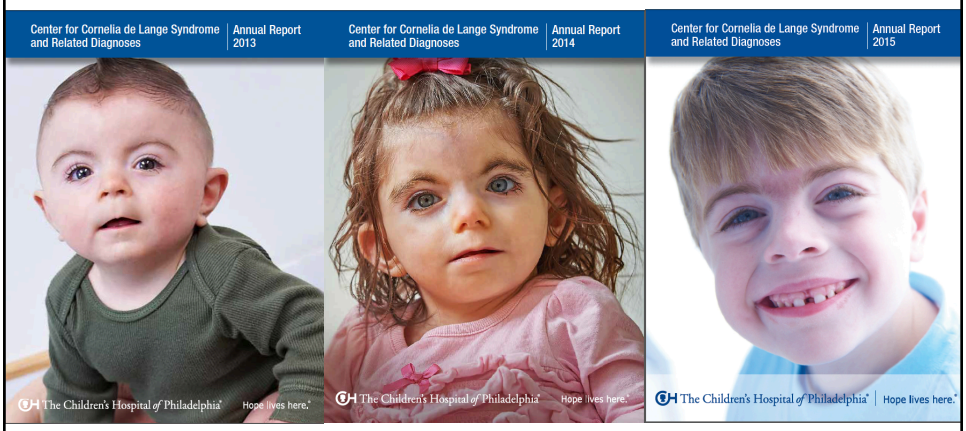
"Genetic Testing and Research for CdLS "

CdLS Meeting, Pesaro, Italy, 2017



Ian D. Krantz, M.D.
The Children's Hospital of Philadelphia and
The Perelman School of Medicine at the University of Pennsylvania

The general reaction to genetics…..

Genetic/Genomic Tests

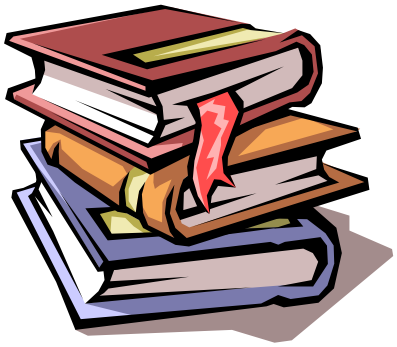
- **All are asking the same question:**

Is there a change in the DNA that is causing or contributing to the observed clinical findings in an individual?

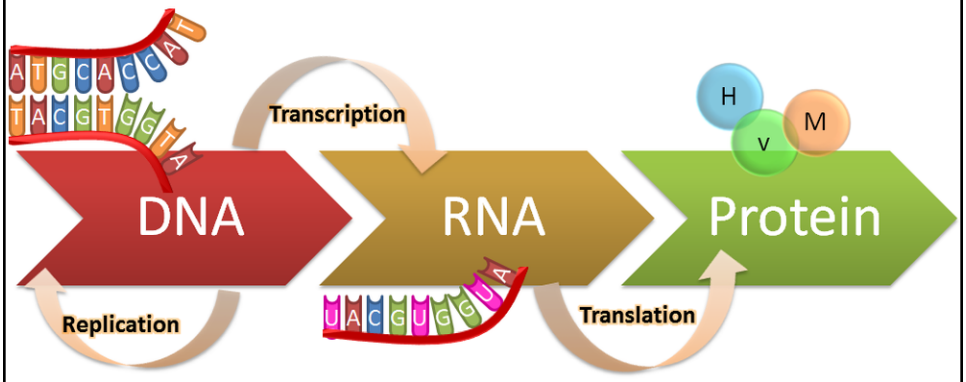
Types of Genetic Testing

- **Chromosome Analysis**
- **Fluorescence in situ hybridization**
- **Single gene**
  - » **PCR single locus & multiplex PCR**
  - » **Southern blot**
  - » **Sanger sequencing**
- **Chromosomal Microarray Analysis**
- **MLPA & aCGH for dup/del of single exons**
- **Next generation sequencing based**
  - » **Panels**
  - » **Exomes**
  - » **Genomes**

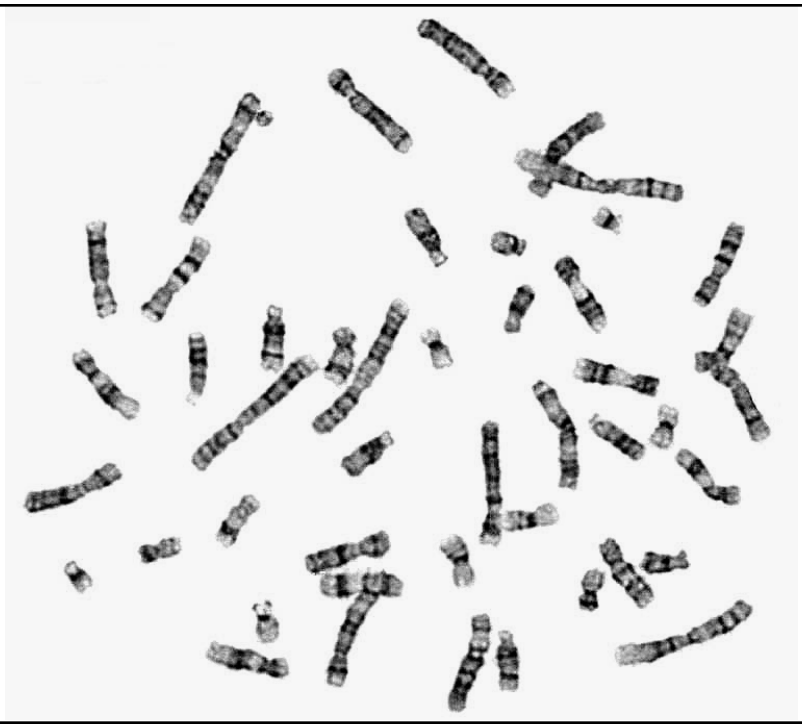
Genetics/Genomics 101



The Central Dogma:

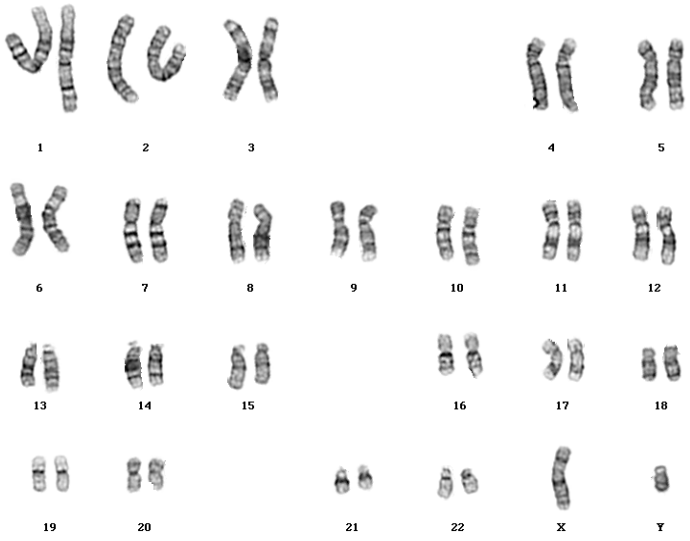



DNA double helix
Histone
DNA
Nucleosomes
200 bp of DNA
Solenoid
Chromatin
Chromatin loop contains approximately 100,000 bp of DNA
Chromatid



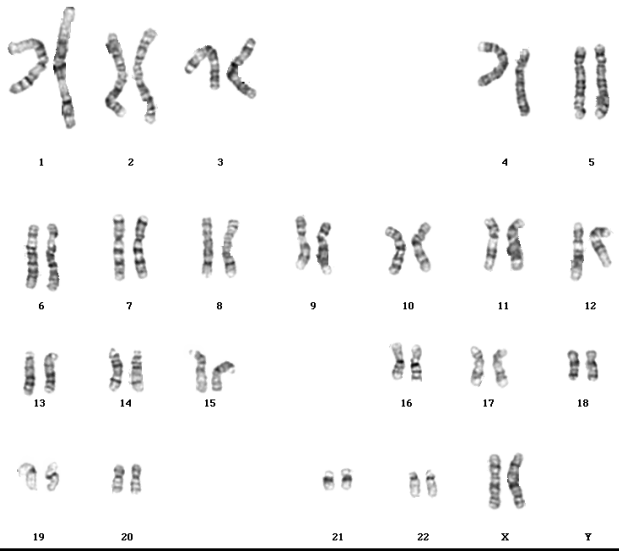
Male - 46, XY

2 copies of all genetic material!



Female - 46, XX

2 copies of all genetic material!

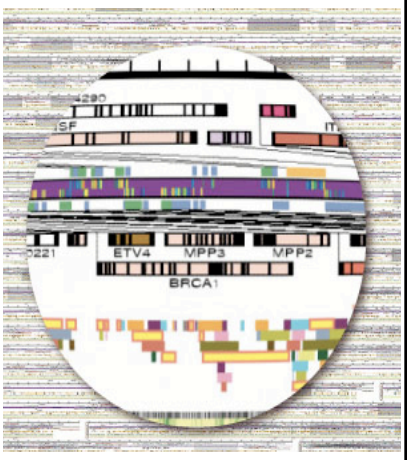


Chromosomal Microarray Analysis (CMA)

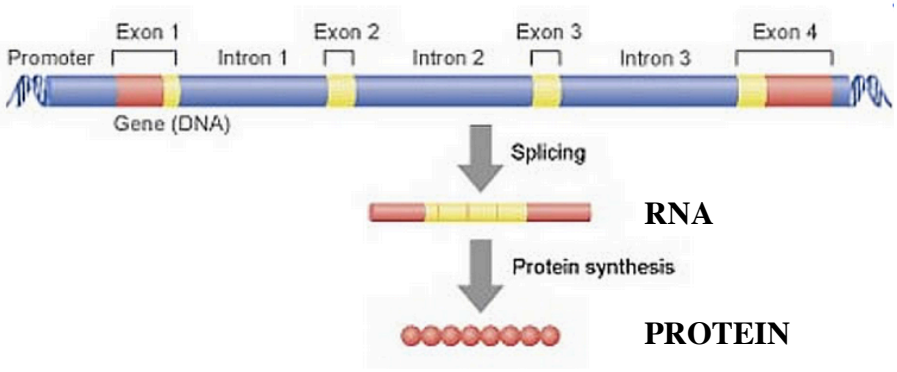
Two Primary Methods

- Comparative Genomic Hybridization ("CGH")
- SNP based testing

- High resolution – can detect very small deletions and duplications



Simplified structure of a gene



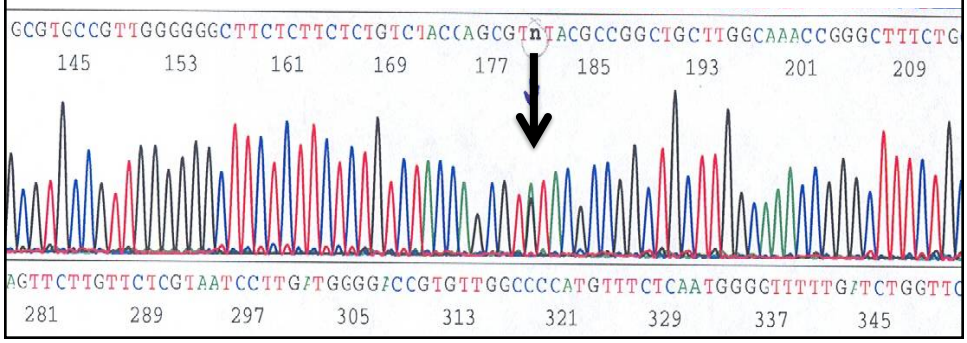
Sequencing Based Tests

- Sanger sequencing
  - Targeted regions-single gene
- Next Generation Sequencing (NGS)

Sanger Sequencing

Gold standard for single gene testing

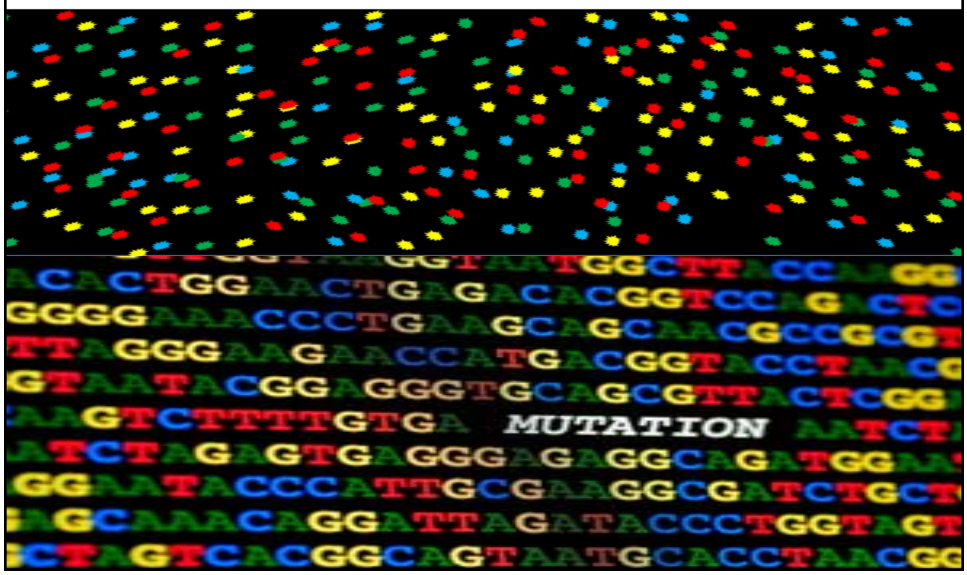
Sequence one genetic region at a time



Sanger sequencing: when to order

- R/O mutations in specific genes based on clinical phenotype
- Limitation:
  - You have to know what you're looking for

Next Generation Sequencing - NGS (2005)
Scalability, Speed, Resolution



NGS Tests
(Massively parallel sequencing)

- Targeted gene panels
  - Collection of genes relevant to a phenotype
- Exome Sequencing
- Genome Sequencing

Targeted Gene Panels

Advantages

- Selected genes with clinical utility
- Complete sequencing of genes of interest
- Cost effective and fast

Limitations

- Adding new genes lags behind discovery
- Upgrade to content = continuous validation
- Diagnosis missed if not on the gene list

NextGen Sequencing

Genome sequencing

- 3 billion base pairs
- 12 million variants per individual
- Advantage: sequence everything
- Disadvantage: expensive, lower fold coverage, data storage, interpretation

Exome sequencing

- 30 Million base pairs (1% of genome)
- 20,000 genes made up of ~180,000 exons
- 50-100,000 variants per exome
- Advantage: higher fold coverage, less expensive
- Disadvantage: incomplete capture

Nancy Spinner, PhD, Ian Krantz, MD

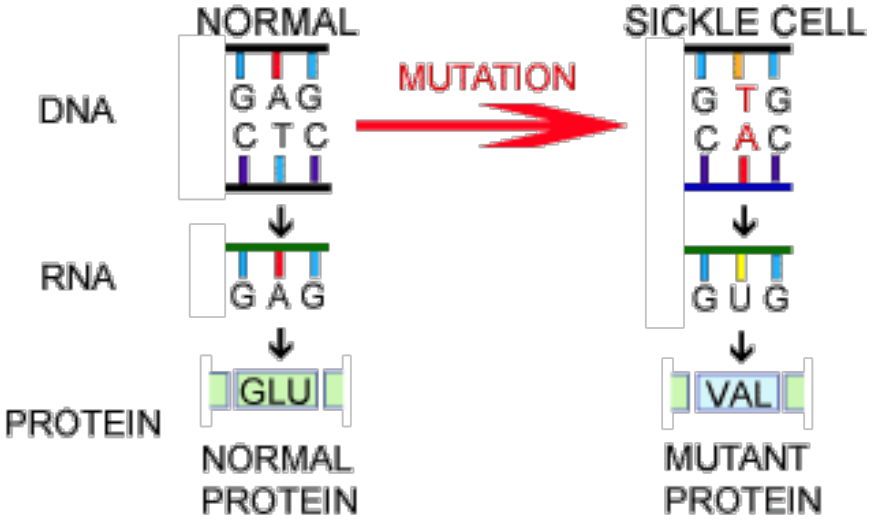
Exome Sequencing: When to Order

- Suspect genetic etiology
- Differential diagnosis → non overlapping disorders
- Genetic heterogeneity (lots of genes)
- Previous genetic testing negative

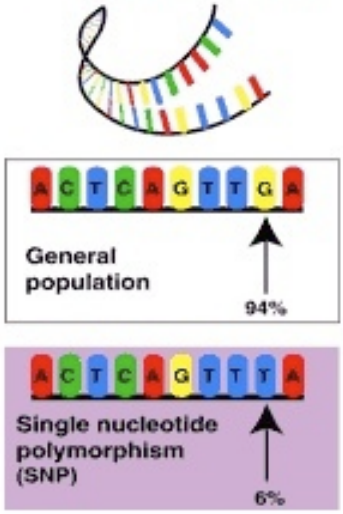
Targeted Testing: e.g. Single gene testing

Genomic level testing: Chromosomal Microarray, Exome/Genome Sequencing

Mutation



Polymorphisms ("poly" many "morphe" form) and Variants of Uncertain Significance (VUS)



Autosomal Dominant Inheritance

	D	d
d	Dd	dd
d	Dd	dd

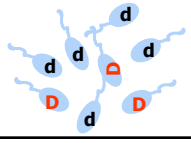
If one of the parents has an AD disorder, there is a 50% recurrence risk with each subsequent pregnancy

	d	d
d	Dd	dd
d	dd	dd

- Often mutations in AD genes arise sporadically
- Recurrence risk ~1%
- Germ line mosaicism

Most common pattern in CdLS

Rare:

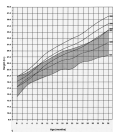


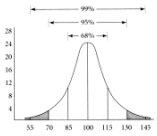
Cornelia de Lange Syndrome (CdLS)

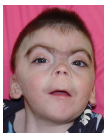
- First description by Vrolik in 1849
- Described by Brachmann in 1916
- Cornelia de Lange described two unrelated infants in 1933
- Prevalence as high as 1 in 10,000
- Most cases are sporadic, but rare familial recurrences occur
- Dominant inheritance with variable expressivity
- Caused by mutations in cohesin structural and regulatory proteins
- Genetically heterogeneous:

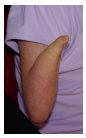
  NIPBL (5p13.1) ~ 60%
  SMC1A (Xp11.2) ~ 5%
  HDAC8 (Xq13) ~ 2%
  SMC3 (10q25) 1 case
  RAD21 (8q24) ~ 1% (atypical)
  unknown ~ 30%

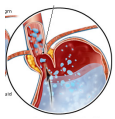
CdLS Multisystem Clinical Manifestations

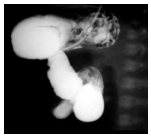

Growth

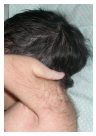

IQ

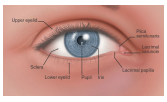

Craniofacial

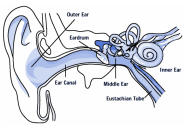

Limbs

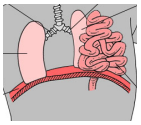

GERD


Malrotation


Hirsutism


Ophtho


Hearing Loss


CDH

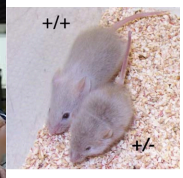

GU

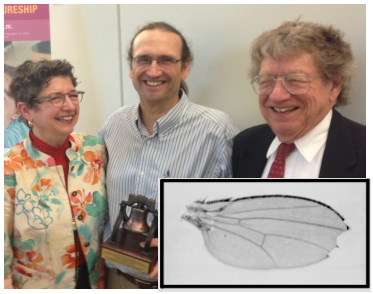

CHD


Hematopoietic

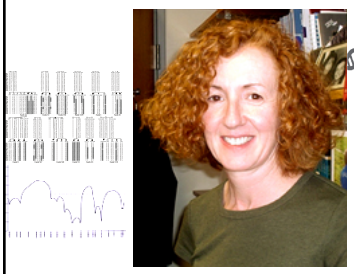
NIPBL (Nipped-B Like)

Drs. Anne Calof and Arthur Lander

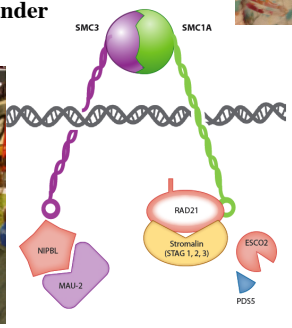




Dr. Dale Dorsett



Dr. Marcella Devoto

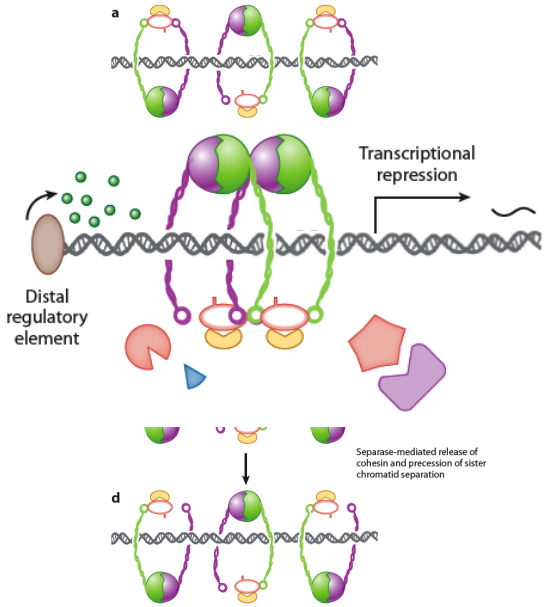


Katsu Shirahige

Cohesin Function

➢**Cohesin function:**

- **Sister chromatid cohesion**
- **Repairing double strand DNA break**
- **Maintaining 3-D genome organization**
- **cell proliferation**
- **maintaining pluripotency of stem cells**
- **gene expression**



NIPBL mutations in CdLS

Found in both Severe and Mild Variants

Found throughout gene

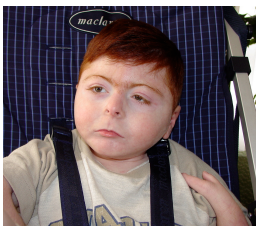
Truncating→more severe

Missense→milder

Overall mutation detection rate of ~ 60%

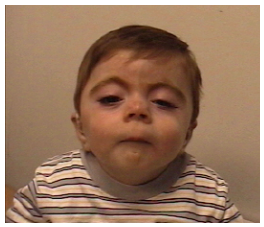
Severe-55/75 (73%)

Missense - 4 (7%)
Nonsense - 18 (33%)
Splicesite - 4 (7%)
Frameshift - 29 (53%)
In Frame Deletion - 0



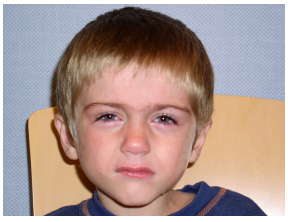
Moderate-37/77 (48%)

Missense - 15 (40%)
Nonsense - 2 (5%)
Splicesite - 7 (20%)
Frameshift - 10 (27%)
In Frame Deletion - 3 (8%)

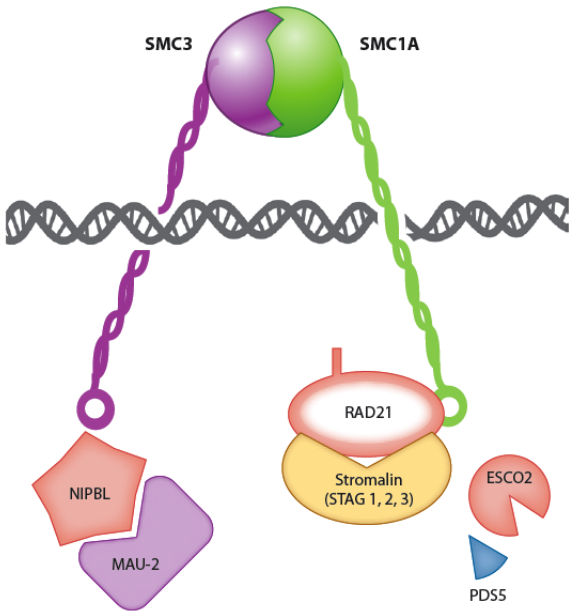


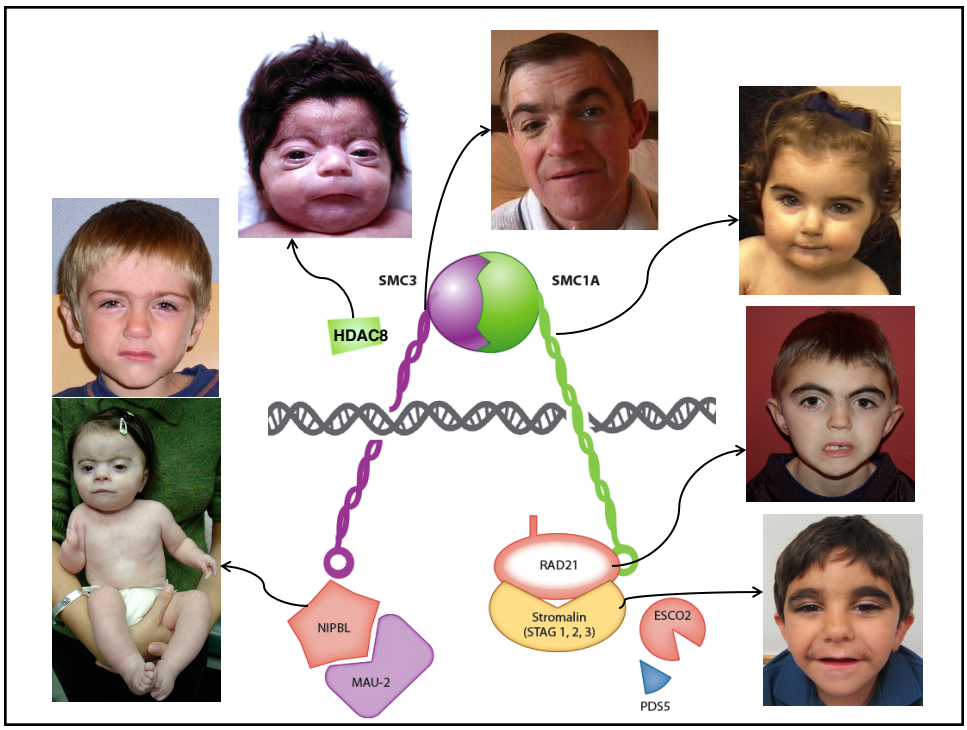
Mild-33/71 (46%)

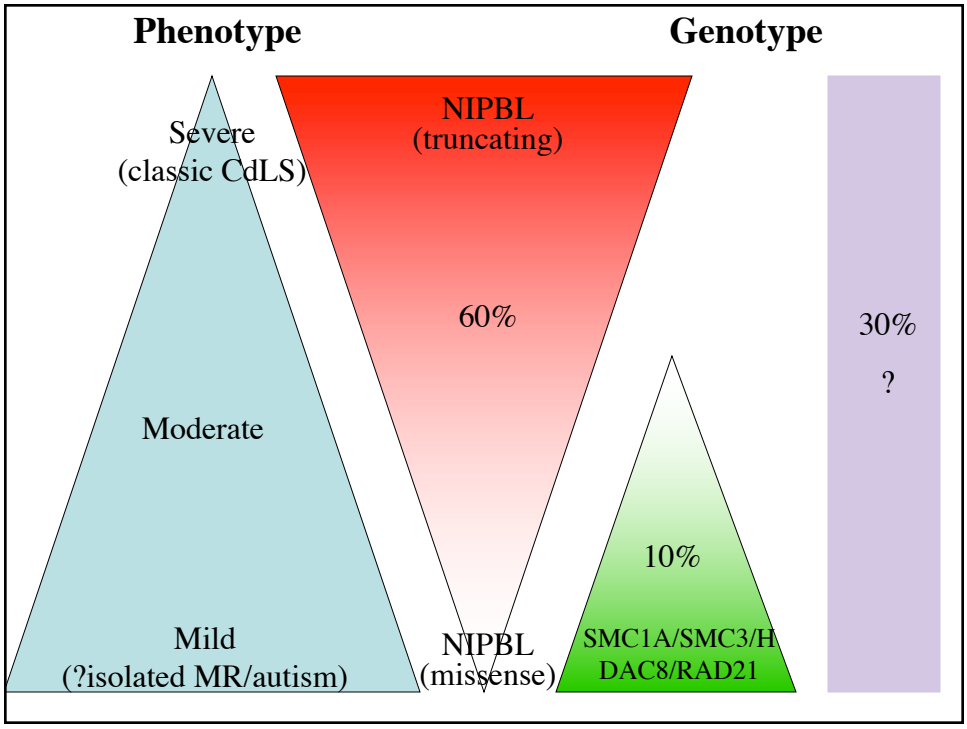
Missense - 21 (64%)
Nonsense - 0
Splicesite - 10 (30%)
Frameshift - 0
In Frame Deletion - 2 (6%)

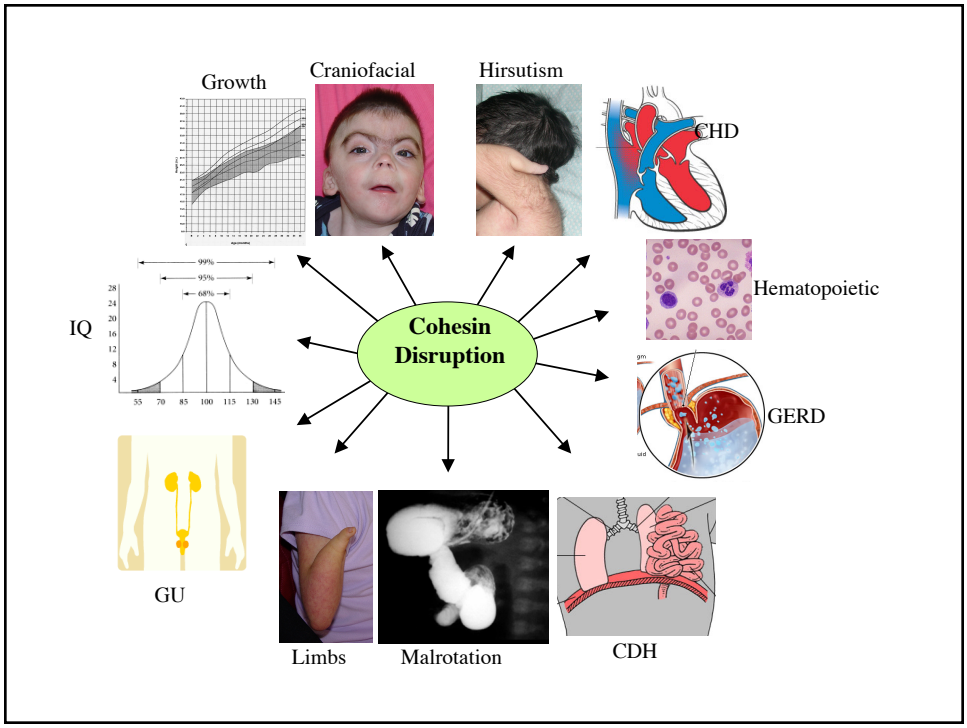


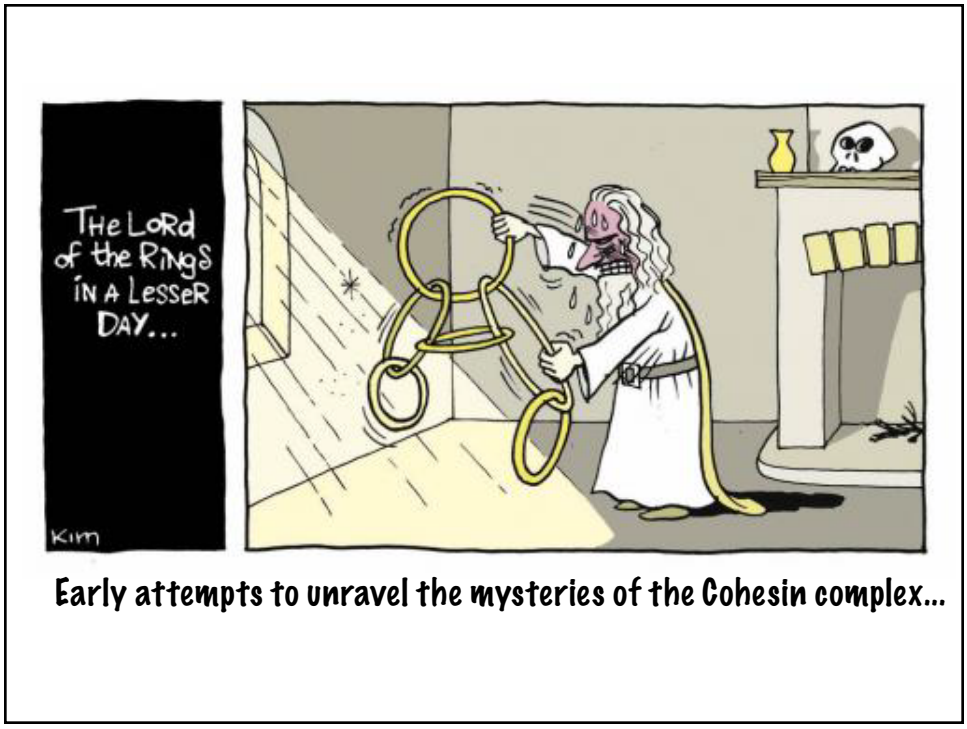
Cohesinopathies

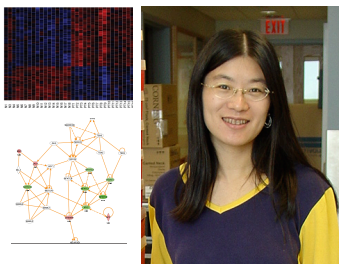


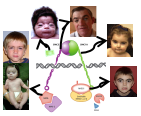
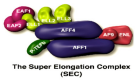

SMC3
SMC1A
HDAC8
RAD21
Stromalin
(STAG 1, 2, 3)
ESCO2
PDS5
NIPBL
MAU-2


Phenotype
Genotype
Severe
(classic CdLS)
Moderate
Mild
(?isolated MR/autism)
NIPBL
(truncating)
60%
NIPBL
(missense)
10%
SMC1A/SMC3/H
DAC8/RAD21
30%
?

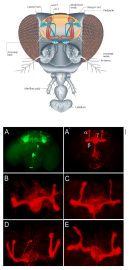
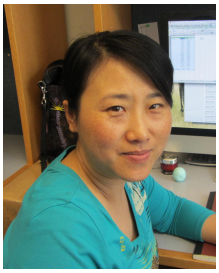

Growth
Craniofacial
Hirsutism
CHD
IQ
Cohesin Disruption
Hematopoietic
GERD
GU
Limbs
Malrotation
CDH

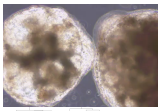
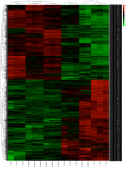

THE LORD of the RINGS IN A LESSER DAY...
Kim
Early attempts to unravel the mysteries of the Cohesin complex...



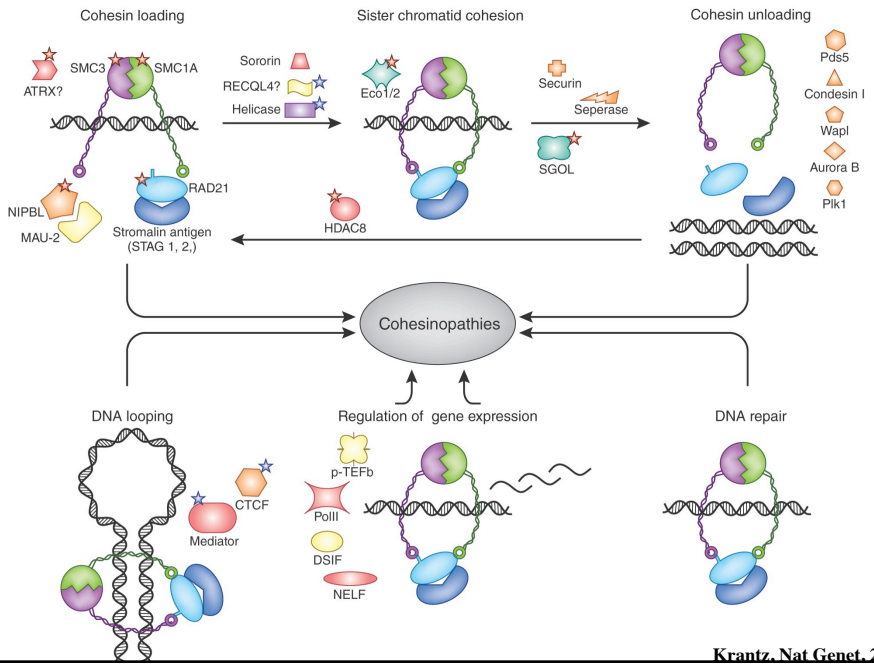
Jinglan Liu, Ph.D. **Matt Deardorff, M.D., Ph.D.** **Kosuke Izumi, M.D., Ph.D.**

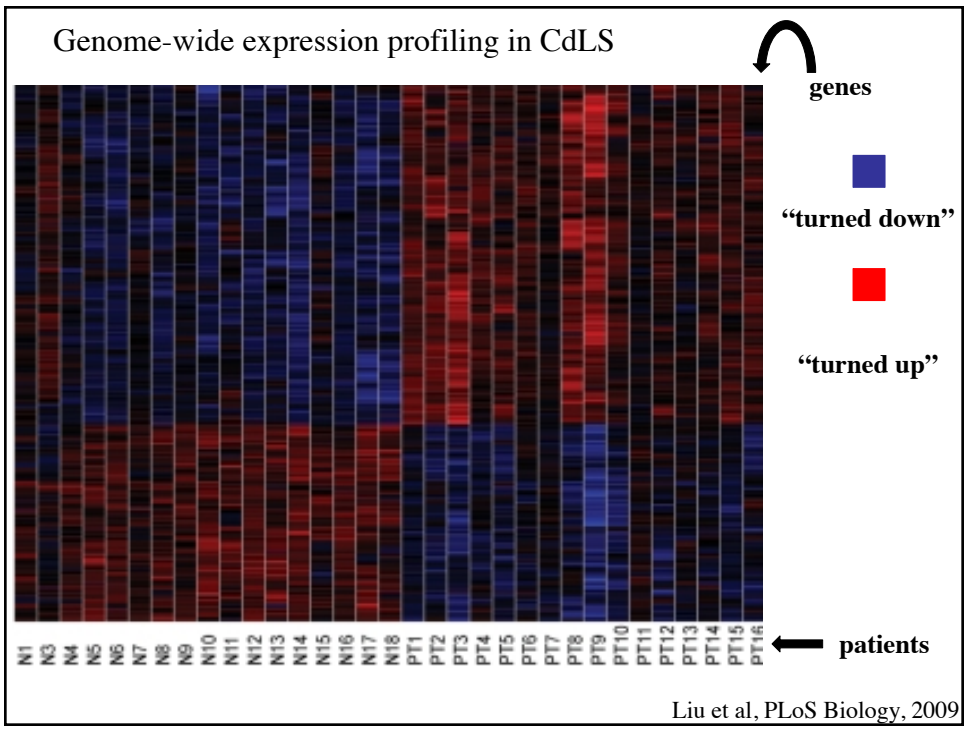



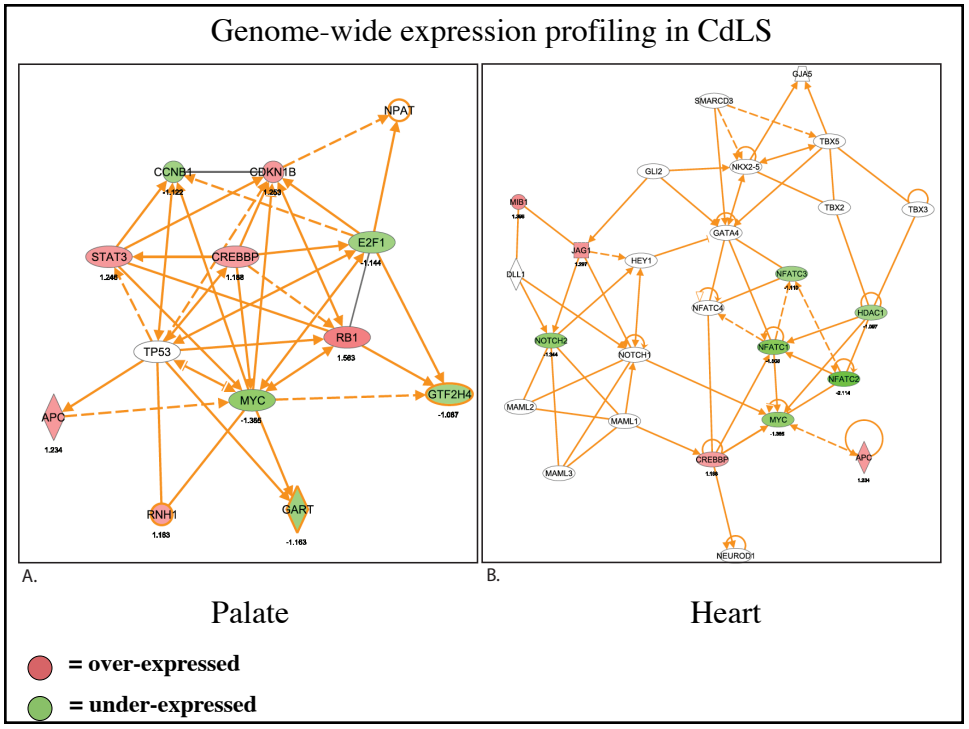
Yaning Wu, Ph.D. **Jay Mills, Ph.D.**

Multiple Roles of Cohesin Complex

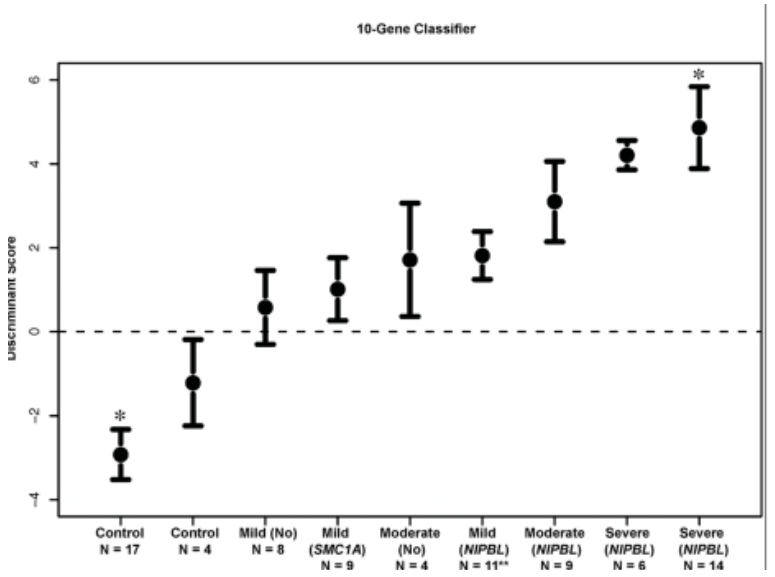


Krantz, Nat Genet, 2014


Genome-wide expression profiling in CdLS
genes
"turned down"
"turned up"
patients
N1 N3 N4 N5 N6 N7 N8 N9 N10 N11 N12 N13 N14 N15 N16 N17 N18 PT1 PT2 PT3 PT4 PT5 PT6 PT7 PT8 PT9 PT10 PT11 PT12 PT13 PT14 PT15 PT16
Liu et al, PLoS Biology, 2009

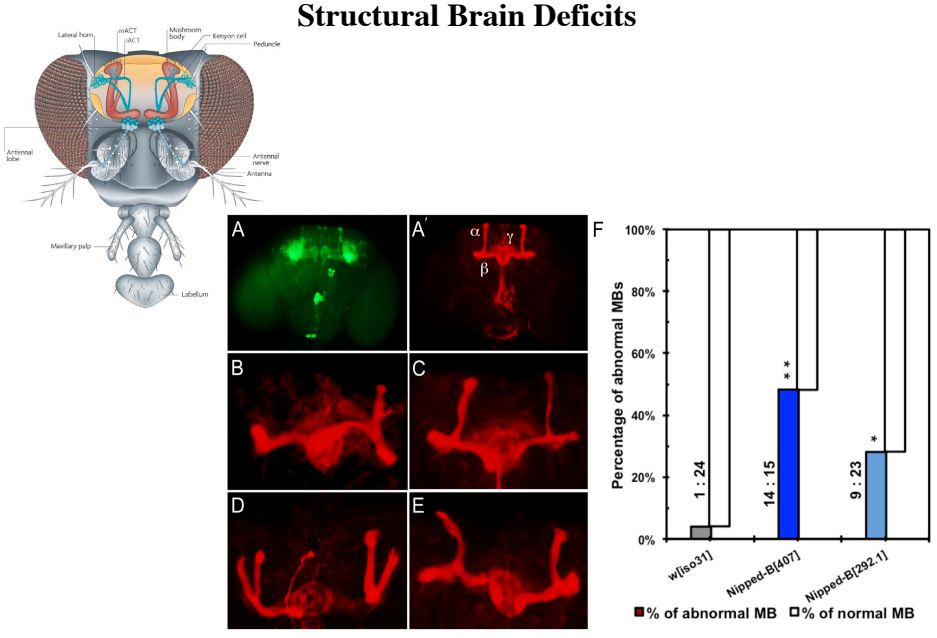

Genome-wide expression profiling in CdLS
NPAT
CCNB1
CDKN1B
STAT3
CREBBP
E2F1
TP53
RB1
MYC
GTF2H4
APC
RNH1
GART
A.
GJA5
SMARCD3
TBX5
GLI2
NKX2-5
MIB1
TBX2
TBX3
GATA4
JAG1
HEY1
DLL1
NFATC3
NFATC4
HDAC1
NOTCH2
NOTCH1
NFATC1
NFATC2
MAML2
MAML1
MYC
MAML3
CREBBP
APC
NEUROD1
B.
Palate
Heart
= over-expressed
= under-expressed

Genome-wide expression profiling in CdLS



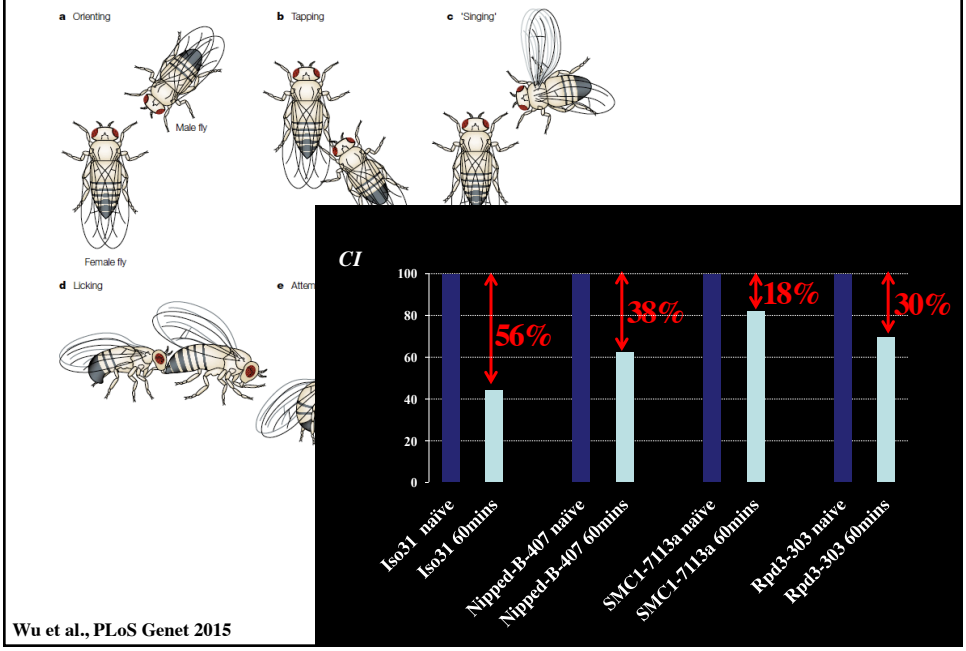
Liu et al, PLoS Biology, 2009

Drosophila Model of CdLS – Heterozygous Cohesin Mutant Flies Structural Brain Deficits



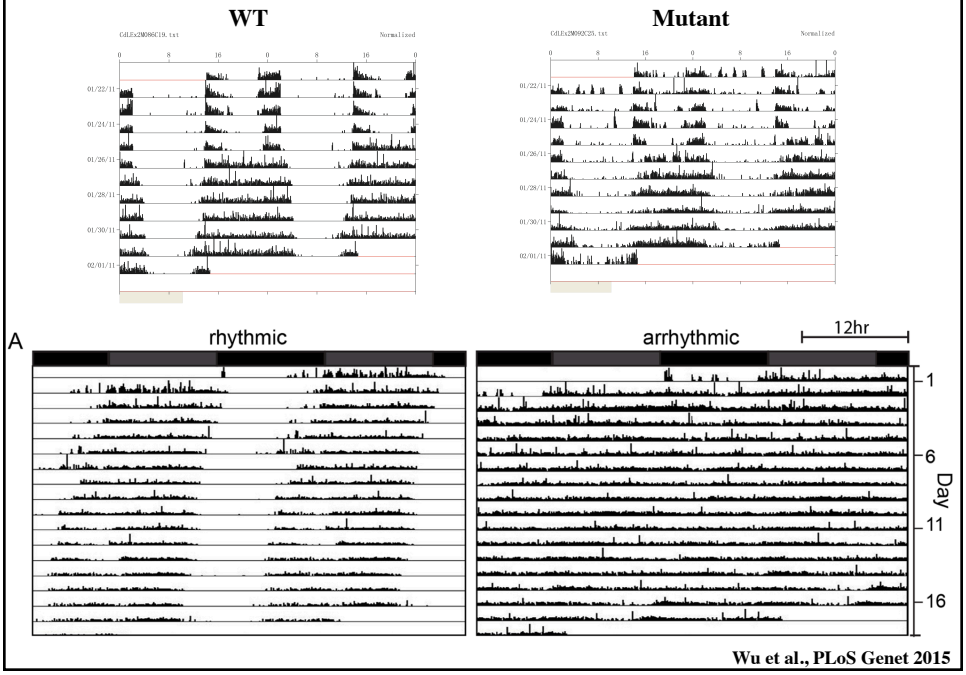
Wu et al., PLoS Genet 2015

Drosophila Model of CdLS – Learning & Memory



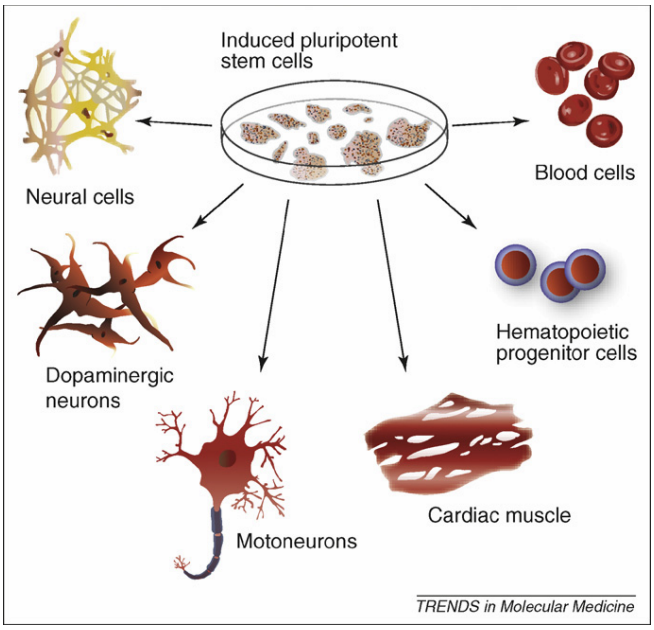
Wu et al., PLoS Genet 2015

Drosophila Model of CdLS – Sleep Disturbance

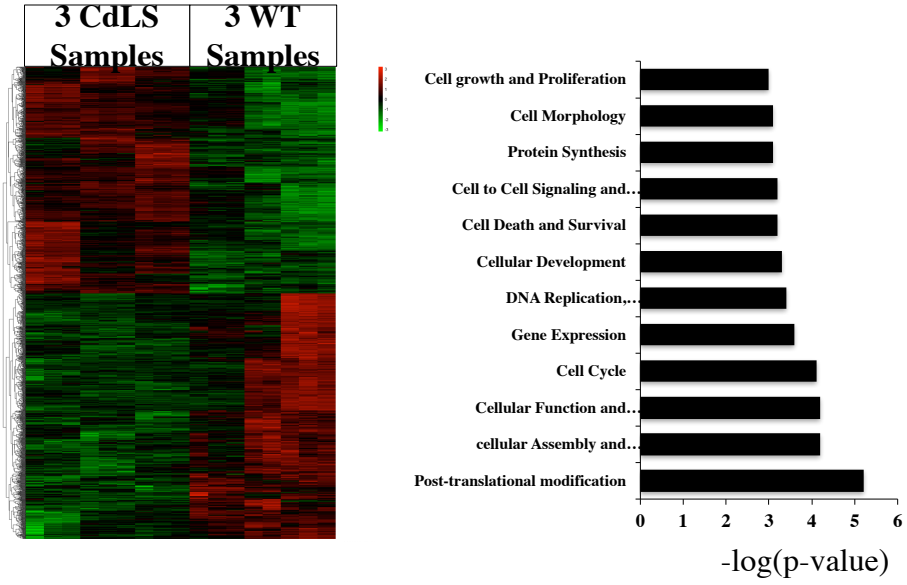


Wu et al., PLoS Genet 2015

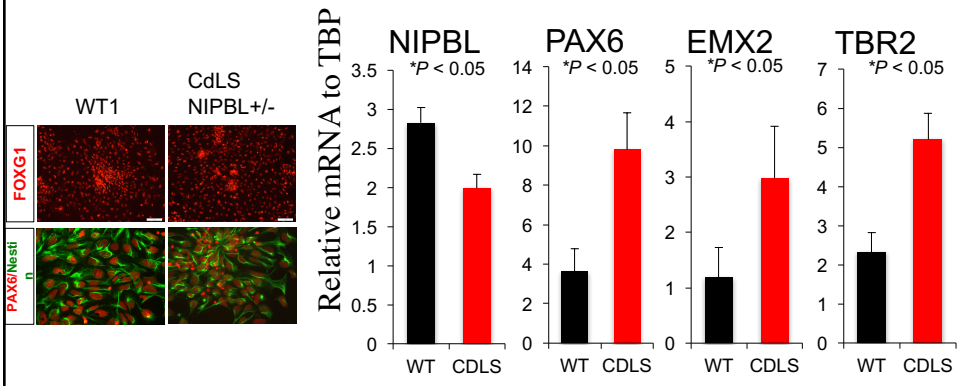
Establishment of Induced Pluripotent Stem Cells (iPSCs)



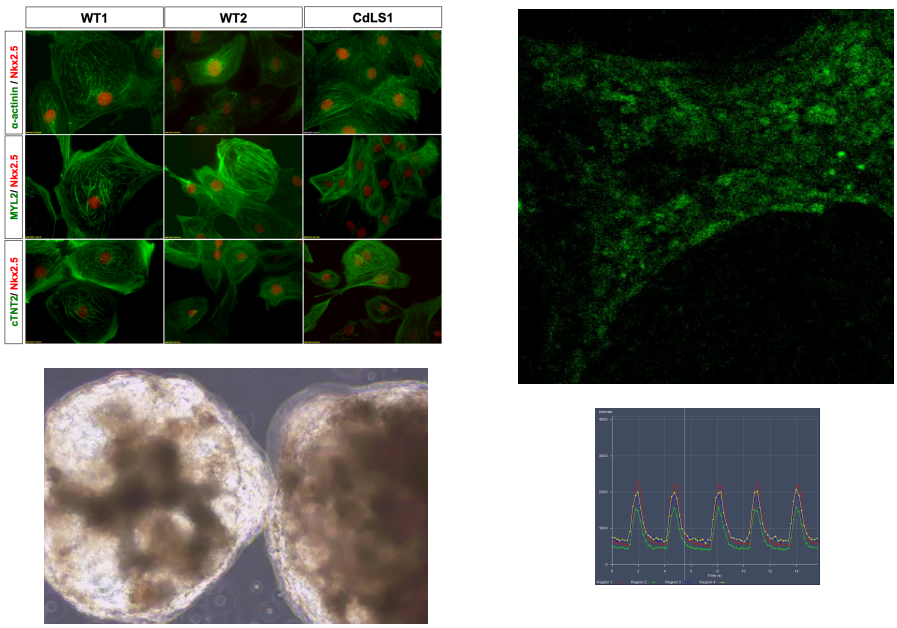
What can a CdLS stem cell tell us?

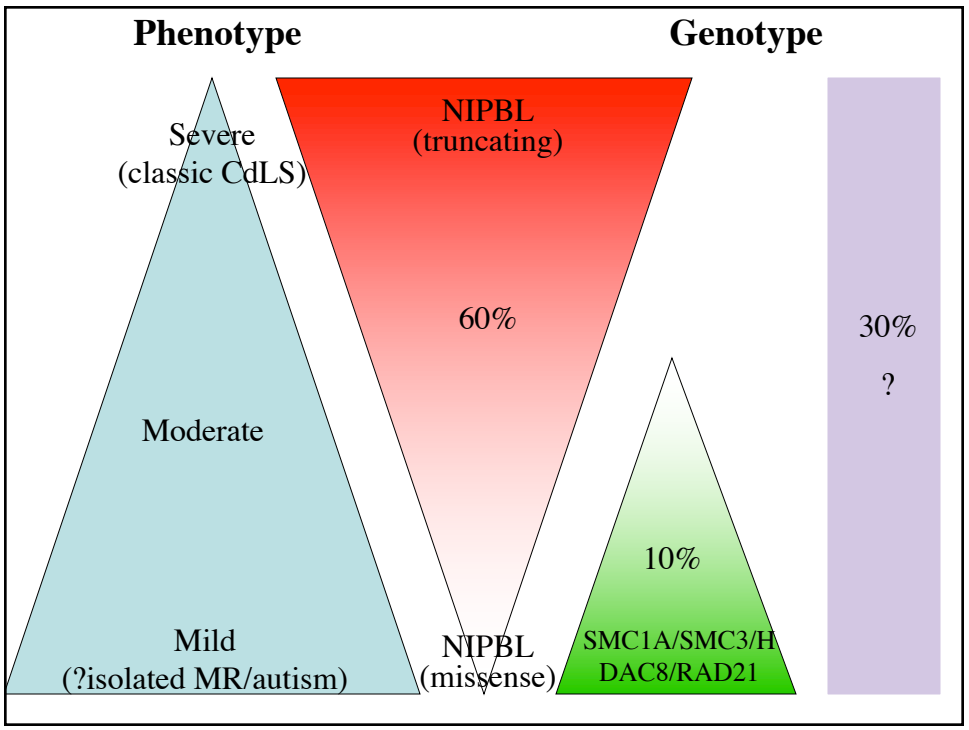


"Neuronal Modeling"



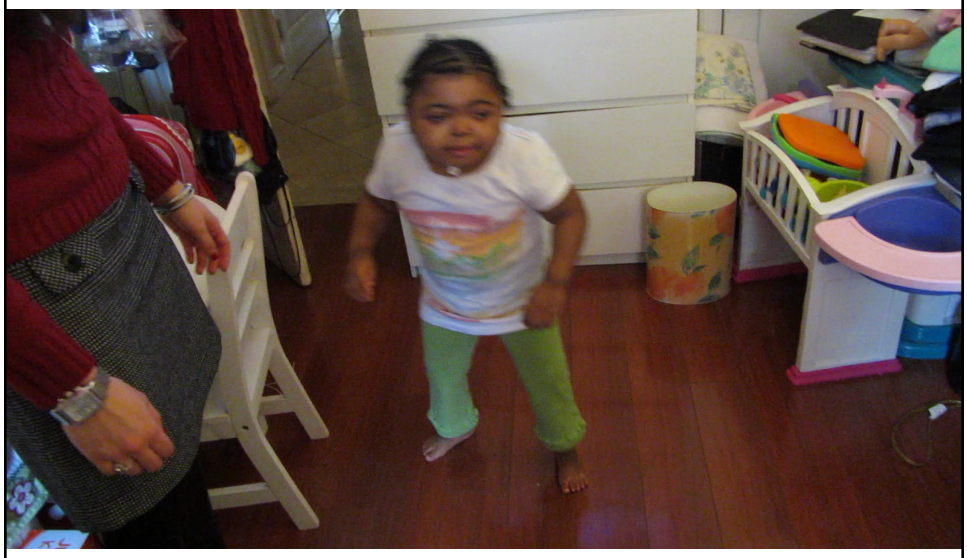
CdLS Patient Derived iPSC Cardiomyocyte Lines





Dr. Kosuke Izumi

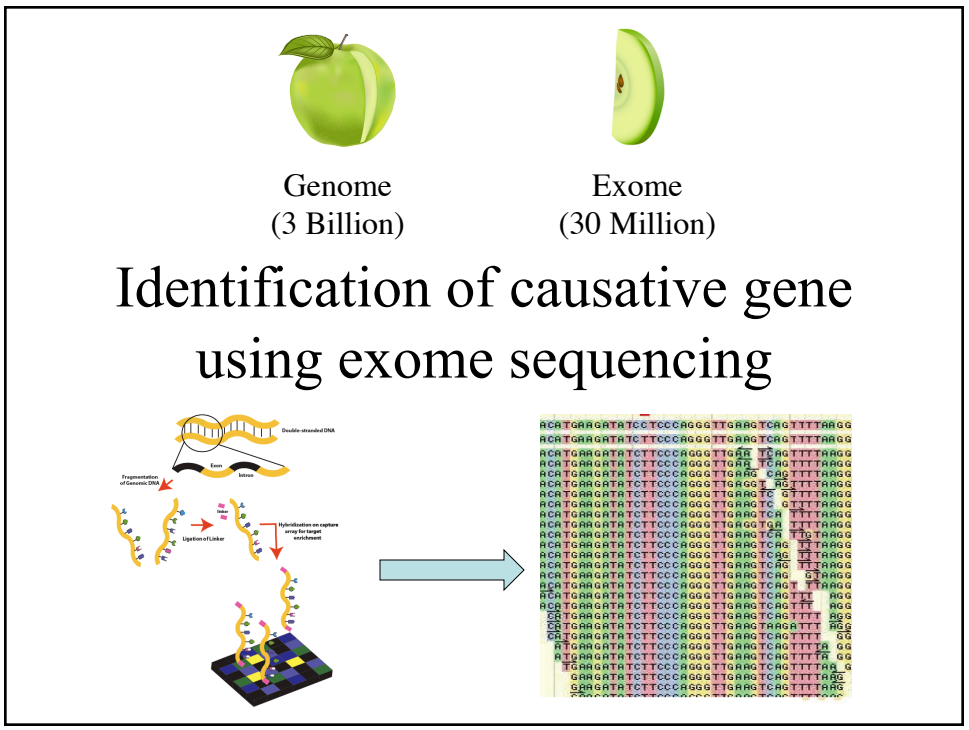

SHKISH
Dept.



CHOPS Syndrome

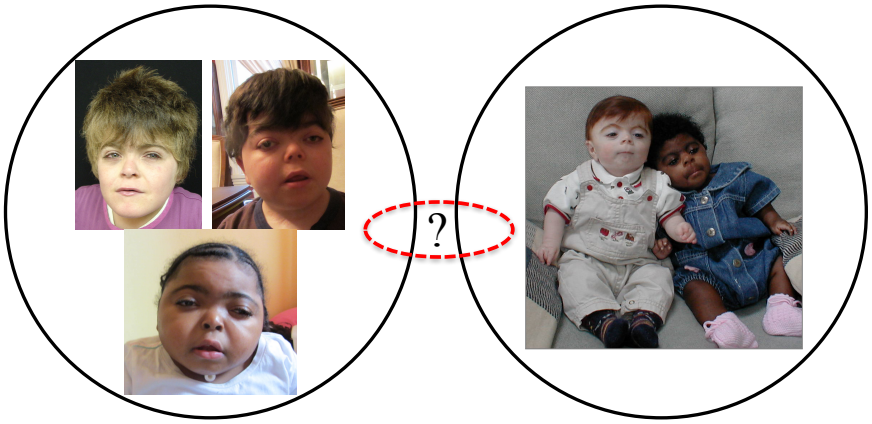
- **C** – Major: Cognitive impairment, Coarse facies,
- **H** – Major: Heart defects, Minor: Hearing loss
- **O** - Major: Obesity
- **P** – Major: Pulmonary involvement (trachea-laryngo malacia, chronic lung dis.)
- **S** - Major: Short stature, Skeletal dysplasia (brachydactyly, vertebral anomalies)

Izumi et al, Nat Genet, 2015


Genome
(3 Billion)
Exome
(30 Million)
Identification of causative gene
using exome sequencing

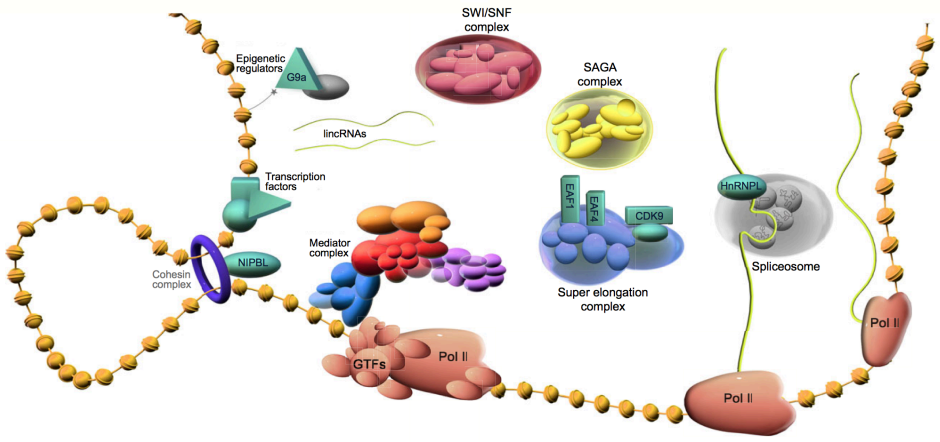

AFF4 missense mutations
b
NHD
ALF
TAD
NLS/NoLS
CHD
H. sapiens KSSSMLQKPIAYVRPMD---------- 256
P. troglodytes KSSSMLQKPIAYVRPMD---------- 256
C. lupus KSNSMLQKPIAYVRPMD---------- 261
B. taurus KSNSMLQKPIAYVRPMD---------- 261
M. musculus KSSSMLQKPIAYVRPMD---------- 261
R. norvegicus KSSSMLQKPIAYVRPMD---------- 261
G. gullus KSSSMLQKPIAYVRPMD---------- 260
D. rerio -SSSMPQKPIAYVRPMD---------- 227
D. melanogaster IAPLVTTPPQASQMPLGGATSGTILAGE 400
760A>G
T254A
761C>G
T254S
772C>T
R258W
WT
T254A
T254S
R258W
AFF4-Flag
1 0.02 0.64 1 0.62 0.83 1 0.79 0.93 1 0.80 1.10
SIAH1-HA
α-tubulin
MG132
SIAH1

Mechanistic link between CHOPS syndrome and Cornelia de Lange syndrome

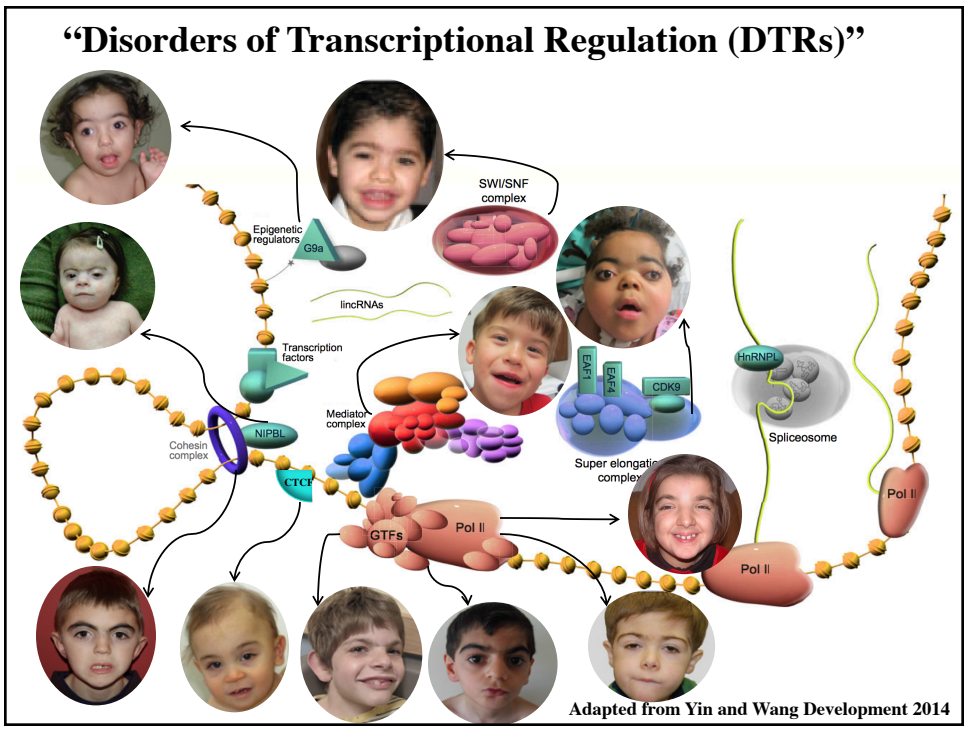


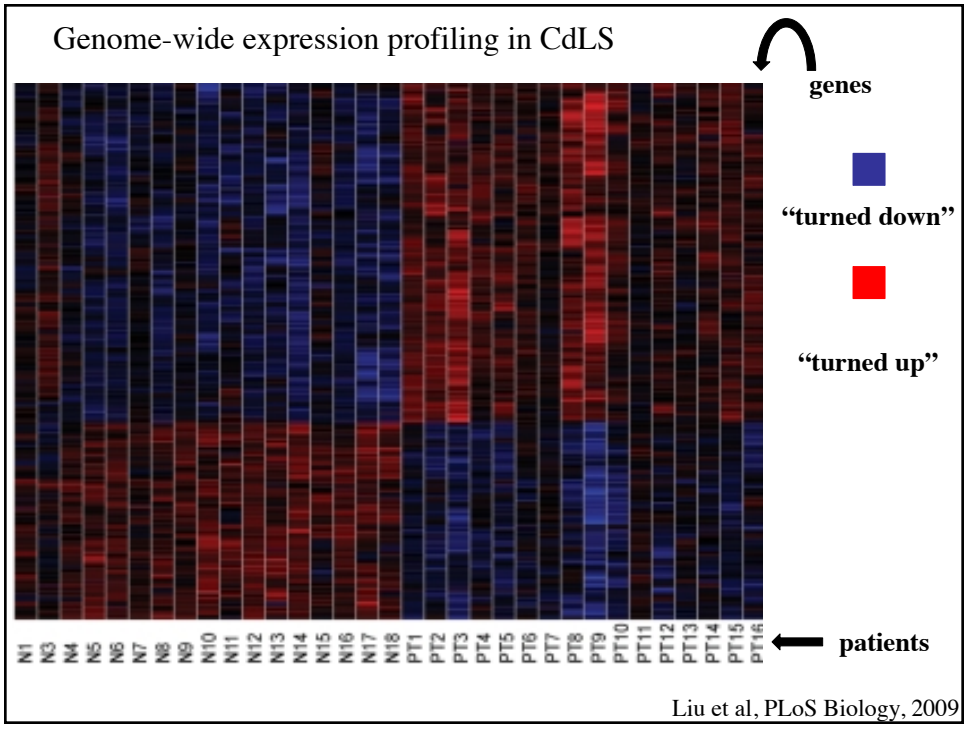
"Disorders of Transcriptional Regulation (DTRs)"

Izumi, Mol Syndromol 2016

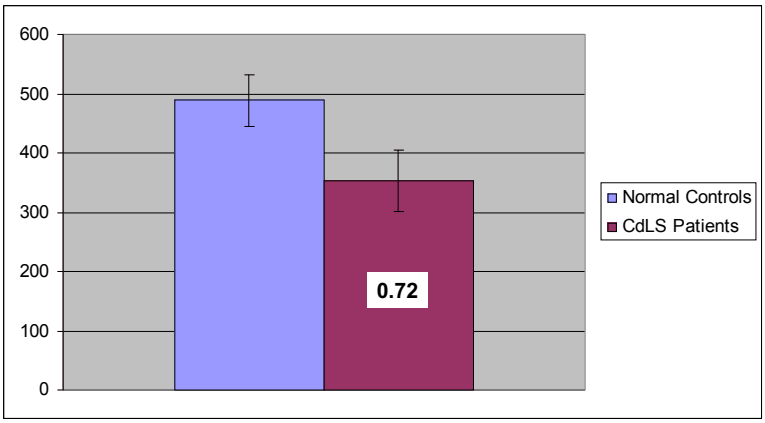


Adapted from Yin and Wang Development 2014

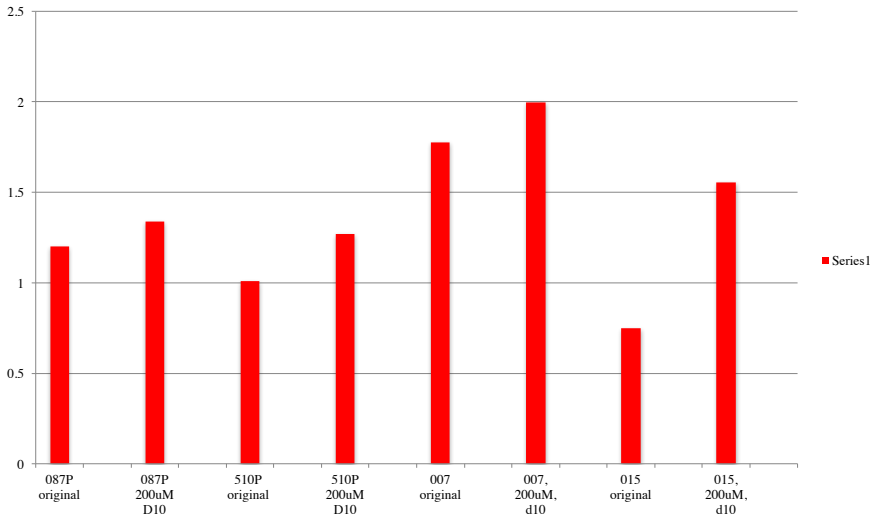

"Disorders of Transcriptional Regulation (DTRs)"
SWI/SNF complex
Epigenetic regulators
G9a
lincRNAs
Transcription factors
Mediator complex
Cohesin complex
NIPBL
CTCF
GTFs
Pol II
EAF1
EAF4
CDK9
Super elongation complex
HnRNPL
Spliceosome
Pol II
Pol II
Adapted from Yin and Wang Development 2014


Genome-wide expression profiling in CdLS
genes
"turned down"
"turned up"
patients
N1 N3 N4 N5 N6 N7 N8 N9 N10 N11 N12 N13 N14 N15 N16 N17 N18 PT1 PT2 PT3 PT4 PT5 PT6 PT7 PT8 PT9 PT10 PT11 PT12 PT13 PT14 PT15 PT16
Liu et al, PLoS Biology, 2009

NIPBL expression in LCLs from 16 normal controls and 15 CdLS patients

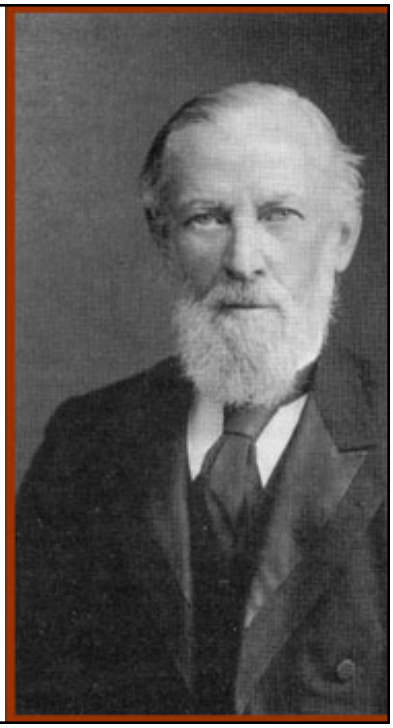


~ 1.38 fold
***p* = 9.38E -09**



'Regarding every disease now incurable, we may entertain the hope - faint it may be with respect to some, stronger in the case of others - that our powerlessness may not be permanent, and that we, or those who come after us, may be able to speak in very different terms.'

William R. Gowers, M.D., 1849-1915



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