The Future of Pediatric Psychopharmacology: Insights from Pediatric Psychosis and the Genome
or
A Clinician and Basic Scientist Team Up: Cat and Joe's Excellent Adventure

Joseph Gonzalez-Heydrich, MD and
Catherine Brownstein, MPH, PhD

Disclosure Statements

Joseph Gonzalez-Heydrich, MD reports having equity in Mightier/Neuromotion Labs as the Founder and Founding Chair of the Scientific Advisory Council, and as a consultant for clinical trial design for Alkermes, Sunovion, and Neurocrine pharmaceutical companies during the past 36 months. In this presentation, he will discuss case studies with the administered medications included for indications and ages not approved by the FDA.

- 14 year old boy
  - Star athlete, good student
  - Over the course of four months, descended into catatonia in with auditory and visual hallucinations, paranoia, aggression, mood dysregulation, and disorganized thoughts.
  - Poor motor coordination
  - CSF found increased protein concentrations; encephalitis test negative
  - Currently responding to Clozapine
  - Child compound het for CAPN1 (Spastic paraplegia 76, autosomal recessive)- doesn't fit phenotype
  - Family history: Paternal aunt has had multiple psychiatric hospitalizations, father has behavioral and anger problems

What Test is Most Likely to Affect Care?

- MRI
- Chromosomal Microarray and Whole Exome Sequencing
- Urine organic Acids
- Serum amino Acids

Stay Tuned!

Learning Objectives

1. Describe the concepts of heterogeneity, pleiotropy, penetrance, and variable expressivity regarding the genetics of severe very early onset psychopathology.

2. Identify the extreme bookends of recent genetic debate about genetic causation for schizophrenia and other serious mental disorders starting with the “Common Disease/ Common Variant Hypothesis” as it applies to findings in psychiatric genetics.

3. Discuss the other extreme bookmark of current psychiatric genetics debate the “Common Disease/Rare Variant” hypothesis of genetic causation in psychiatry and how it might lead to new treatments.

SPOILER ALERT!

Current Psychopharmacology	Future Psychopharmacology
Diagnosis: Disorders based on constellations of symptoms without reference to cause (exception PTSD) and do not track with biology or treatment response.	**Diagnosis:** Diseases defined mechanistically (e.g. gene defect, resulting physiology and environmental interaction)
Treatments: Found by accident and ameliorate only symptoms.	**Mechanisms:** Found by tracing effects of genes to RNA to proteins to cells to brain networks to symptoms
Mechanisms: inferred from accidentally found treatments and so do not lead to any breakthroughs.	**Treatments:** translated from precise knowledge of pathophysiology, halts or reverses disease progression not just decreases symptoms
Outcomes for serious mental illness: poor with 80-90% rates of disability	**Outcomes for serious mental illness:** good by preventing the unfolding of serious mental illnesses

HOW DO WE GET THERE

- **<u>Embrace the opportunities of new technology</u>:**
  - Genetic sequencing
  - Induced pluripotent stem cell (iPSC) derived brain cells
  - Genome editing (e.g. CRISPR)
- **“<u>Treasure your exceptions</u>”-William Bateson, 1908.**
- **<u>Enjoy new golden age of clinical description</u>**
  - These are newly discovered genetic diseases
  - Genes first approach describes the varied outcomes of a mutation
  - **The indispensable partnership: Clinicians + Basic scientists**

Phenotype First Approach Leads to Genes First Approach

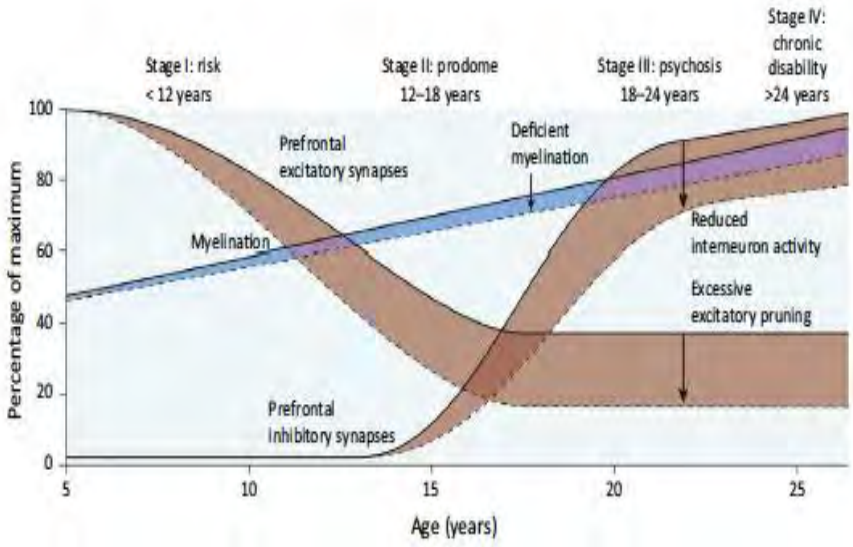
- Phenotype first approach has discovered:
  - Heterogeneity
    - Multiple causal paths to same common disorder
    - Common disorders are actually multiple diseases
  - Very early onset forms lead to gene discovery
- Genes first approach has discovered:
  - Pleiotropy: same mutation ➡ multiple differing symptom manifestations/disorders
  - Variable expressivity/penetrance (severity)?

Example: Very Early Onset Psychosis (<14 yrs)

"Typical" Schizophrenia

Symptoms

- **Cognitive impairments:** Deficits include working memory, verbal fluency, social cognition
- **Deficit symptoms:** Loss of motivation, blunted affect, impoverished thought and speech
- **Psychotic symptoms:** Hallucinations, delusions,



Course of Illness

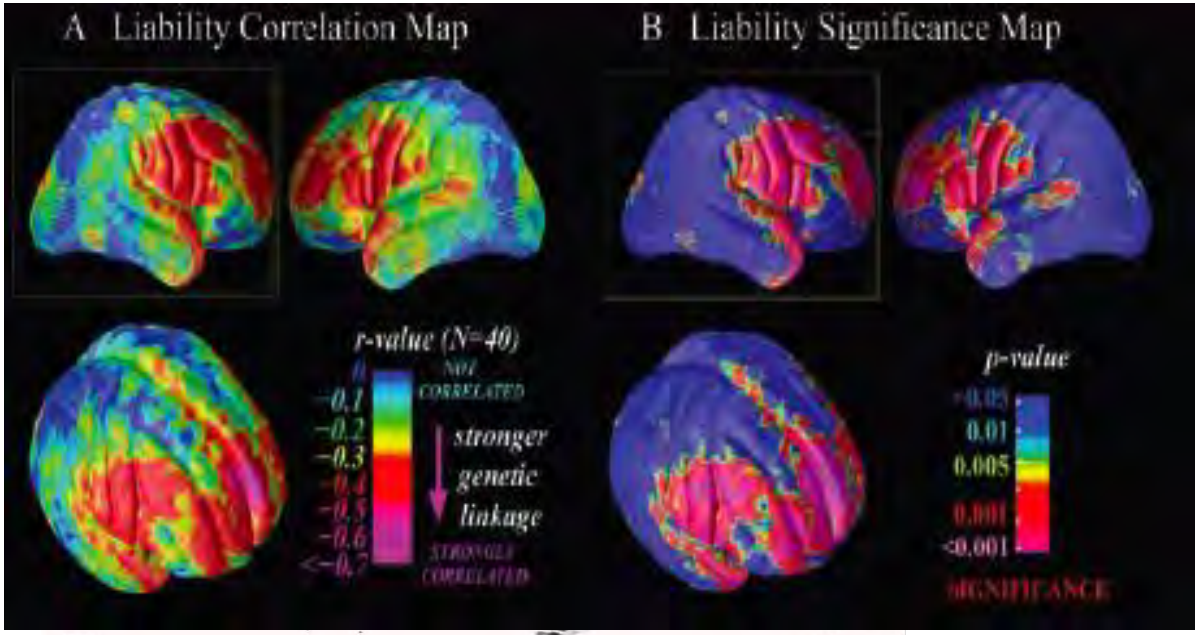
- **Onset:** Cognitive and deficit symptoms in mid-teen years; psychosis follows in late teens, 20's.
- **Trajectory:** Cognitive and deficit symptoms unremitting; superimposed pattern of relapsing and remitting acute psychotic episodes
- **Treatment**: Response to antipsychotic drugs deteriorates over time

Very Early Onset Psychosis:

- Similar symptoms (but more visual)
- More baseline neurodevelopmental disorders
- Long-term diagnosis variable, not all schizophrenia

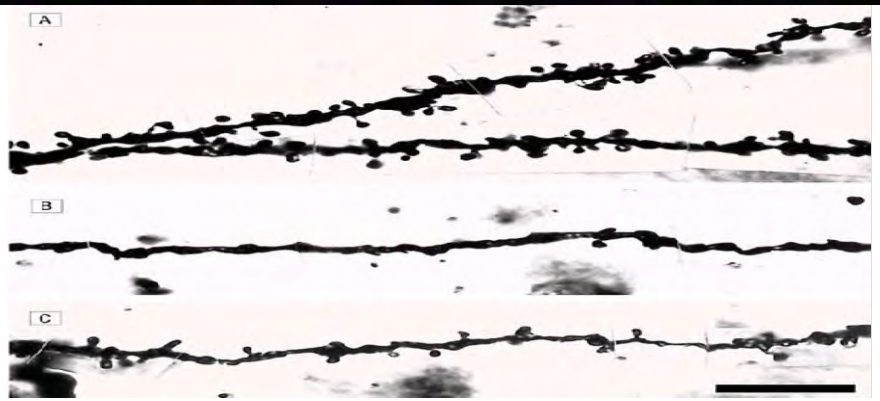
Adapted from slide from Steve Hyman

Schizophrenia: anatomic pathology--but no molecular mechanisms



- Excessive cortical thinning during adolescence
- Location, timing consistent with cognitive impairments

Cannon et al. Proc Natl Acad Sci U S A. 99:3228-33, 2002



Control

Glantz and Lewis, 2000

Schizophrenia

Slide from Steve Hyman

High heritabilities mean that molecular clues to pathogenesis are contained within our genomes

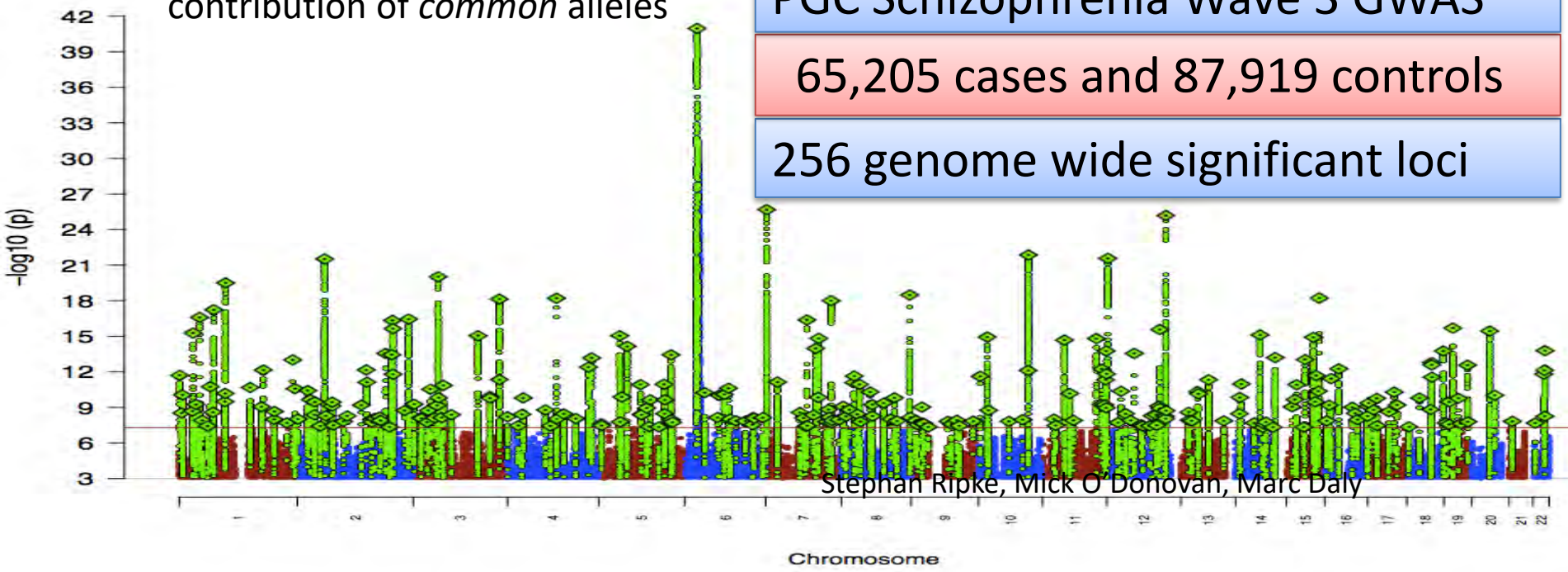
Disorder	Heritability (h^2)
Autism Spectrum	0.8
Schizophrenia	0.8
Bipolar Disorder	0.7-0.8
Major Depression	0.35

Heritability estimates based on comparing concordance of MZ Vs. DZ twins

Slide from Steve Hyman

Common Disease Common Variant Hypothesis:

Manhattan plot showing contribution of *common* alleles



- Schizophrenia, MDD, bipolar disorder, anxiety disorder, and ADHD correlated and share common variant risk ➡ "the Genome didn't get the DSM manual in the mail" –Steve Hyman
- Each gene has small effect (<1.4 RR)
- Polygenic risk scores (**PRS: $\Sigma\beta_i * g_i$**) explains ~7% of risk of a schizophrenia ➡ missing heritability

SCHEMA Consortium: Rare Variant Association 25,033 cases / 51,507 controls find only two genes with exome-wide levels of significance

TJ Singh

Transmitted rare exome variants associated with typical onset schizophrenia have modest effect sizes.

TRIO

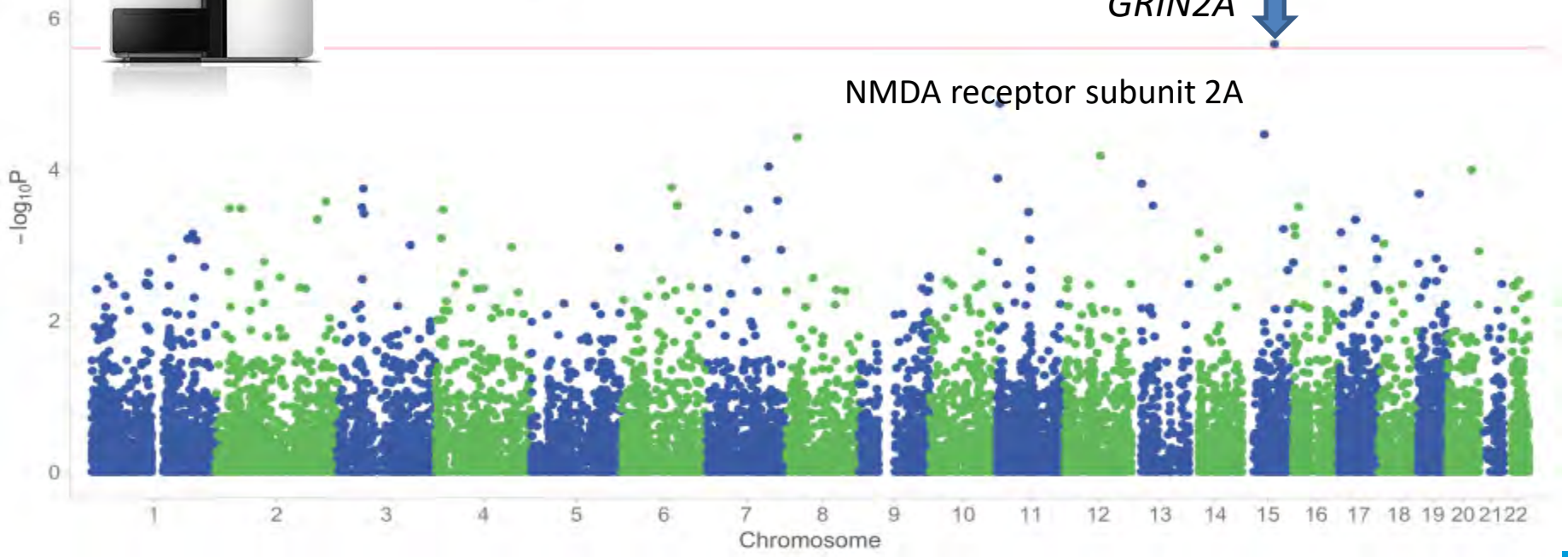
Affects dendritic branching

Component of histone methyltransferase

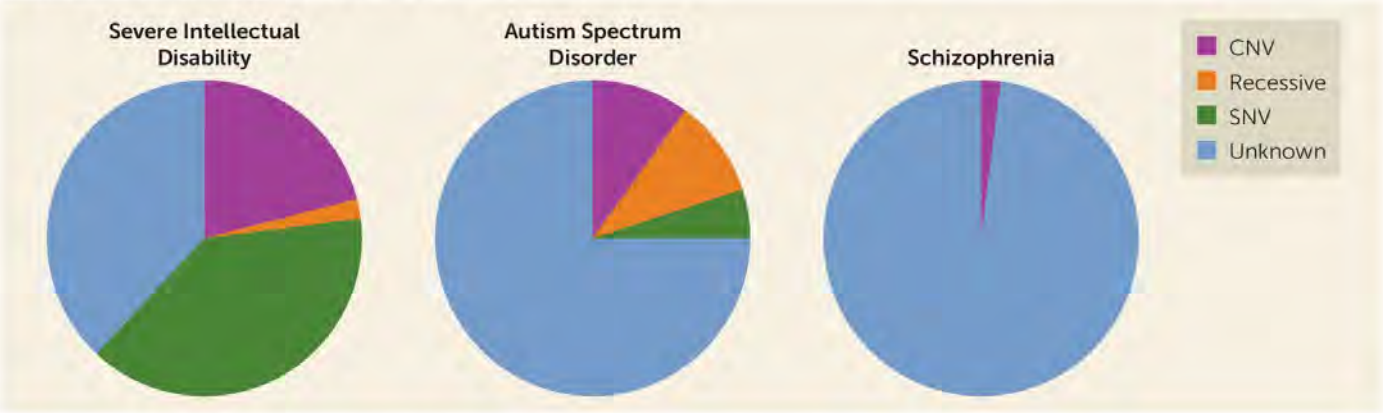
SETD1A

GRIN2A

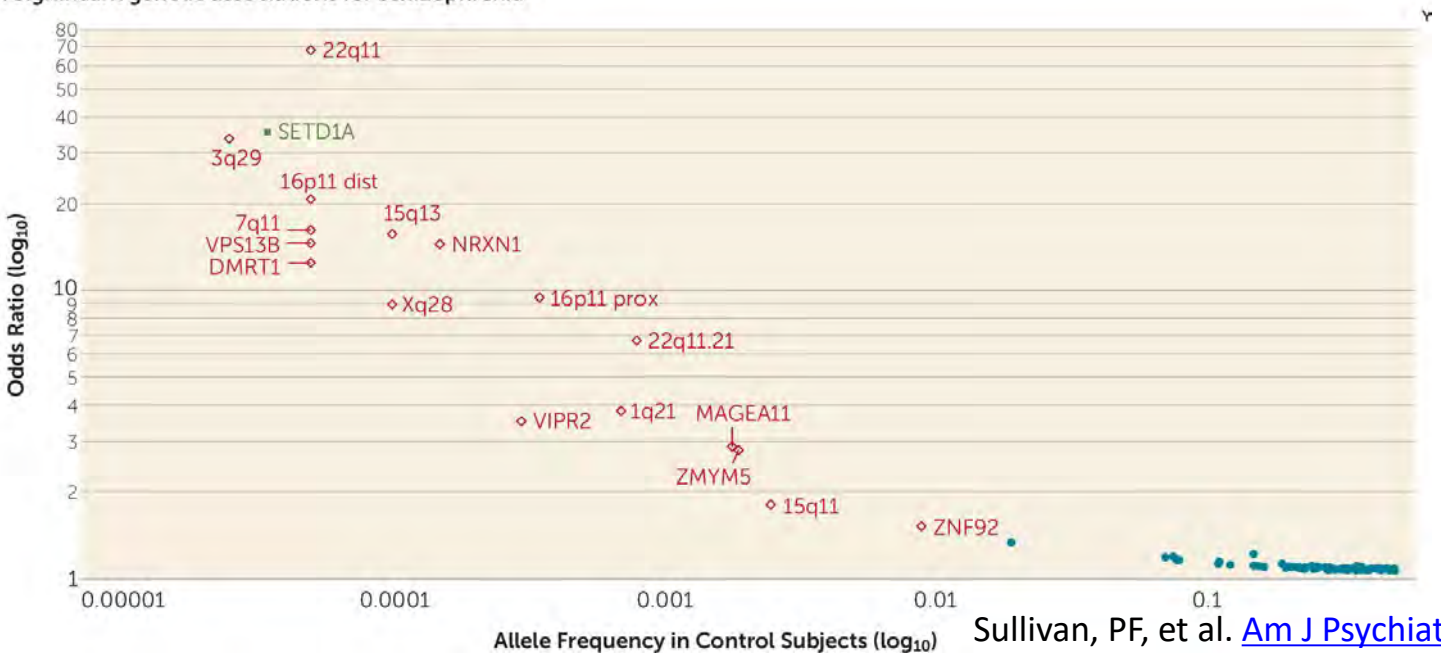
NMDA receptor subunit 2A



A. Genetic causes of severe psychiatric disorders[a]



B. Significant genetic associations for schizophrenia[b]



Sullivan, PF, et al. Am J Psychiatry. 2018 Jan 1; 175(1): 15–27.

DEVELOPMENTAL NEUROPSYCHIATRY RESEARCH CLINIC

Identify New Treatments & Outcome Measures for Children with Early psychosis and Psychosis risk

- **Genetic High Risk for schizophrenia**
  - Copy number variation (CNV) associated with Schizophrenia
- **Clinical High Risk for schizophrenia**
  - Prodrome, ages 7-18 years
  - Hallucinations, paranoia but retain insight
- **Early Psychosis**
  - Especially under age 14 at onset (VEOP)
  - Genetics-CMA & whole exome Sequencing looking for coding region mutations
  - Whole genome sequencing looking for non-coding region mutations
  - Long read sequencing – structural variation missed with above

- **Since 2011**
  - Over 500 children evaluated for early psychosis or high risk
  - 30% are ≤13 years old

Collaborations helping start parallel research clinics:

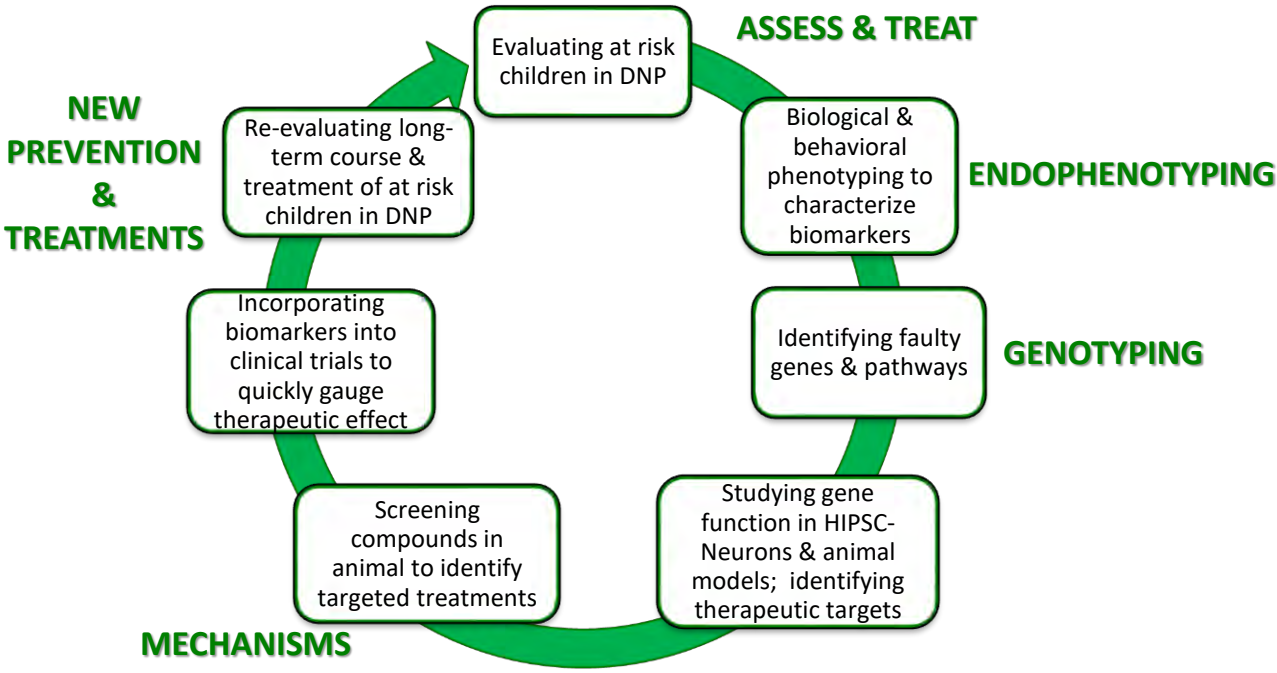
China: Shenzhen Kangning Hospital

Mexico: Instituto Nacional de Medicina Genómica (INMEGEN)

Developmental Neuropsychiatry Program Discovery Cycle

A Road Map for Translational Neuropsychiatric Research

- Growing practice in identification and treatment of childhood psychotic disorders
- >100 children/year evaluated in the Developmental Neuropsychiatry Clinic
- >30% confirmed with psychotic disorder diagnosis
- >50% under age 14 years—too young for any of the other early psychosis programs in the state



Genetic Sequencing Has Great Promise, But How to Get Started.

- BCH's "Gene Partnership" introduced me to Catherine Brownstein, PhD
- Catherine got The Manton Center for Orphan Diseases at BCH to support a "very early onset psychosis" (VEOP) cohort.

Manton Center For Orphan Disease Research

Alan Beggs, PhD
Director, Manton Center

Pankaj Agrawal, MD
Division of Newborn Med.

- Has approved IRB,
- infrastructure for consenting families,
- gathering (samples DNA, RNA, cells for iPSC creation) and
- banking these.

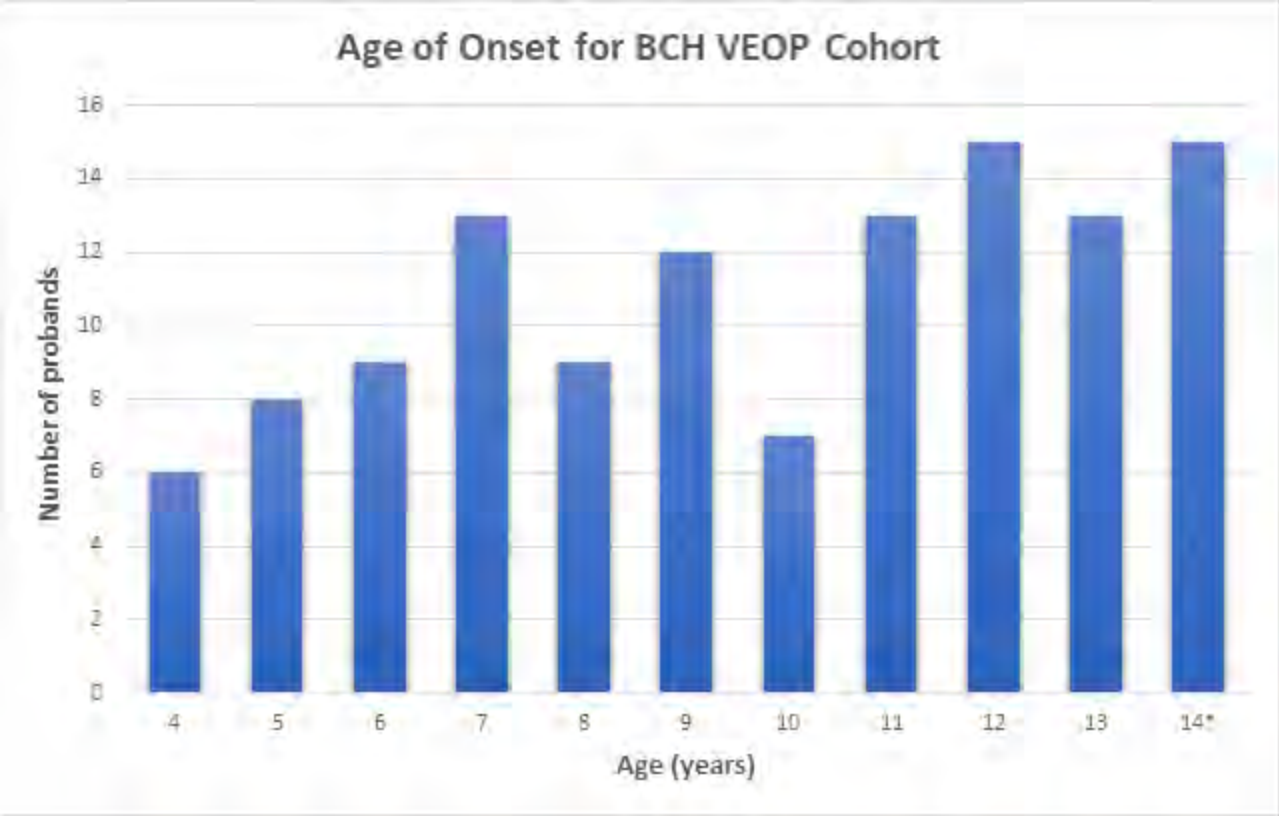
Very Early Onset Psychosis at BCH

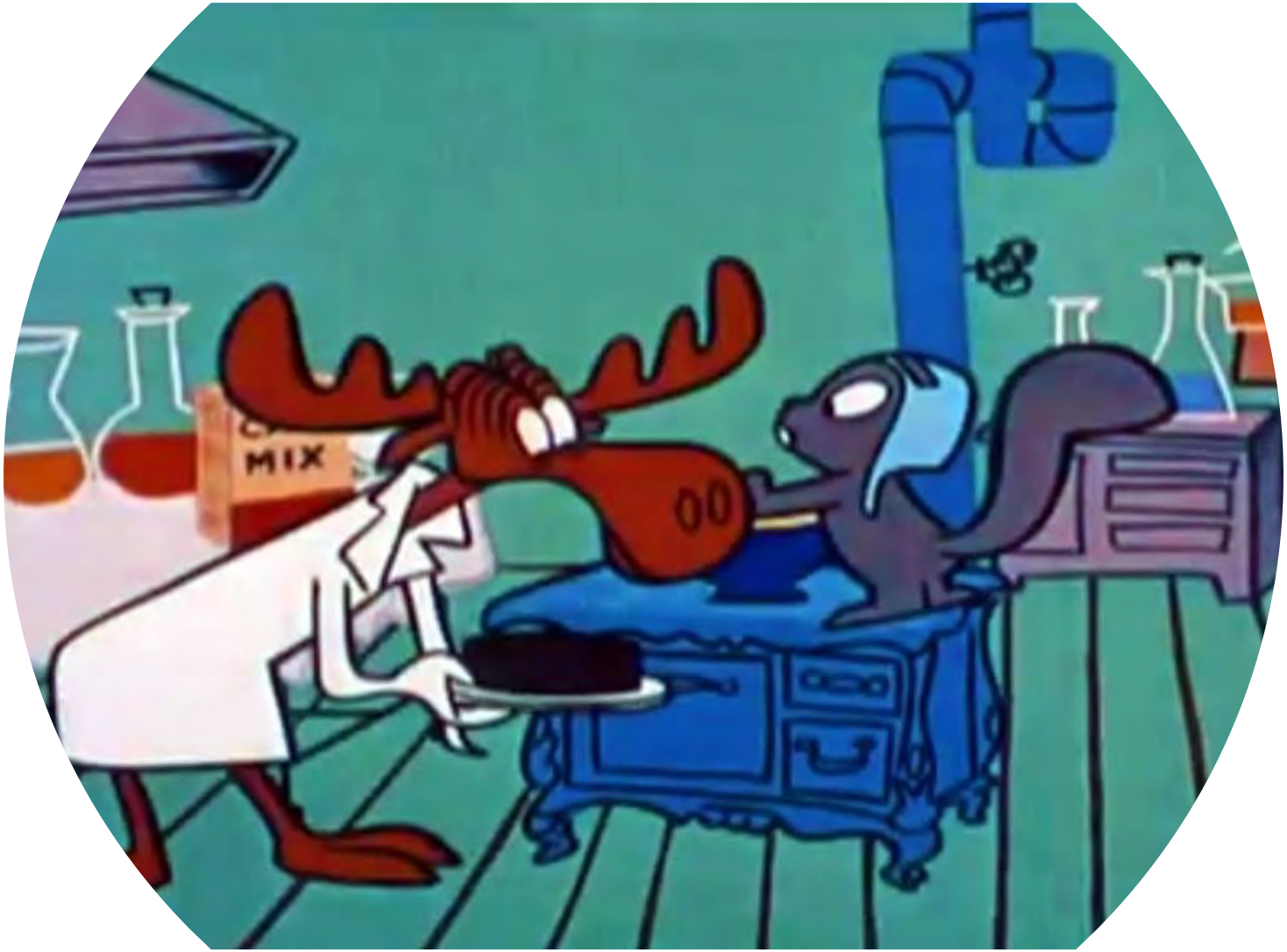
Identify New Genetic Variants, Treatments & Outcome Measures to Support Therapeutic Interventions

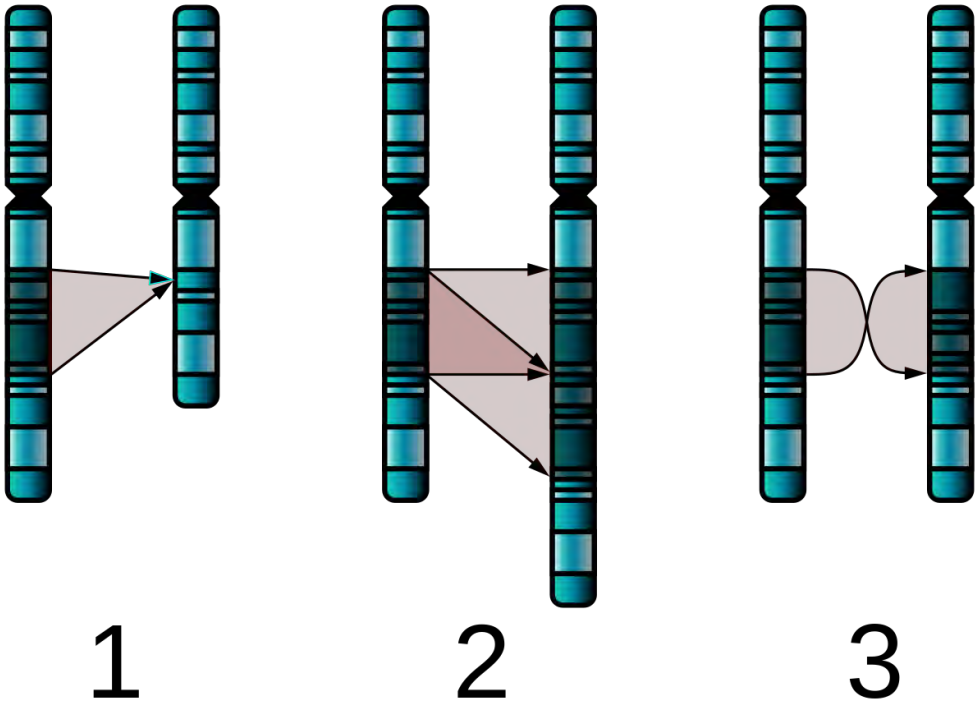
225 patients with early onset psychosis Enrolled .

Biological sample collection on 191 patients.

- We have Chormosomal Microarray data looking for copy number variants on 138 subjects a
- Whole exome sequencing on 151 patients.
- Whole Gemome sequencing on 43 patients
  - we have analyzed these genomes for repeat expansions and retrotransposon insertions.
- We banked samples from which iPSC can be created (skin cells or blood cells) for 102 EOP patients.


Age of Onset for BCH VEOP Cohort
Number of probands
16
14
12
10
8
6
4
2
0
4
5
6
7
8
9
10
11
12
13
14*
Age (years)


MIX



- Copy Number Variant (CNV)
- 1.Deletion
- 2. Duplication
- 3. Inversion or Translocation

BCH VEOP Cohort Compared To NIMH COS Cohort: Neurodevelopmental CNV Rate

	BCH VEOP	NIMH COS (Ahn et al., 2014)
# of probands	92	126
Neurodevelopmental CNVs from Ahn et al., 2014	11/92 probands (12.0%)	15/126 probands (11.9%)
Neurodevelopmental CNVs also on Psychiatric Genomics Consortium (PGC) significant CNV list (Marshall et al., 2017)	2 out of 7 CNVs (28.6%)	4 out of 10 CNVs (40.0%)

BCH VEOP Cohort Compared to Adult SZ Cohort

	BCH VEOP Cohort	Adult Schizophrenia Cohort (Bergen et al., 2018)	Controls (Bergen et al., 2018)
Schizophrenia CNVs	7 (7.6%)	407 (1.9%)	115 (0.57%)
No Schizophrenia CNVs	85	20,681	20,107
Totals	92	21,088	20,222

*The Fisher Exact test value is **p=0.0022** after comparing the BCH VEOP cohort to the Adult Schizophrenia population. The value is p**<0.00001** after comparing the BCH VEOP cohort to controls.

CNVs and Very Early Onset Psychosis (n=92)

- 12% of childhood onset schizophrenia patients have one of 46 CNVs (Ahn, 2014)
  - 12% in BCH VEOP probands
- 2% of adults with schizophrenia have one of 11 CNVs (Bergen, 2019)
  - 8% in BCH VEOP probands
- 11-12% of population cohort adolescents have a CNV>250kb (Huguet, 2018)
  - 24% in BCH VEOP probands

Unpublished results

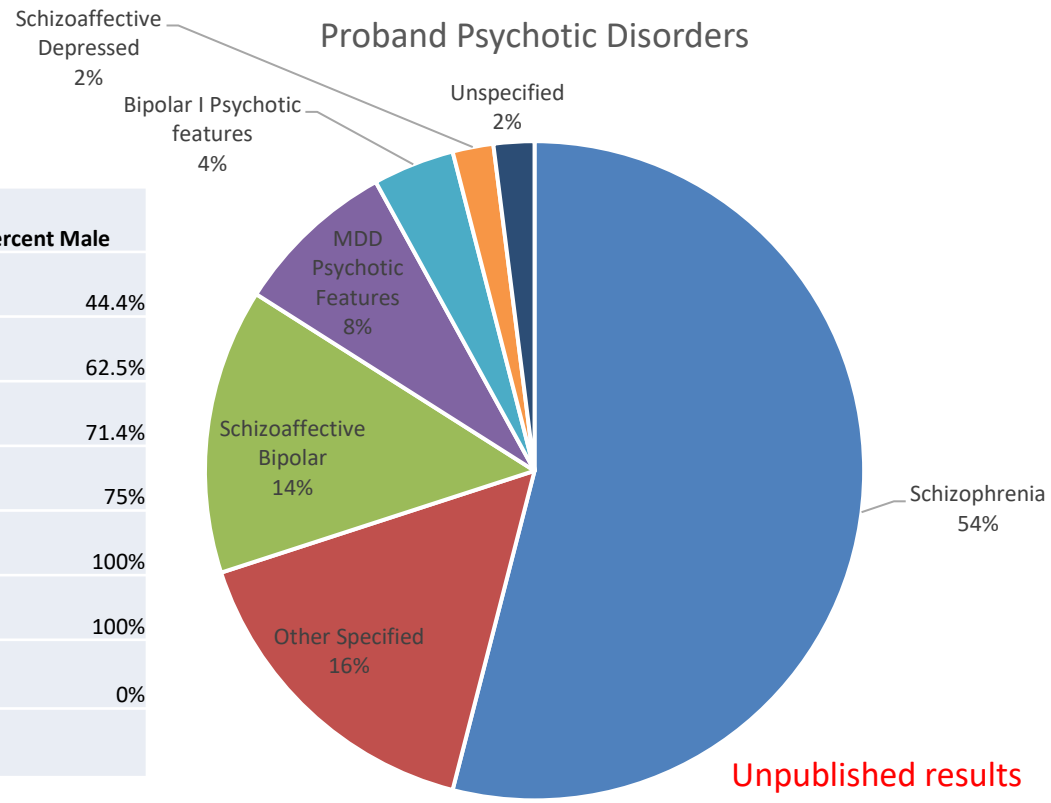
Early Psychosis Investigation Center (EPICenter)

- David Glahn, PhD – Director
- The EPICenter created 2019 integrates clinical, translational and basic research with clinical care for children and adolescents with early or very early onset psychosis (EOP) and their families.
- We will create a cohort of well characterized EOP probands and family members with extensive phenotyping and available biological samples to facilitate future research
- Our aims for the first two years include (1) recruiting and assessing 120 EOP probands; (2) conducting follow-up assessments with probands at 6-month intervals; and (3) assess 510 relatives of probands with identical phenotypic scales
- David Glahn enlisted his collaborators to help us compare rates of rare mutations in the BCH VEOP cohort to that in other large cohorts

David Glahn, PhD

Psychotic Disorders of Probands per SCID-5

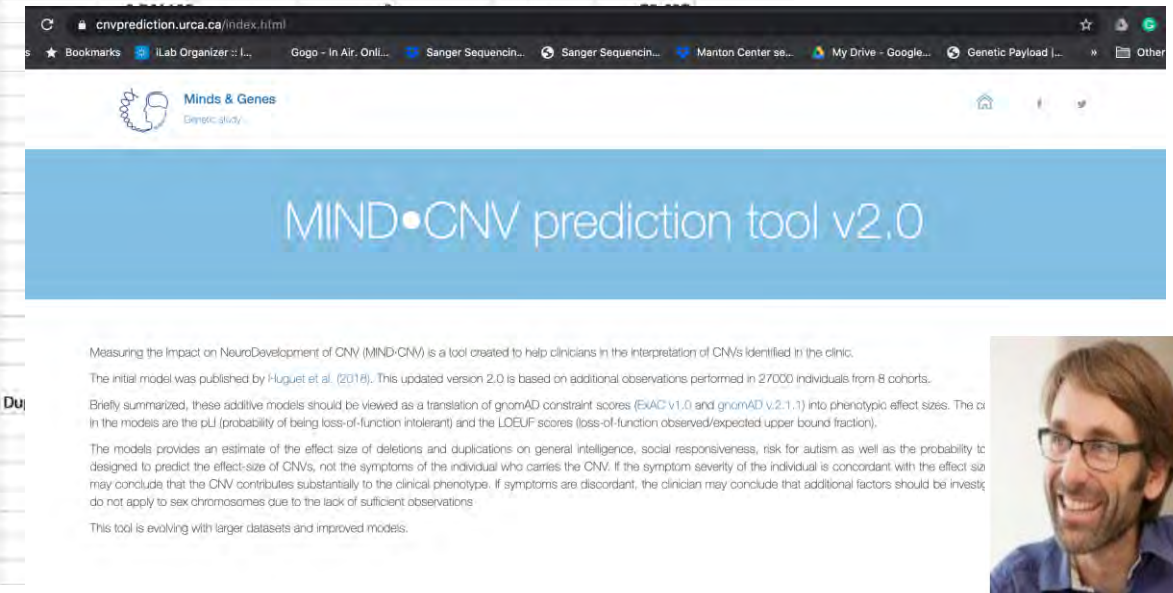
Psychotic Disorder	Percent of Sample	Count	Percent Male
Schizophrenia	54%	27	44.4%
Other Specified	16%	8	62.5%
Schizoaffective Bipolar	14%	7	71.4%
MDD Psychotic Features	8%	4	75%
Bipolar I Psychotic Features	4%	2	100%
Schizoaffective Depressed	2%	1	100%
Unspecified	2%	1	0%
Total	100%	50	



Unpublished results

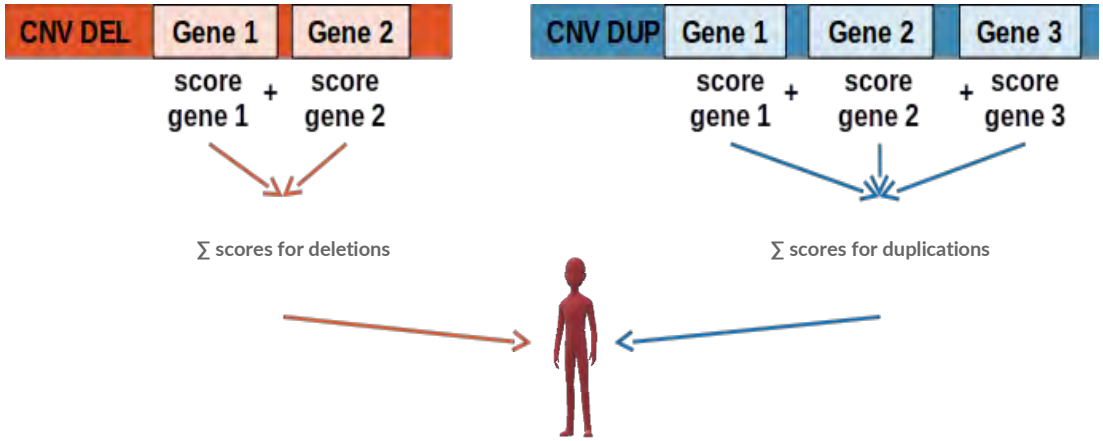
Comparison of CNVs to larger cohorts

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Aberration	Chr	Cytoban	Start	Stop	#Probe	Amplificat	Gain	Loss	Deletio	pval	Gene Nan	size	Notes
2	0529-01	normal											0	
3	0567-01	chr10	q21.1	55538186	56676426		Duplication (4 copies)		0	0		PCDH15	1138.24	
4	0587-01	chr22	q11.22 - q11	23301480	24995964			0.430054		0		RTDR1, GNA	1694.484	
5	0590-01	chr7	q31.33	126457718	126536646				-0.888408	0		GRM8	78.928	
6	0630-01	chr8	q23.3	112317029	112392656									
7	0641-01	normal												
8	0642-01	chr16	p13.11	14897761	16276117									
9	0642-01	chX	q28	152955334	152961664									
10	0643-01	chr7	q34	141921825	142017021									
11	0644-01	chr6	q12	63543898	64025806									
12	0644-01	chr19	q13.42 - q13	56238724	56515068									
13	0647-01	chr6	p22.2	25419199	26457539									
14	0744-01	chr20	q13.2	53396513	53490076									
15	0806-01	chr4	q28.3	134943258	135195162									
16	0806-01	chr6	q24.2	144328804	144329441									
17	0806-01	chr9	q33.3	129373899	129379296									
18	0806-01	chr10	q26.3	135270324	135377390									
19	0806-01	chr16	q24.1	86600972	86602522									
20	0826-01	chr14	q31.1	79943567	80411918									
21	0838-01	chr7	p21.3	8201938	8520075									
22	0840-01	chr8	q24	125977314	126,458,921		Du							
23	0841-01	chr3	p26.3	270300	283433									
24	0841-01	chr9	q33.3	129373899	129379296									
25	0841-01	chr17	q25.3	79792272	79953020									
26	0841-01	chrX	p11.1	58543266	58544060									
27	0847-01	chr3	p14.2	60217711	60395355									
28	0849-01	chrX	p22.33	3313959	3912069									
29	0849-01	chrX	p22.2	15950430	16439709									



Sebastien Jacquemont, MD

Genetic Enrichment Analysis: How important is a particular CNV?



Probability of being loss-of-function intolerant (pLI)

Analysis of protein-coding genetic variation in 60,706 humans

18 AUGUST 2016 | VOL 536 | NATURE | 285

Measuring intolerance to mutation in human genetics

Zachary L. Fuller[1*], Jeremy J. Berg[1], Hakhamanesh Mostafavi[1], Guy Sella[1,2,3] and Molly Przeworski[1,2,3]

NATURE GENETICS | VOL 51 | MAY 2019 | 772–776 | www.nature.com/naturegenetics

Measuring and Estimating the Effect Sizes of Copy Number Variants on General Intelligence in Community-Based Samples

Guillaume Huguet, PhD; Catherine Schramm, PhD; Elise Douard, MSc; Lai Jiang, PhD; Aurélie Labbe, PhD; Frédérique Tihy, PhD; Géraldine Mathonnet, PhD; Sonia Nizard, MD; Emmanuelle Lemyre, MD; Alexandre Mathieu, MSc; Jean-Baptiste Poline, PhD; Eva Loth, PhD; Roberto Toro, PhD; Gunter Schumann, PhD; Patricia Conrod, PhD; Zdenka Pausova, MD; Celia Greenwood, PhD; Tomas Paus, MD, PhD; Thomas Bourgeron, PhD; Sébastien Jacquemont, MD; for the IMAGEN Consortium

JAMA Psychiatry May 2018 Volume 75, Number 5

Over representation of functional mutations in EOP Deletions

		Deletions		Duplications	
Contrast	Scores	OR [95%CI]	p-value	OR [95%CI]	p-value
EOP vs. Unselected	N genes	1.12 [1.03-1.18]	**$7x10^{-4}$**	1.02 [0.94-1.07]	0.50
	pLI	1.88 [1.37-2.59]	**$1x10^{-4}$**	1.23 [0.89-1.46]	0.07
ASD vs. Unselected	N genes	1.19 [1.18-1.21]	**$4x10^{-102}$**	1.04 [1.03-1.05]	**$4x10^{-23}$**
	pLI	3.11 [2.75-3.54]	**$2x10^{-70}$**	1.26 [1.21-1.32]	**$1x10^{-24}$**
EOP vs. ASD	N genes	0.96 [0.88-1.02]	0.31	0.98 [0.91-1.03]	0.59
	pLI	1.05 [0.78-1.23]	0.65	0.98 [0.70-1.13]	0.84

EOP n=139; ASD n= 13,590; Unselected n=16,515

Significant results (p<0.025) are bolded

Unpublished results

Schizophrenia & Neurodevelopmentally Implicated CNVs in BCH VEOP Cohort (Schizophrenia CNVs are marked *, genome wide significant Schizophrenia CNV are marked **)

Schizophrenia & Neurodevelopmental CNVs	BCH VEOP Cohort Probands	Potential System Dysfunction/Conditions to Monitor:
1q21.1 Duplication*	**1468-01,** 1464-01	Heart disease; Epilepsy; Cataracts; Neuroblastoma
2q13 Duplication	**1110-01**, 1231-01	Liver disorder; Kidney; Heart disease (CHD); Hypotonia; Cranial dysmorphisms
15q11.2 Deletion*	1325-01	Epilepsy
16p11.2 Duplication**	**1384-01**	Kidneys
16p13.11 Duplication*	**0642-01**, 1104-01	Heart disease; Skeletal abnormalities; Vision; Epilepsy
16p13.11 Deletion	**0602-01, 1125-01**	Epilepsy
22q11.2 Deletion**	**1430-01**	Heart disease; Immune; Pulmonary; Kidney; Gastrointestinal

De novos in EOP

Avg pLi of de novo			
Category	EOP (N=32)	Control patient (N=33)	Mixed neurologic phenotype genetic cases (N=100)
All chromosomes	0.35	0.28	0.42
X chromosome only	0.86	0.3	0.77
chromosomes without X	0.33	0.28	0.38

Comparison	t-test
All chromosomes	
EOP vs control	t=2.68, df=1065, p=0.007
EOP vs mixed genetic cases	t=-2.68, df=1070, p=0.007
X chromosome only	
EOP vs control	t= +5.39, df= 24, p<.0001
EOP vs mixed genetic cases	t= +0.84, df=97, p=0.403
chromosomes without X	
EOP vs control	t=+1.96m df=1039, p=0.05
EOP vs mixed genetic cases	t= -1.69, df=971, p= 0.09

Unpublished results

Very Early Onset Psychosis: High Rate of Rare Mutations, Trauma, Suicidality

- Schizophrenia associated CNV rate ~4 x higher than in adult onset schizophrenia ($p<0.001$)
- High rate of Traumatic Events
- High rate of suicidality
- iPSC brain cells as bridge to understanding interaction

Early Intervention IN PSYCHIATRY

Received: 6 November 2017 | Revised: 9 January 2018 | Accepted: 4 February 2018

DOI: 10.1111/eip.12565

WILEY

ORIGINAL ARTICLE

Potentially traumatic events in youth with and at clinical high risk for psychosis

Nicholas Morelli[1] | Jason Fogler[1] | Sahil Tembulkar[1,2] | Kelsey Graber[1] | Sarah H. Lincoln[3,4] | Michelle Bosquet Enlow[1] | Joseph Gonzalez-Heydrich[1,3] | Eugene J. D'Angelo[1,3]

ELSEVIER

Comprehensive Psychiatry

Volume 78, October 2017, Pages 31-37

Suicidal behaviors and their relationship with psychotic-like symptoms in children and adolescents at clinical high risk for psychosis

Eugene J. D'Angelo [a, b], Sarah Hope Lincoln [b, c], Nicholas Morelli [a], Kelsey Graber [a], Sahil Tembulkar [a], Joseph Gonzalez-Heydrich [a, b]

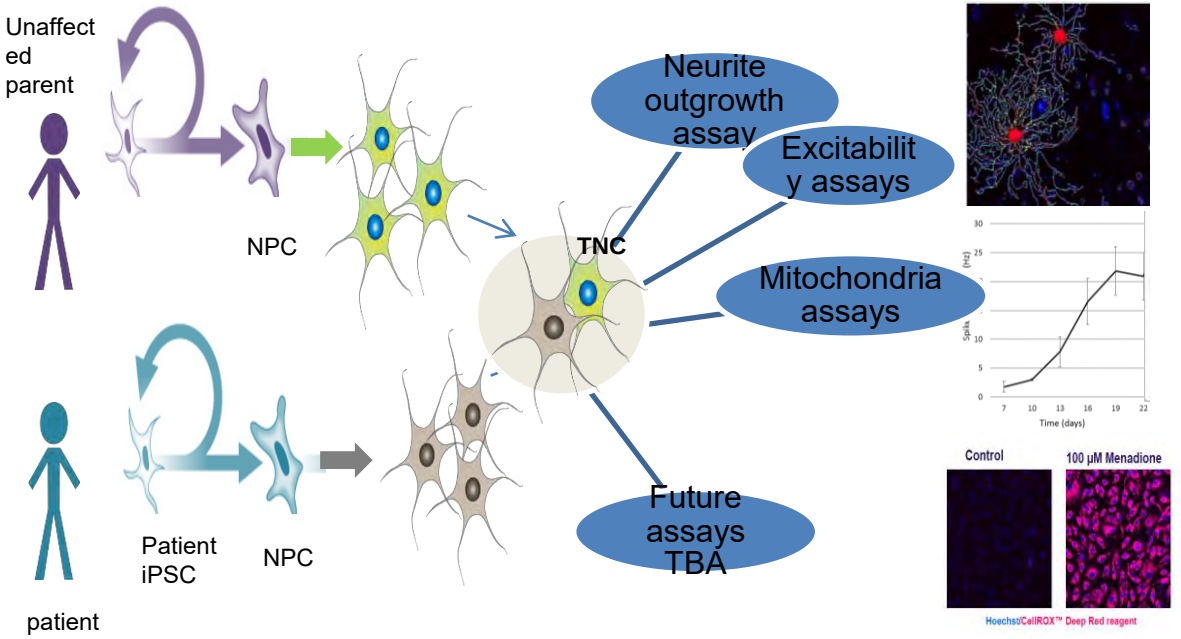
Clinical Take Away Points

- VEOP have a higher rate of CNV than adult onset schizophrenia (**p=0.0022**).
- The majority (~60%) of CNVs identified in VEOP are not accepted SZ-associated CNVs.
- All of the CNVs in the BCH VEOP cohort indicated the need for medical monitoring.
- **Routine testing for CNV and vigilance for trauma and suicidality is indicated for patients presenting with VEOP.**

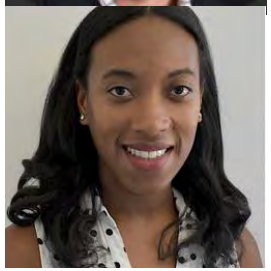
Cellular phenotyping via the Translational Neuroscience Center's Human Neuron Core

Director Mustafa Sahin, MD PhD

Assistant Director
Elizabeth Buttermore, PhD

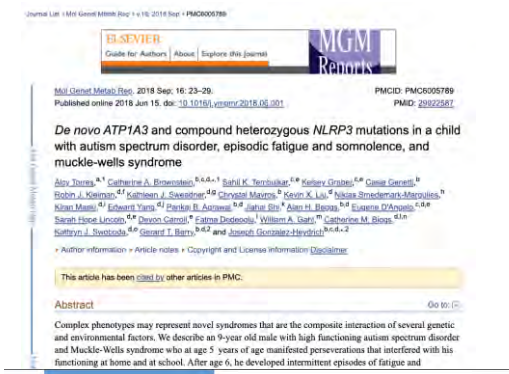


Infrastructure for gene discovery and functional characterization



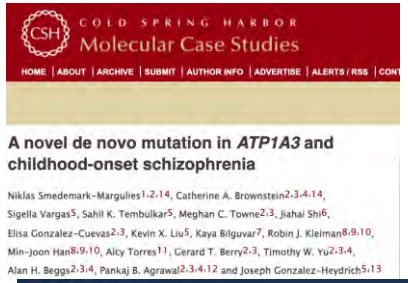
Research programs on:

- *ATP1A3*
- *TRRAP*
- RCL1
- ZMYM2
- ATP1B1
- FOXP1
- ASXL3
- CMIP
- 16p13.11 del/dup
- KCNQ3



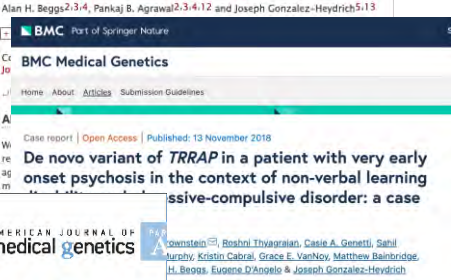
MGM Reports

De novo ATP1A3 and compound heterozygous *NLRP3* mutations in a child with autism spectrum disorder, episodic fatigue and somnolence, and muckle-wells syndrome



COLD SPRING HARBOR Molecular Case Studies

A novel de novo mutation in *ATP1A3* and childhood-onset schizophrenia

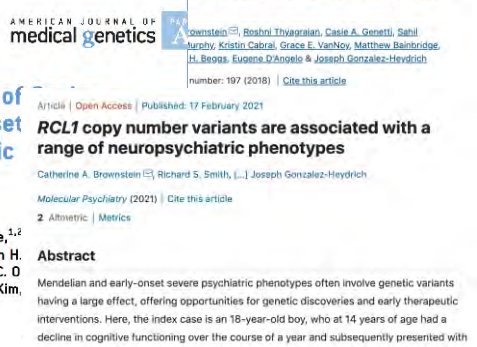


BMC Medical Genetics

De novo variant of *TRRAP* in a patient with very early onset psychosis in the context of non-verbal learning

ORIGINAL ARTICLE

Overlapping 16p13.11 Deletion and Gain of Variations Associated With Childhood Onset Psychosis Include Genes With Mechanistic Implications for Autism Associated Pathways: Two Case Reports



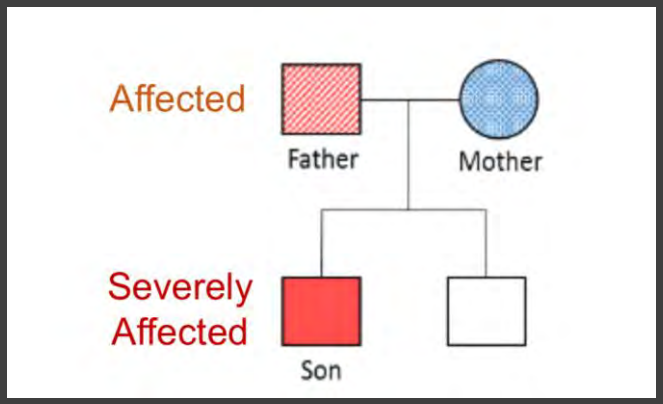
AMERICAN JOURNAL OF medical genetics

RCL1 copy number variants are associated with a range of neuropsychiatric phenotypes

Abstract

Mendelian and early-onset severe psychiatric phenotypes often involve genetic variants having a large effect, offering opportunities for genetic discoveries and early therapeutic interventions. Here, the index case is an 18-year-old boy, who at 14 years of age had a decline in cognitive functioning over the course of a year and subsequently presented with

Case 1: Investigating RCL1



- 14 year old boy
  - Star athlete, good student
  - Over the course of four months, descended into catatonia in with auditory and visual hallucinations, paranoia, aggression, mood dysregulation, and disorganized thoughts.
  - Poor motor coordination
  - CSF found increased protein concentrations, encephalitis test negative
  - Currently responding to Clozapine
  - Child compound het for CAPN1 (Spastic paraplegia 76, autosomal recessive)- doesn't fit phenotype
  - Family history: Paternal aunt has had multiple psychiatric hospitalizations, father has behavioral and anger problems

RCL1 as a candidate EOP gene

Variant Information

Gene: RCL1
Variant: 9:4827019C>T
Strand: +

Ensembl Transcript Data

ENST00000381750 4:c.370C>T, p.Gln124Ter, 370/1122

Mutation Type

Annovar RefSeq: Stopgain

Involved in production of ribosomal RNA

Original Article OPEN Published: 21 March 2017

A rare missense variant in *RCL1* segregates with depression in extended families

N Amin, F M S de Vrij, M Baghdadi, R W W Brouwer, J G J van Rooij, O Jovanova, A G Uitterlinden, A Hofman, H L A Janssen, S Darwish Murad, R Kraaij, J Stedehouder, M C G N van den Hout, J M Kros, W F J van IJcken, H Tiemeier, S A Kushner & C M van Duijn

Molecular Psychiatry **23**, 1120–1126 (2018) | Download Citation

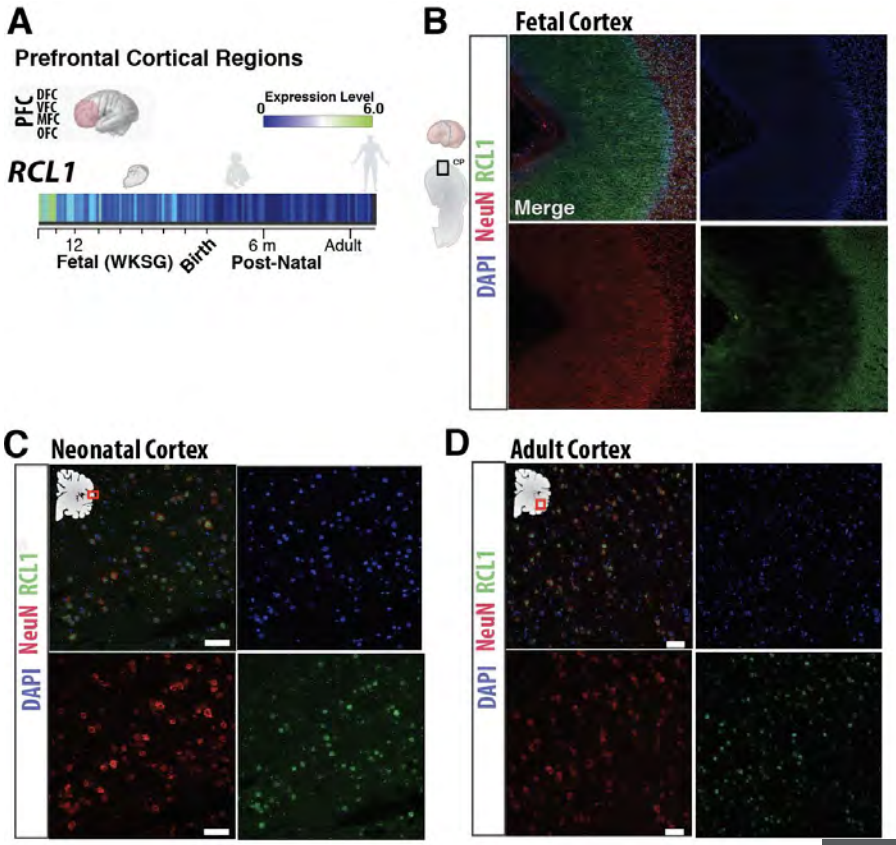
Investigating RCL1

***RCL1* expression is highest during fetal and early post-natal human brain development**

A) Bulk transcriptome analysis of prefrontal cortical regions revealed *RCL1* transcripts are enriched during fetal gestational weeks and decrease postnatally.

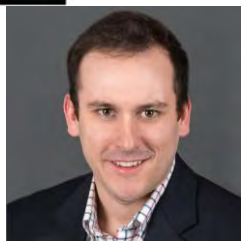
B-D) Immunohistochemistry of an anti-body probe against RCL1 and corresponding confocal fluorescence imaging of fetal, 9-month-old, and adult human cortex.

Cell type specific neuronal marker NeuN and nuclei marker (DAPI) show *RCL1* present in both neurons and non-neuronal cell types



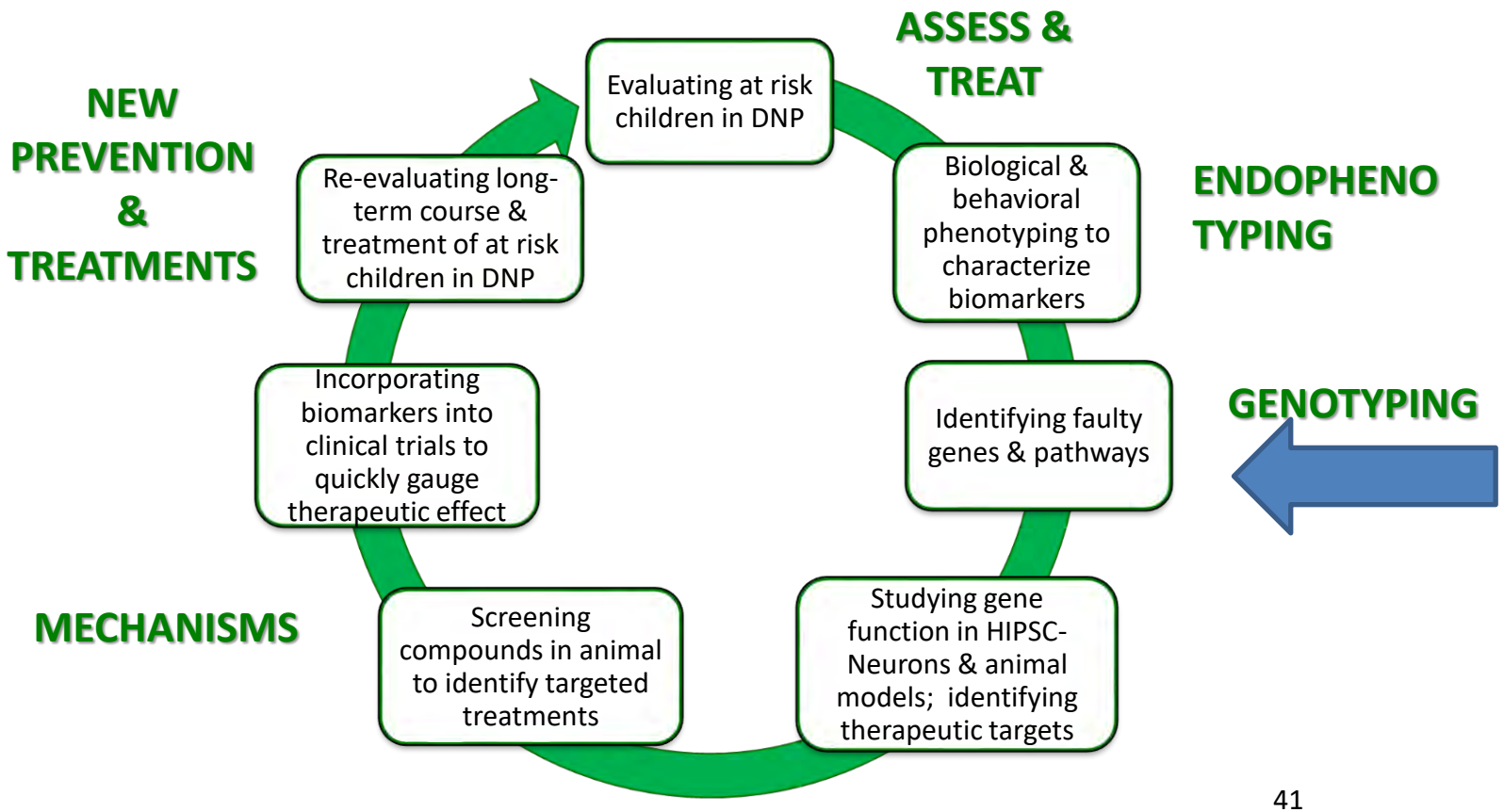
Brownstein, C.A., Smith, R.S., Rodan, L.H. *et al. RCL1* copy number variants are associated with a range of neuropsychiatric phenotypes. *Mol Psychiatry* **26,** 1706–1718 (2021). https://doi.org/10.1038/s41380-021-01035

Richard Smith, PhD
Instructor HMS



Developmental Neuropsychiatry Program Discovery Cycle

A Road Map for Translational Neuropsychiatric Research



Case 2: TRRAP Variant in Childhood Onset Psychosis

- The proband is a 12-year old Caucasian boy who first presented to neurology at age 5 for concerns about school performance
- Evaluation showed delays in gross motor skills and some behavioral concerns but above average intelligence
- At age 7, concern for ADHD and compulsive behaviors (severity decreased over time) and mild social delays.
- At age 9, proband referred for psychotherapy evaluation for paranoia and hallucinations. Reports that he started hearing voices at age 7.
- Diagnosed with major depression with psychotic features in the context of NVLD (non-verbal learning disability) and OCD
  - Fear of being hunted, voices listening to him, mortality
  - Treated with Fluoxetine and responded

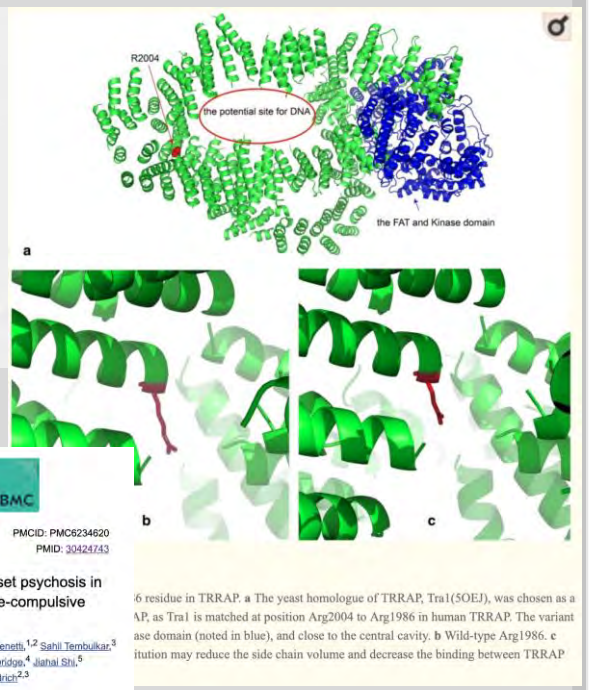
TRRAP: involved in histone acetyltransferase activity (HAT), epigenetic transcription activation, DNA repair


Variant Information

Gene: TRRAP
Variant: 7:98553863G>A
Strand: +

Refseq Transcripts
XM_005250617.1:c.6032G>A
NM_001244580.1:c.6011G>A
NM_003496.3:c.5957G>A
XM_005250618.1:c.6011G>A
XM_005250619.1:c.5957G>A

Ensembl Transcript Data
ENST00000359863.4:c.6011G>A, p.Arg2004Gln, 6011/11580, ENST00000355540.3:c.5957G>A, p.Arg1986Gln, 5957/11493



6 residue in TRRAP. a The yeast homologue of TRRAP, Tra1(5OEJ), was chosen as a AP, as Tra1 is matched at position Arg2004 to Arg1986 in human TRRAP. The variant ase domain (noted in blue), and close to the central cavity. b Wild-type Arg1986. c itution may reduce the side chain volume and decrease the binding between TRRAP


BMC Medical Genetics

BMC Med Genet. 2018; 19: 197.
Published online 2018 Nov 13. doi: 10.1186/s12881-018-0711-9
PMCID: PMC6234620
PMID: 30424743

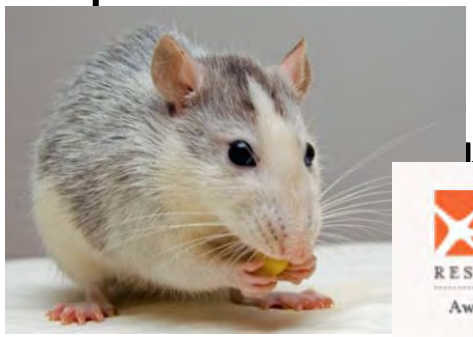
De novo variant of *TRRAP* in a patient with very early onset psychosis in the context of non-verbal learning disability and obsessive-compulsive disorder: a case report

Chrystal F. Mavros,[#1,2] Catherine A. Brownstein,[#1,2] Roshni Thyagrajan,[1,2] Casie A. Genetti,[1,2] Sahil Tembulkar,[3] Kelsey Graber,[3] Quinn Murphy,[1,2] Kristin Cabral,[1,2] Grace E. VanNoy,[1,2] Matthew Bainbridge,[4] Jiahai Shi,[5] Pankaj B. Agrawal,[1,2] Alan H. Beggs,[1,2] Eugene D'Angelo,[3] and Joseph Gonzalez-Heydrich[2,3]

Author information · Article notes · Copyright and License information Disclaimer

Mouse model

- Using CRISPR/Cas9 to create a mouse model with the exact amino acid change as the patient

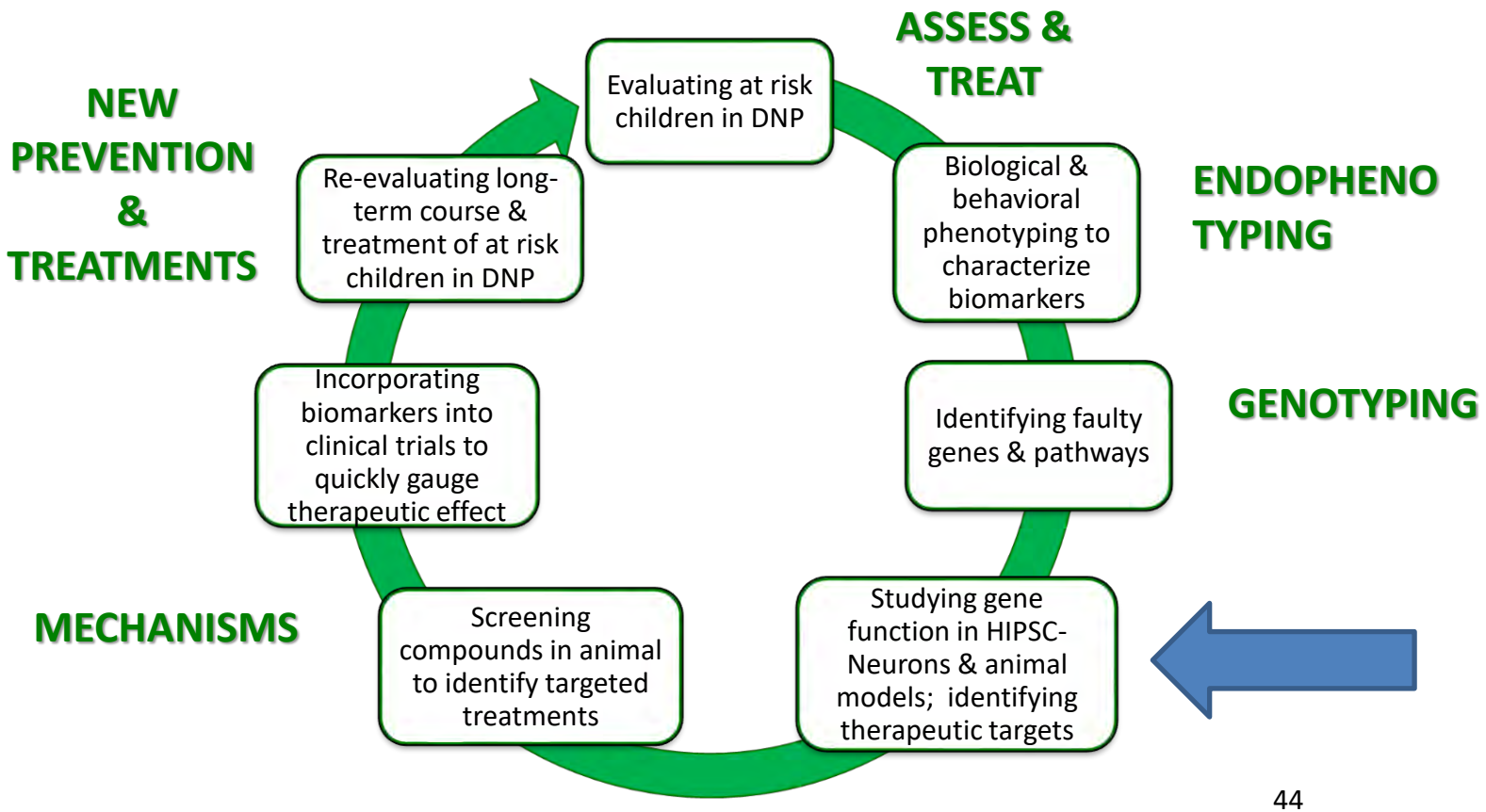


screening

"Sorry, kiddo. Your old man has to work so you can go to the best drug trials in the country."

Developmental Neuropsychiatry Program Discovery Cycle

A Road Map for Translational Neuropsychiatric Research



Role for the Na^+/K^+ ATPase subunit (*ATP1A3*) in Childhood Onset Schizophrenia and Beyond

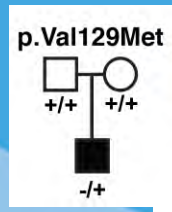
Proband

-Diagnosed at age 3 with selective mutism and depression

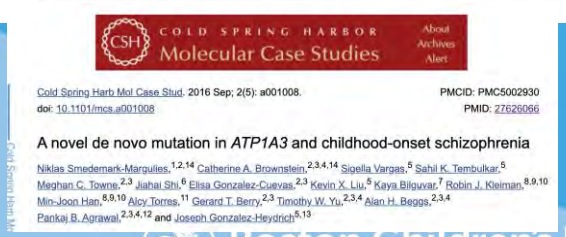
-Described as having mood swings, lack of emotional control, and severe anxiety

-Severe self-injurious behaviors

-Presented with command hallucinations and behavioral worsening meeting full DSM 5 criteria for COS at 6 years of age (now 10 y.o.)



Gene	Chr	HGVS DNA	HGVS protein	Variant type	Variant allele fraction	SIFT score	PolyPhen-2 score	Genotype	ExAC MAF	ExAC constraint z-score
ATP1A3	19	c.385G>A	p.V129M	SNV	42% of 40 reads	0	0.999	Het	0%	7.38


COLD SPRING HARBOR Molecular Case Studies

Cold Spring Harb Mol Case Stud. 2016 Sep; 2(5): a001008. doi: 10.1101/mcs.a001008 PMCID: PMC5002930 PMID: 27626066

A novel de novo mutation in *ATP1A3* and childhood-onset schizophrenia

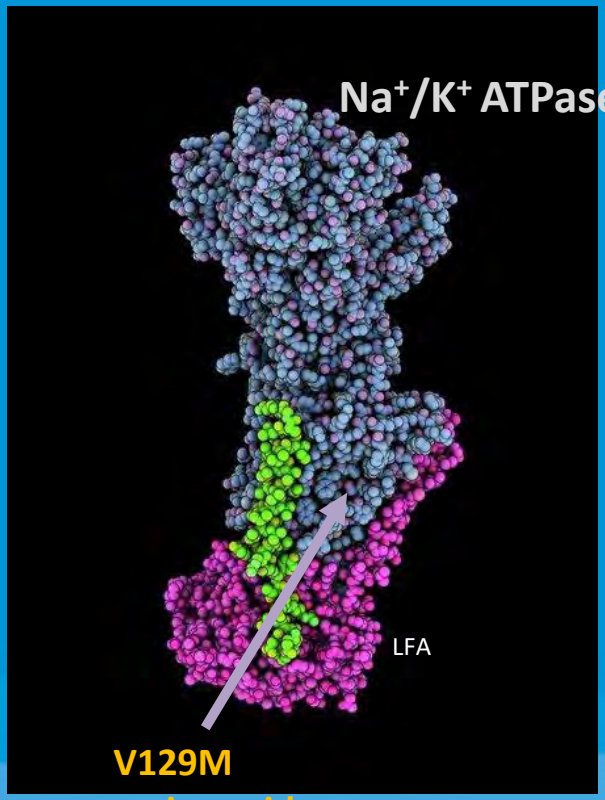
Niklas Smedemark-Margulies,[1,2,14] Catherine A. Brownstein,[2,3,4,14] Sigella Vargas,[5] Sahil K. Tembulkar,[5] Meghan C. Towne,[2,3] Jiahai Shi,[6] Elisa Gonzalez-Cuevas,[2,3] Kevin X. Liu,[5] Kaya Bilguvar,[7] Robin J. Kleiman,[8,9,10] Min-Joon Han,[8,9,10] Alcy Torres,[11] Gerard T. Berry,[2,3] Timothy W. Yu,[2,3,4] Alan H. Beggs,[2,3,4] Pankaj B. Agrawal,[2,3,4,12] and Joseph Gonzalez-Heydrich[5,13]


ELSEVIER MGM Reports

Mol Genet Metab Rep. 2018 Sep; 16: 23–29. Published online 2018 Jun 15. doi: 10.1016/j.ymgmr.2018.06.001 PMCID: PMC6005789 PMID: 29922587

De novo ATP1A3 and compound heterozygous *NLRP3* mutations in a child with autism spectrum disorder, episodic fatigue and somnolence, and muckle-wells syndrome

Alcy Torres,[a,1] Catherine A. Brownstein,[b,c,d,1] Sahil K. Tembulkar,[c,e] Kelsey Graber,[c,e] Cassie Genetti,[b] Robin J. Kleiman,[d,f] Kathleen J. Sweadner,[d,g] Chrystal Mavros,[h] Kevin X. Liu,[c] Niklas Smedemark-Margulies,[h] Kiran Maski,[d,i] Edward Yang,[d,i] Pankaj B. Agrawal,[b,d] Jiahai Shi,[h] Alan H. Beggs,[b,d] Eugene D'Angelo,[c,d,e] Sarah Hope Lincoln,[d,e] Devon Carroll,[e] Fatma Dedeoglu,[f] William A. Gahl,[m] Catherine M. Biggs,[d,l,n] Kathryn J. Swoboda,[d,n] Gerard T. Berry,[b,d,2] and Joseph Gonzalez-Heydrich[b,c,d,,2]



Boston Childrens Hospital
Until every child is well

HARVARD MEDICAL SCHOOL
TEACHING HOSPITAL

Patient #1: Genetic Finding

- **WES identified a *de novo* missense change in ATP1A3**: NM_152296.4:c.385G>A and **p.V129M** (hg19).
  - This variant has not been previously described.
  - The gene is highly conserved and the variant is predicted to damage the protein's function.
- The *ATP1A3* gene codes for neuron specific isoform 3 of the alpha subunit of the Na+/K+-ATPase (NKA) complex which is expressed in GABA projection neurons of basal ganglia.
- ATP1a3 mutations are linked to Alternating Hemiplegia of Childhood, Rapid Dystonia Parkinsonism, and CAPOS syndrome (Brashear et al., 2008; Ozelius, 2012; H Rosewich et al., 2014; Sweney, Newcomb, & Swoboda, 2015).
- **3 additional unrelated cases with rare ATP1a3 mutations and COS identified subsequently** (Chaudette et al., 2018)

ATP1A3 in neuropsychiatric disorders

PMC full text: Lancet Neurol. Author manuscript; available in PMC 2015 May 1.
Published in final edited form as:
Lancet Neurol. 2014 May; 13(5): 503–514.
doi: 10.1016/S1474-4422(14)70011-0
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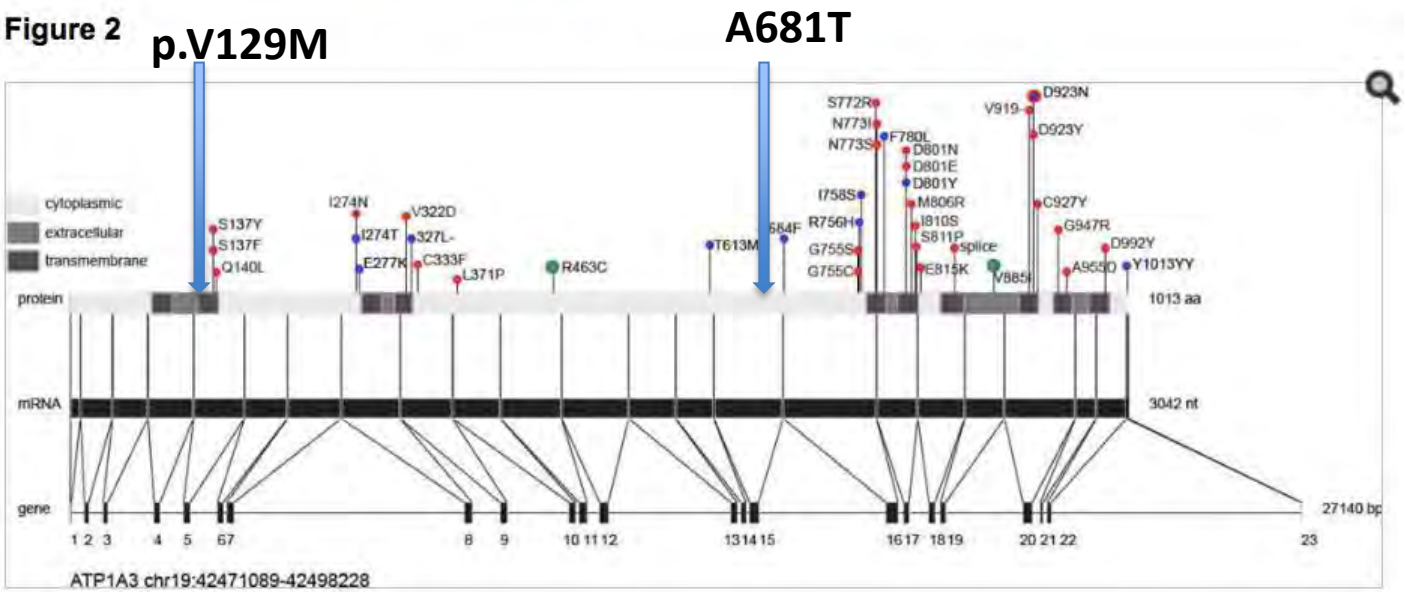
<< Prev Figure 2 Next >>

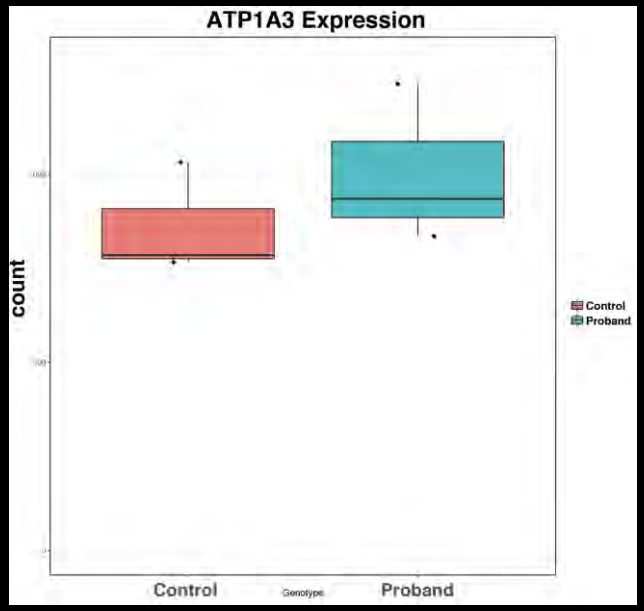
Figure 2



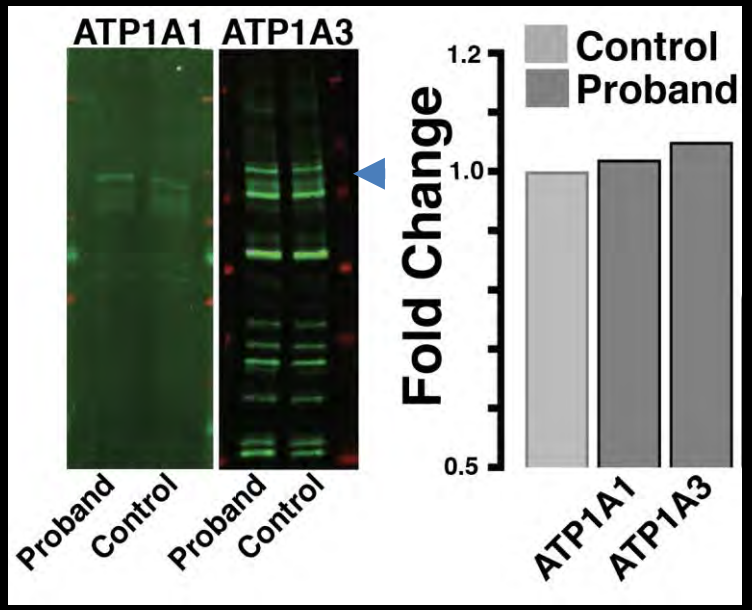
Schematic depicting the location of AHC-causing (red dots) and RDP-causing (blue dots) mutations in *ATP1A3*, mRNA and protein. The one mutation shared between disease phenotypes is located at D923N (blue dot with a red dot inside). Two rare polymorphisms identified in the general population are indicated by the green dots. Amino acid modifications are provided to the right of the dots.

ATP1A3 level is comparable between proband and control neurons

RNA

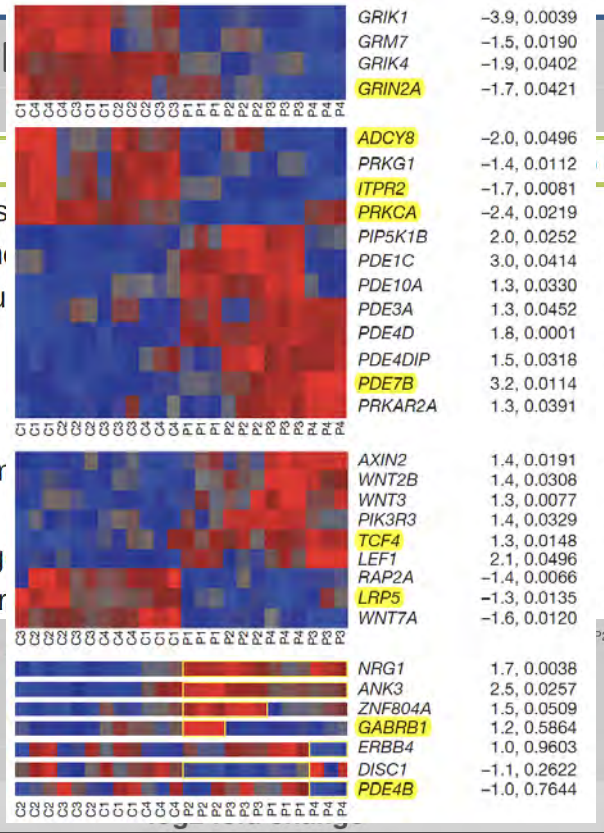


LI-COR Western



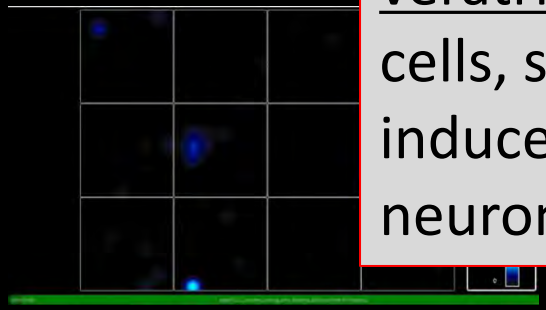
Proband is enriched for pathways differentially enriched in SCZ lines

Category	ID		BgRatio	pvalue
DO	DOID:0060040	pervas	197/7649	1.06466457404575e-06
DO	DOID:0060037	developm	365/7649	1.55380755847366e-06
DO	DOID:0060041	au	187/7649	3.02746235501074e-06
DO	DOID:12849		187/7649	3.02746235501074e-06
DO	DOID:543		42/7649	8.90410782787124e-06
DO	DOID:3312		156/7649	5.96958561144847e-05
DO	DOID:2621	autonom	375/7649	7.66201439835284e-05
DO	DOID:769		375/7649	7.66201439835284e-05
DO	DOID:1342	cong	19/7649	0.000117320158906067
DO	DOID:1192	peripher	396/7649	0.00013590141296756



Brennard et al, 2011

Spontanous and evoked spiking activity unchanged between patient & Control

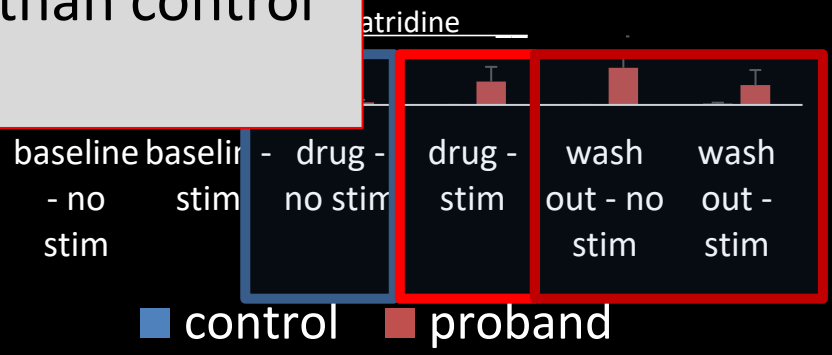


PATCH CLAMP VALIDATION

COS neurons were more resistant to veratridine challenge than controls cells, showing smaller veratridine induced depolarization than control neurons.

Action Potential bursts: proband recovers from Veratridine treatment better than proband

Veratridine- Blocks sodium channel inactivation (more excitability)

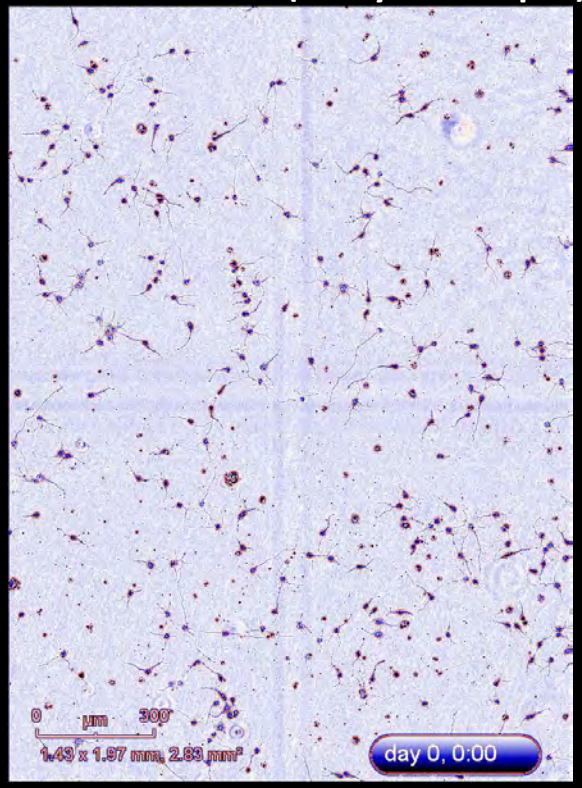


-Mean firing NOT changed between proband and control

-Bursts per well NOT changed between proband and control

Live cell tracking of differentiated COS neurons

Proband neurons (3 Day time-lapse)



Phenotype Testing	Effect (N=2 clones, 2 differentiations)
Cell Viability / Short-term survival	Increased 60% in Proband
Neurite Length	Mild Decrease (large variability)
Neurite Branching	No Change (large variability)
Cell Body Size	No Change

Proband neurons has increased rates of survival post plating

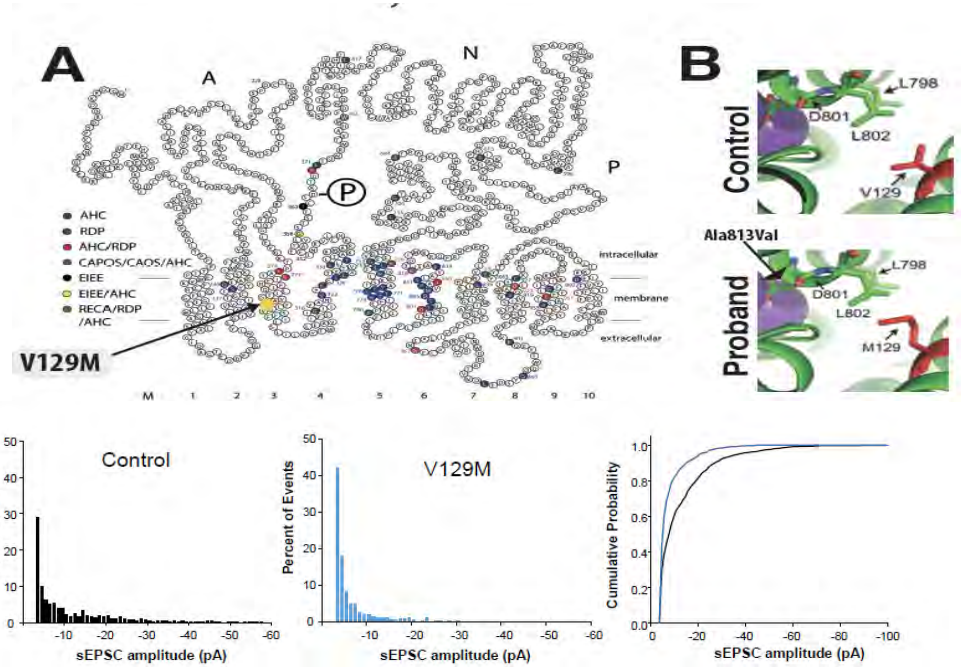
Front Genet. 2019; 10: 1137.
Published online 2019 Dec 18. doi: 10.3389/fgene.2019.01137
PMCID: PMC6930680
PMID: 31921276

Childhood-Onset Schizophrenia: A Systematic Overview of Its Genetic Heterogeneity From Classical Studies to the Genomic Era

Arnaud Fernandez,[1,2,3,*] Malgorzata Marta Drozd,[3] Susanne Thümmler,[1,2] Emmanuelle Dor,[1,2] Maria Capovilla,[3] Florence Askenazy,[1,2,*,†] and Barbara Bardoni[3,4,*,†]

and/or function have been found in 12 autosomes and one sex chromosome (X). We also describe five SNVs in X-linked genes inherited from a healthy mother, arguing for the X-linked recessive inheritance hypothesis. Moreover, *ATP1A3* (19q13.2) is the only gene carrying more than one SNV in more than one patient, making it a strong candidate for COS. Mutations were distributed in various chromosomes illustrating the genetic heterogeneity of COS. More than 90% of CNVs involved in COS are also involved

BCH ATP1a3 Mutation in COS: Probable Gain of Function leads to Psychosis



Additional side-chain bulk from the V129M mutation may alter pump function

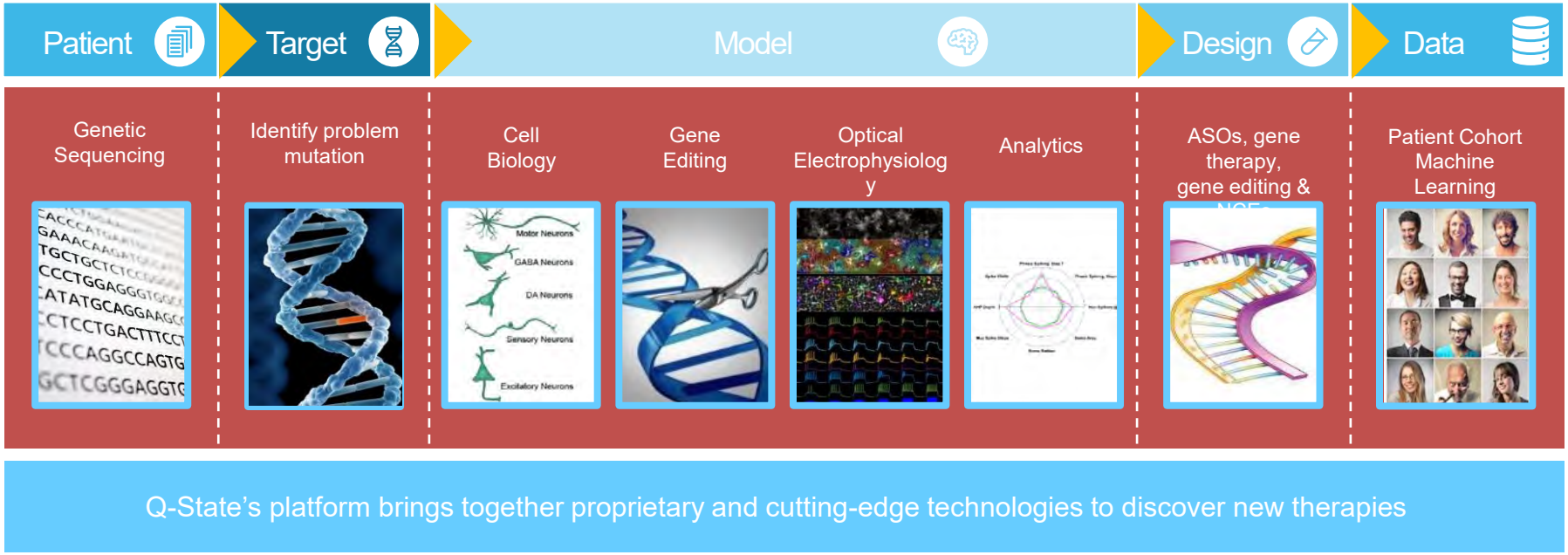
EPSP amplitude decreased in Cortical neurons from ATP1a3 patient-derived iPSCs

CRISPR correction of mutations reverses observed electrophysiologic cell phenotype

Hypothesis: Under stress abnormal pump function impacts neuronal function more

Q-State precision medicine platform

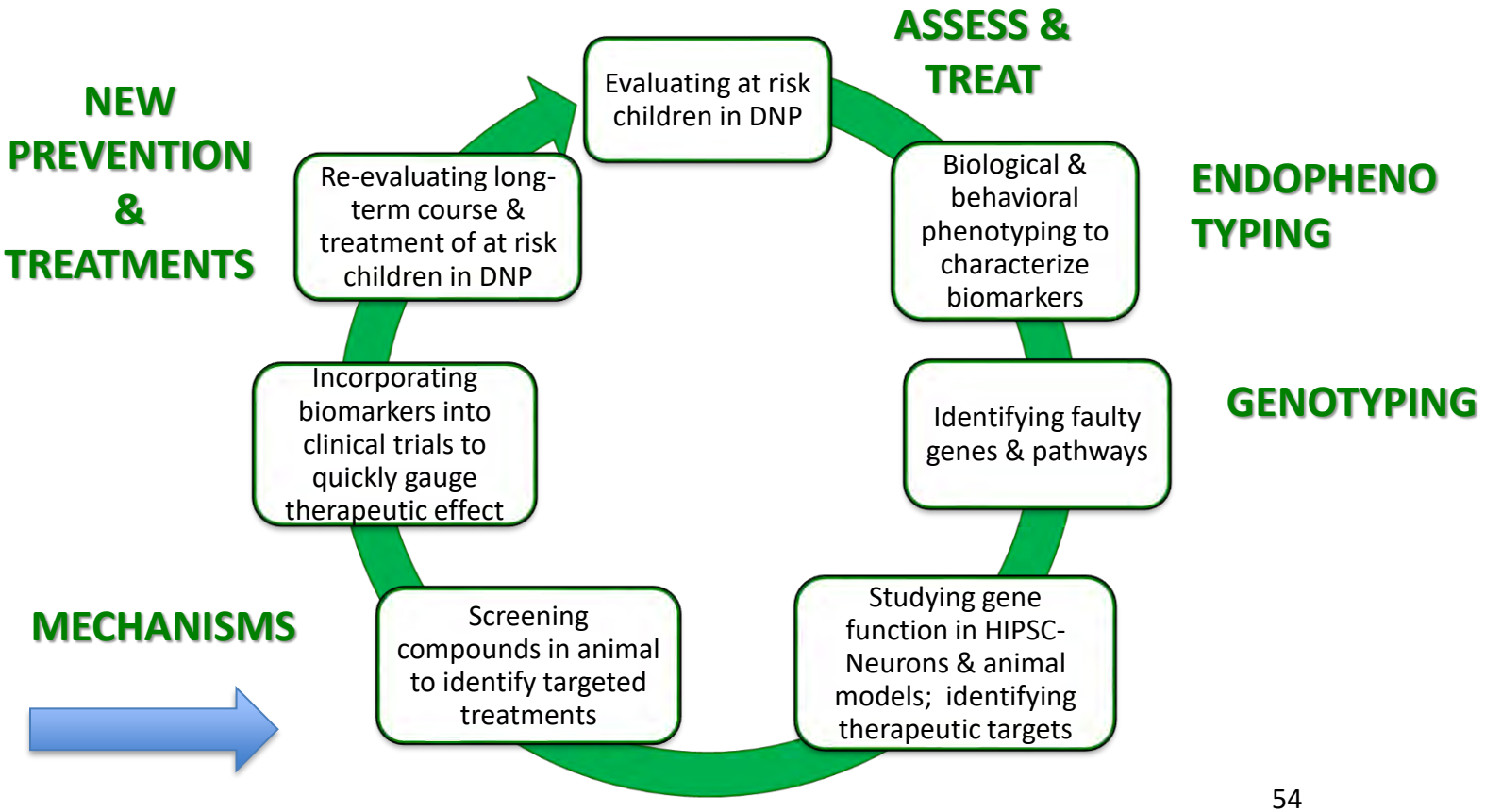
Q-State uses proprietary technologies to create a world-class precision medicine platform.
Building **patient-specific neuronal models** of disease allows Q-State to identify the best therapeutic candidates.
The ability to develop human models is critical in neuroscience, where animal models may yield limited insights.



Q-State's platform brings together proprietary and cutting-edge technologies to discover new therapies

Developmental Neuropsychiatry Program Discovery Cycle

A Road Map for Translational Neuropsychiatric Research



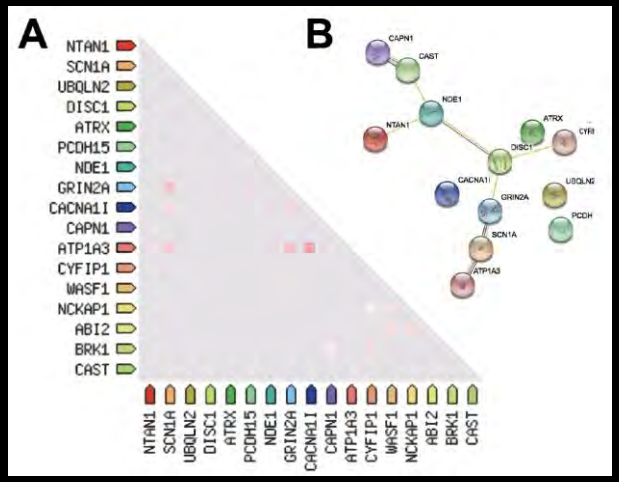
Future Directions

Total VEOP Manton Recruitment

Participant Type	Enrolled	DNA	PBMCs	RNA
Probands	138	130	66	52
Family Members	265	239	92	73
Total	403	369	158	125

As of 9/12/2019. *There are an additional 21 probands with early onset psychosis/clinical high risk, 3 probands with a genetic risk factor for psychosis, and 27 probands with the 16p13.11 deletion or duplication without psychosis (51 additional probands).

Gene	Function	VEOP	iPSCs	RNAseq	Inheritance
CACNA1I	T-type calcium channel	Yes	Sent, Arriving Sept 2019	Dec 2019	Unknown- adopted proband. +/-
KCNQ3	Potassium voltage-gated channel	Yes	Yes, proband & unaf. father	Dec 2019	Maternally Inherited. +/-
PCDH15	Protocadherin-15; Ca^{++}-dependent	Yes	Yes, male proband	Partial, Panel	Paternally Inherited +/-
PCDH19 & FUS	Protocadherin-15, Ca^{++}-dependent, interacts with FUS.	Yes	Sent, 2 probands (1 M. VEOP and one EOBP- expected Sept 2019	Dec 2019	PCDH19 inherited from mother; FUS likely inherited from father who is mentally ill.
ATP1A3	ATPase subunit alpha-3	Yes	Yes from male Proband, unaffected father control, Cas9 KI, Cas9 Control	Yes	*De novo +/-*
SCN1A	Sodium channel Alpha 1	Yes	Sent proband, maternal carrier and unaffected father, exp. Sept 2019	Dec 2019	+/-
16p.13.11 del and duplication	Excitation related (NTAN1—see below, NDE1- strong epilepsy candidate)	YES	Yes, 3 del, 2 dups; Sent 4 del and 1control exp. Sept 2019	Yes	Both paternally and maternally inherited in two probands, unknown in adopted proband, all +/-, for 7 probands, typical onset psychosis in 1 proband and late onset for 1 proband
NTAN1	protein degradation through the N-end rule pathway.	Yes	Yes, cas9 single and double knock out	Exp. July 2019	Created with Cas9 on background of one of the 16p13.11 unaffected parent controls, +/- and -/-
CAPN1	Ca^{++}-activated protease	VEOP at 14 years	Sent compound het proband and parents each with one mutated allele, exp. Sept 2019	Dec 2019	Compound heterozygous male proband with each parent providing one mutated allele.
ATRX and LRRK2	ATRX ATP-dependent helicase	VEOP	Sent on proband and con. mother, expected Sept 2019	Dec 2019	ATRX is denovo +/-, LRRK2 is either paternally inherited or denovo +/-
GRIN2A	GluN2A protein, subunit of NMDA glutamate-gated ion channels.	VEOP	Sent on male proband and unaffected mother exp. Sept 2019	Dec 2019	Inheritance either paternal or denovo -/+
15q11.2 del	a cause of idiopathic generalized epilepsy. Has gene CYFIP1	VEOP	Sent proband, carrier father and unaffected mother, exp. Sept 2019	Dec 2019	Paternally inherited -/+
UBQLN2	Ubiquilin-2	Yes	Yes, identical twin females; Sent second family (female proband) exp. Sept 2019	Dec 2019	One family Maternally Inherited +/- The other family is an adopted proband, inheritance unknown +/-



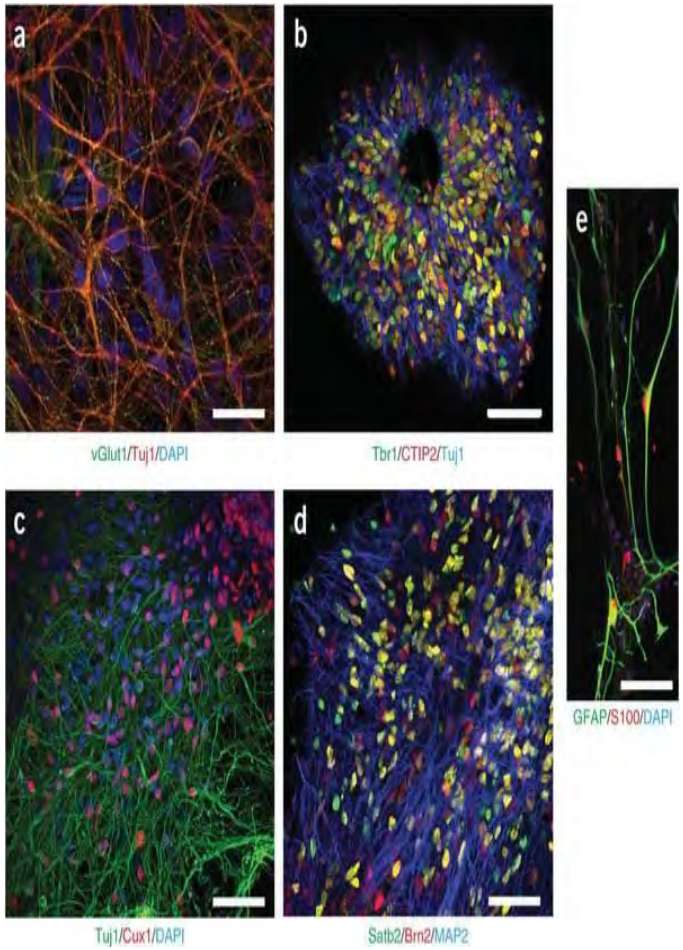
Clinical Take Way Points

- Very Early Onset Psychosis (VEOP) broadly defined, not just very onset schizophrenia has a high rate of discoverable rare likely causative genetic variation.
- The rate of de novo rare predicted damaging variants in highly conserved genes is considerable, ~6% in our sample.
- Many of the genes implicated are implicated in other central nervous system diseases.
- Whole Exome Sequencing of VEOP patients and parents is justifiable to
  - highlight additional medical risks
  - Eventually give families answers about causality and risk to other family members
  - Inform field

NEXT STEPS

Unprecedented Opportunity to Develop Therapeutics to Prevent Schizophrenia

- **Genes**
  - Youngest affected children are helping us find highly penetrant genes
- **Neurons**
  - Neuronal cell cultures to identify cell autonomous effects
- **Networks in Living Brain**
  - From rodent knock out models to human clinical trials



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