **Gene Silencing**

H3K9me/me2

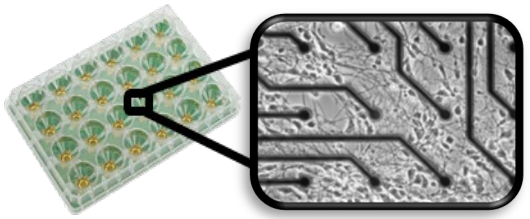




DNA methylation



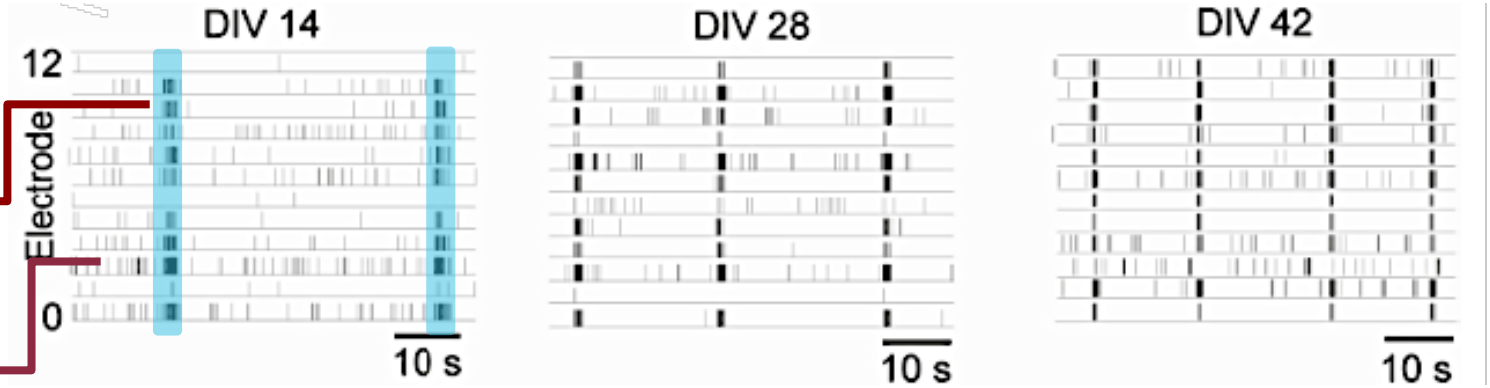
MEAs as a tool to follow neuronal network development



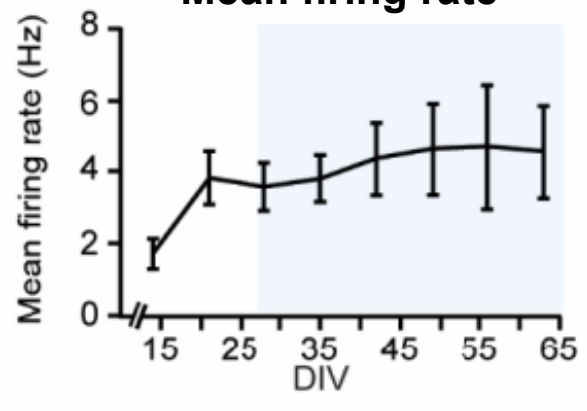
Development (Days in vitro)

Network burst

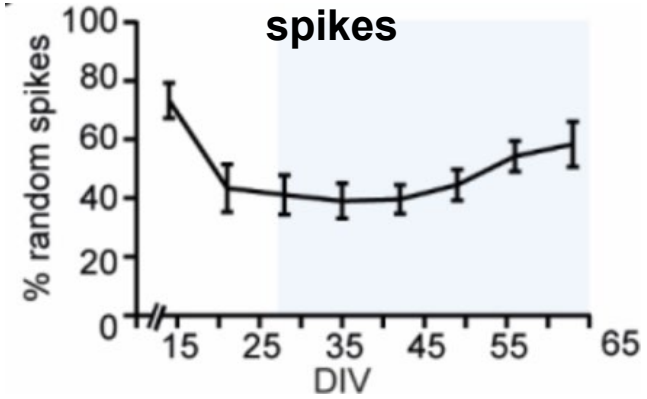
Random spikes



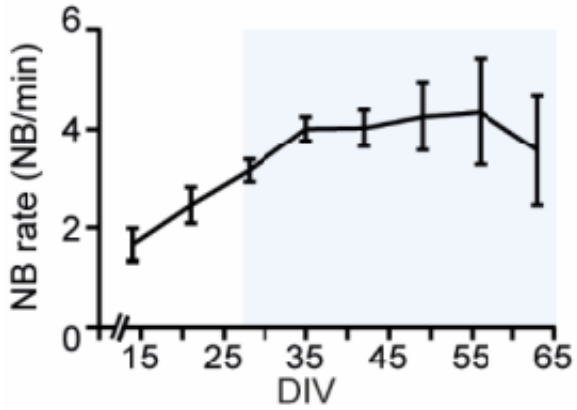
Mean firing rate



Percentage of random spikes



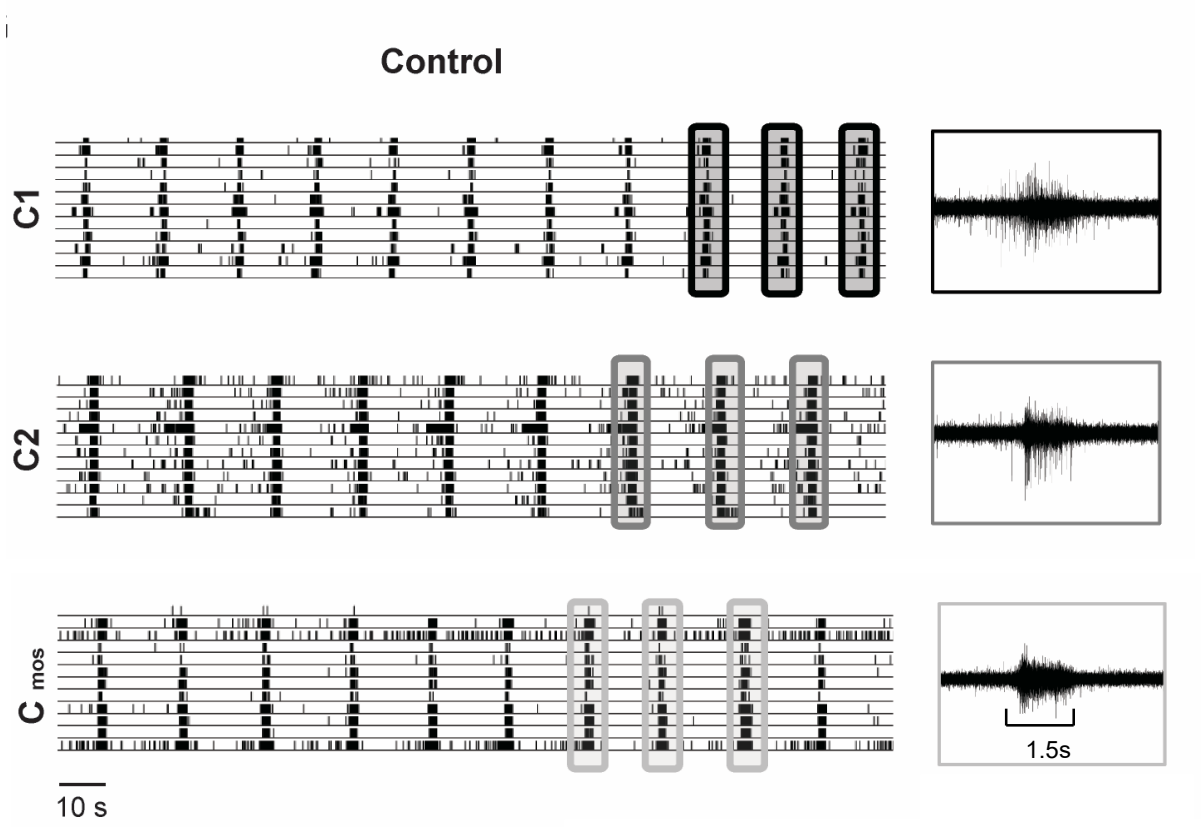
Network burst rate

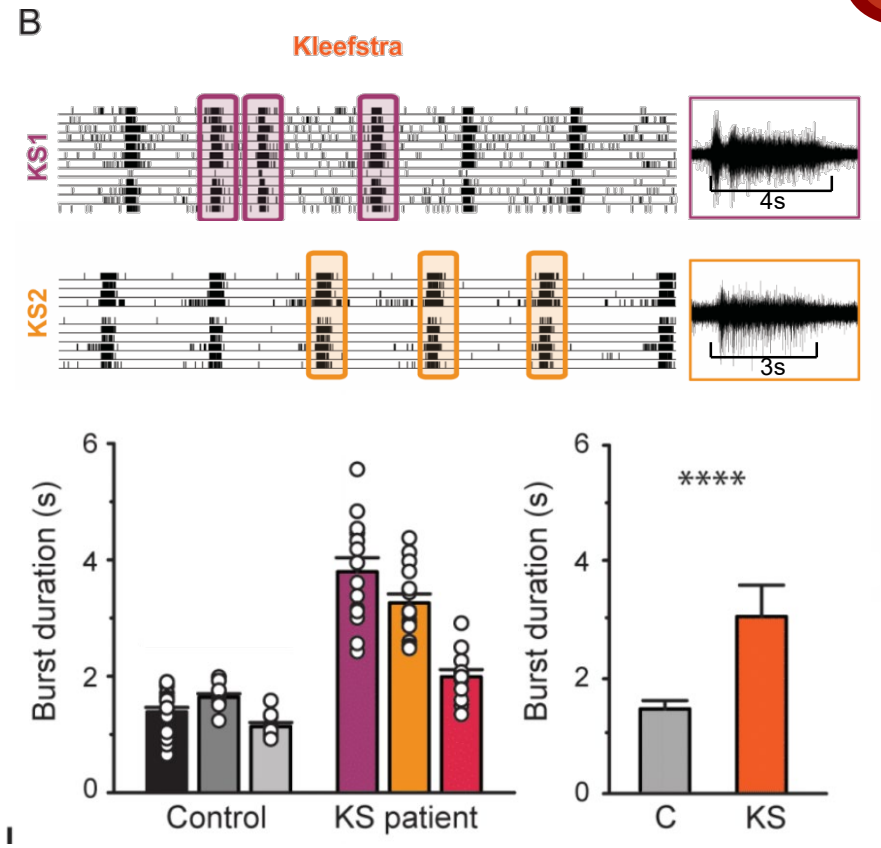


Mossink et al, Stem Cell Reports 2021

Disease-specific neuronal network phenotypes

Excitatory

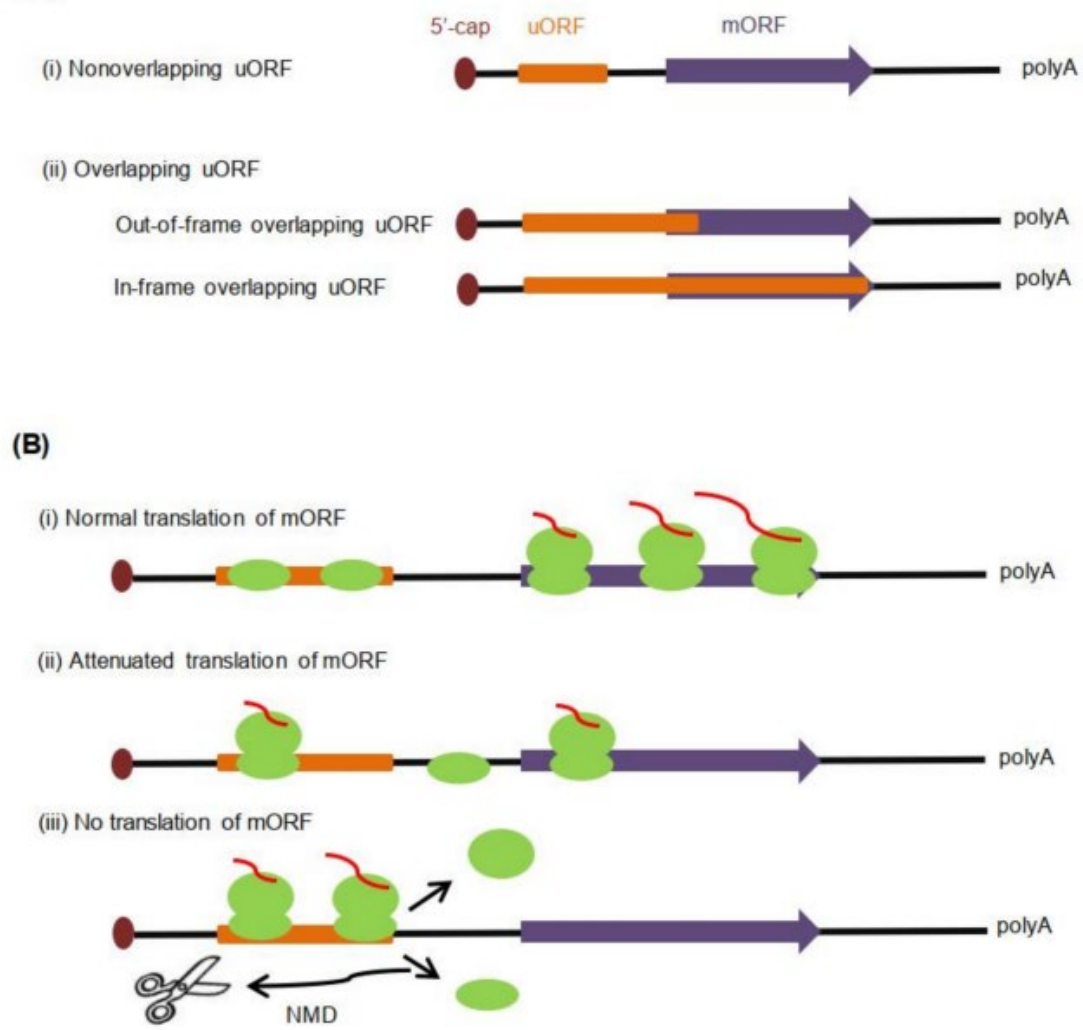




Control iNeuron networks show very similar network phenotype

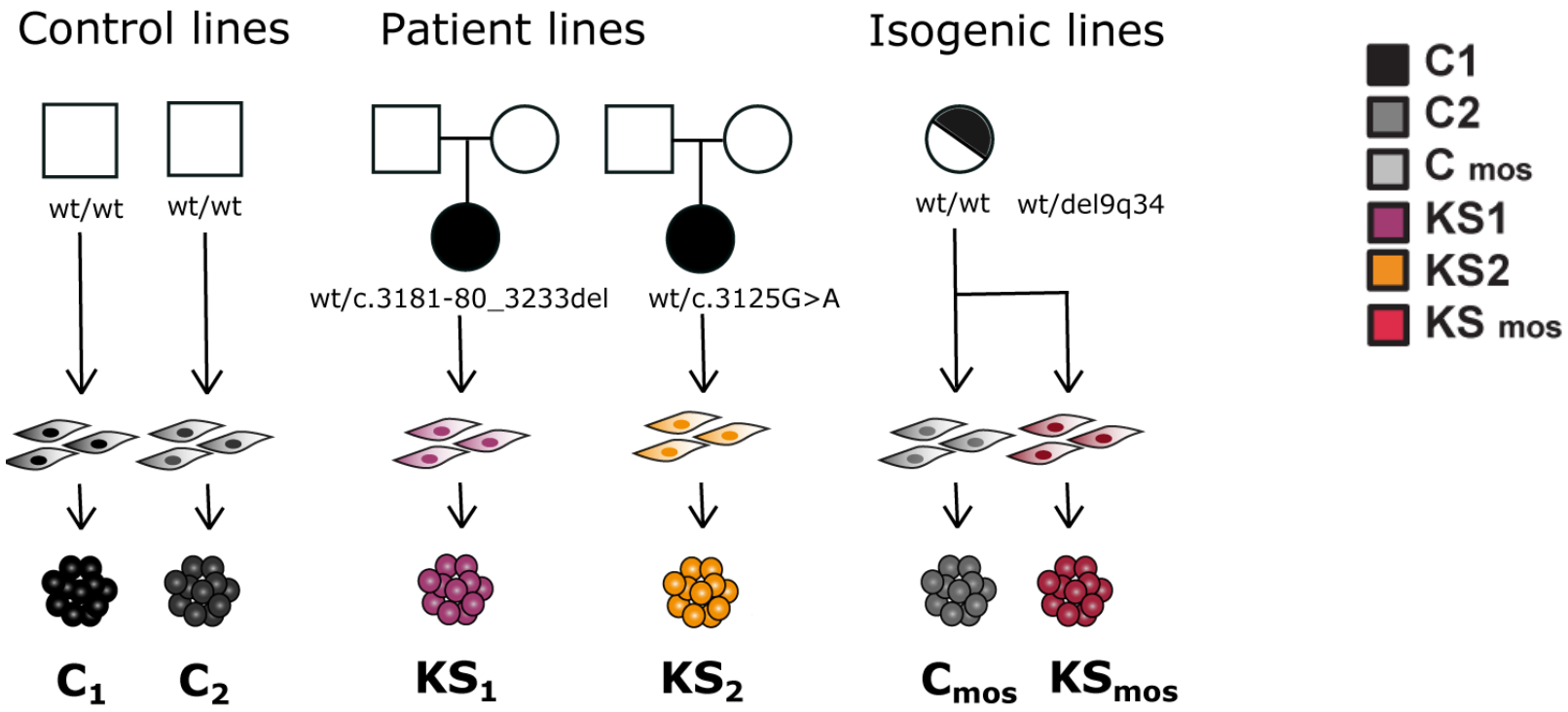
Hyperactivity & Long bursts

Frega et. al., Nat Commun. 2019



Zhang T et al. Int J Mol Sci. 2020

Control and Kleefstra iPSC lines



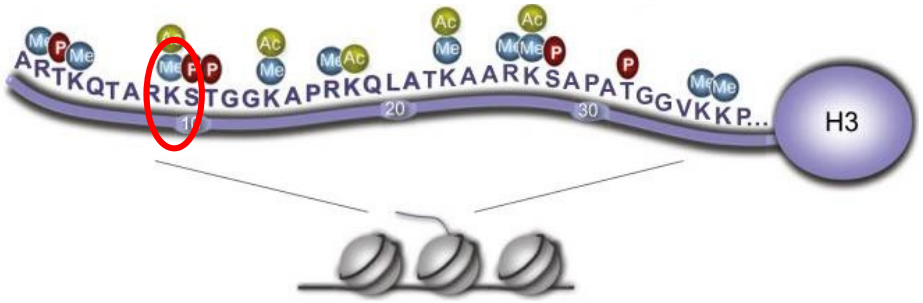
Frega et. al., 2019

Kleefstra Syndrome

- **Haploinsufficiency of the *EHMT1* gene**

Euchromatine Histone Methyltransferase 1

EHMT1 catalyze the mono and dimethylation of histone H3 at lysine 9



Core phenotype:

- **Developmental Delay**
- **Intellectual Disability (ID)**
- **Autism spectrum disorder**
- **Childhood hypotonia**
- **Facial characteristics**
- **Regression during adolescence**

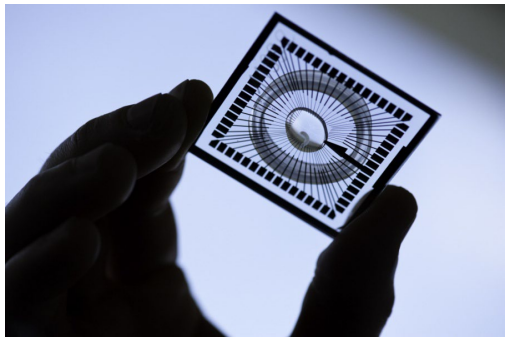
Various features:

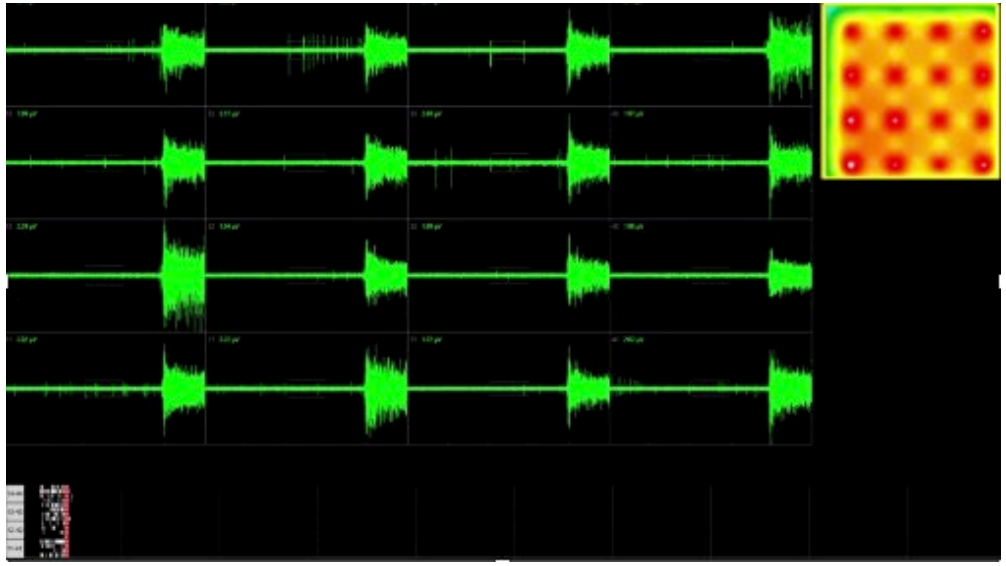
- Obesity
- Heart defects
- Seizures
- Behavioral problems
- Hearing/vision loss

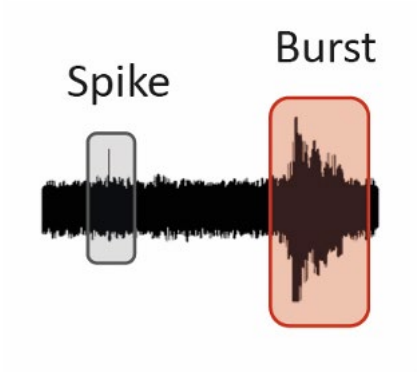
Samuel, diagnosed with Kleefstra syndrome due to an *EHMT1* L.o.f. variant

Measuring neural network activity of iPSC-derived networks

Micro-electrode arrays







Development

Days In Vitro