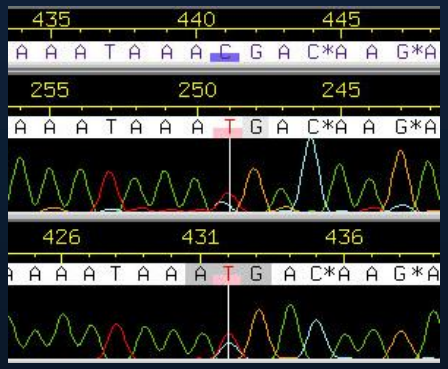
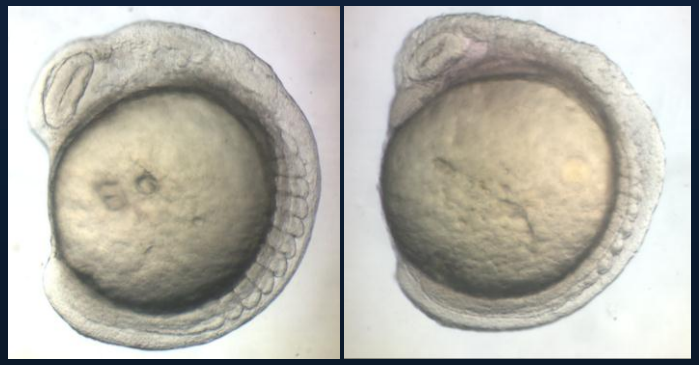
Coupling sequencing and functional studies in neonates





Nicholas Katsanis, Ph.D.
Center for Human Disease Modeling
Duke University Medical Center

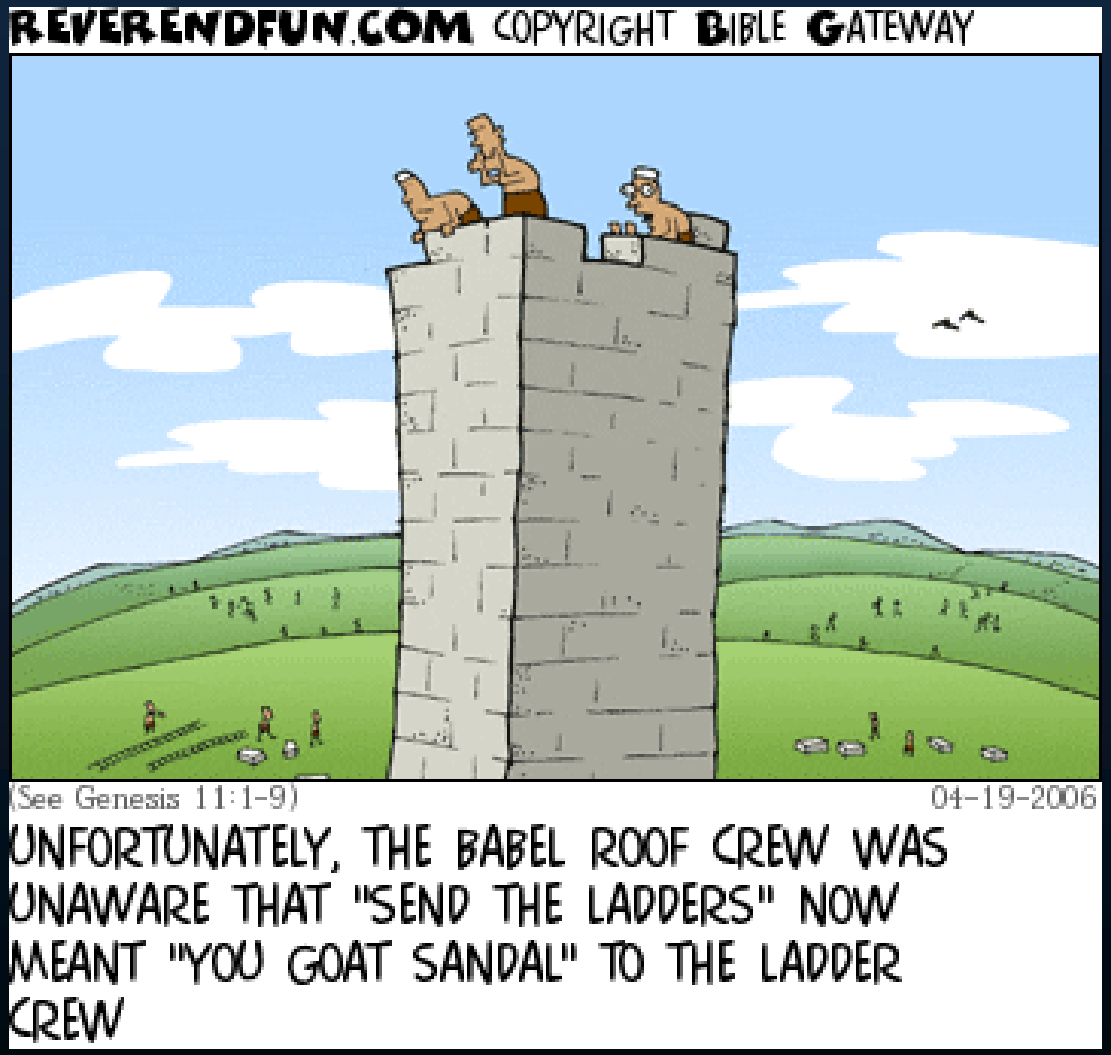
Why babies (apart from the obvious!)

1. Birth defects: 21% of infant deaths
2. Mortality rates plateaued
3. 5-10% of repeat admissions

Why (part II)

1. Early diagnosis/prognosis
2. High-value information content (families)
3. Focused clinical investment
4. Maximum therapeutic window

1. Communication across disciplines



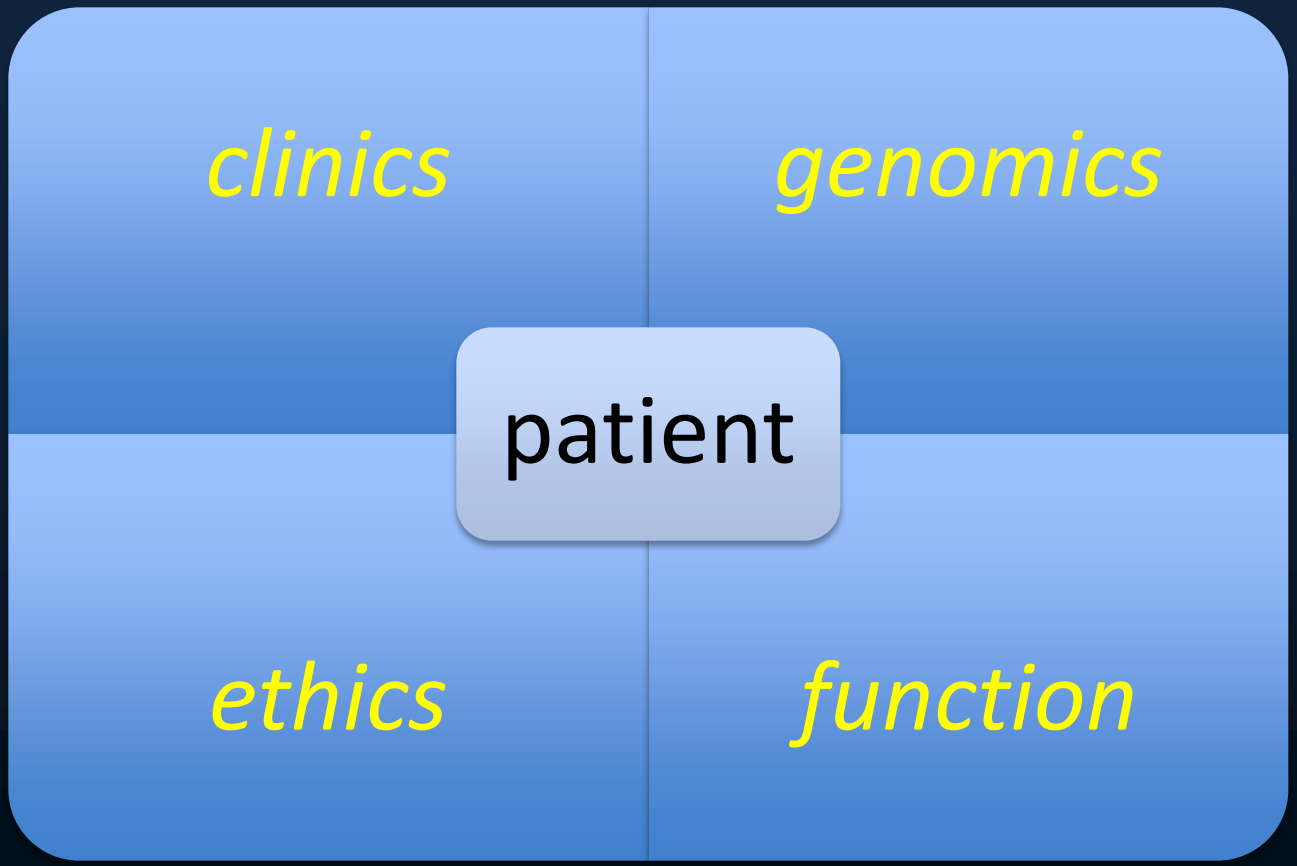
2. Information management

3. Ethical transmission of information

A bold new activity

Duke University Task Force for Neonatal Genomics

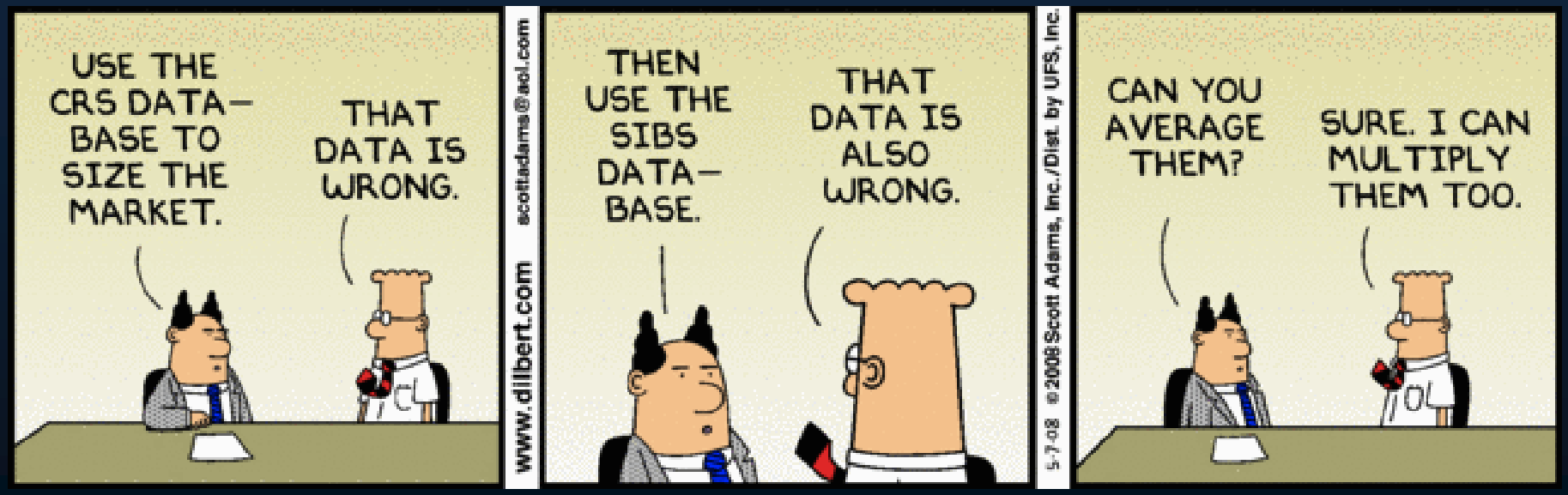
synthesis of disciplines around patient needs



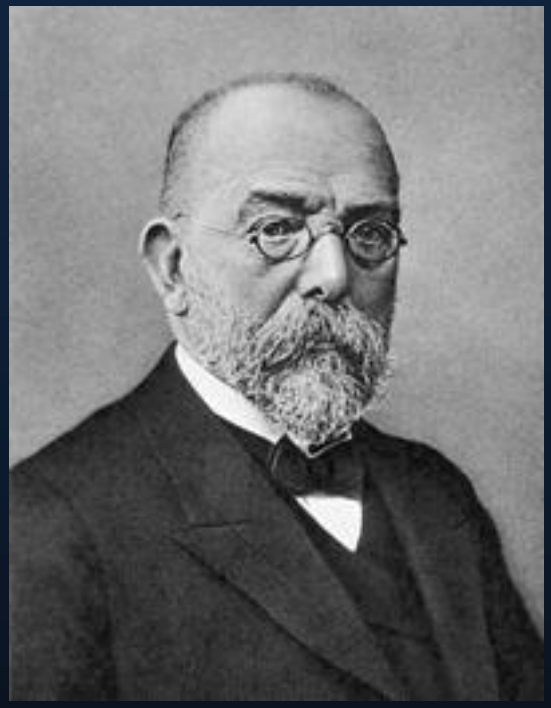
The process

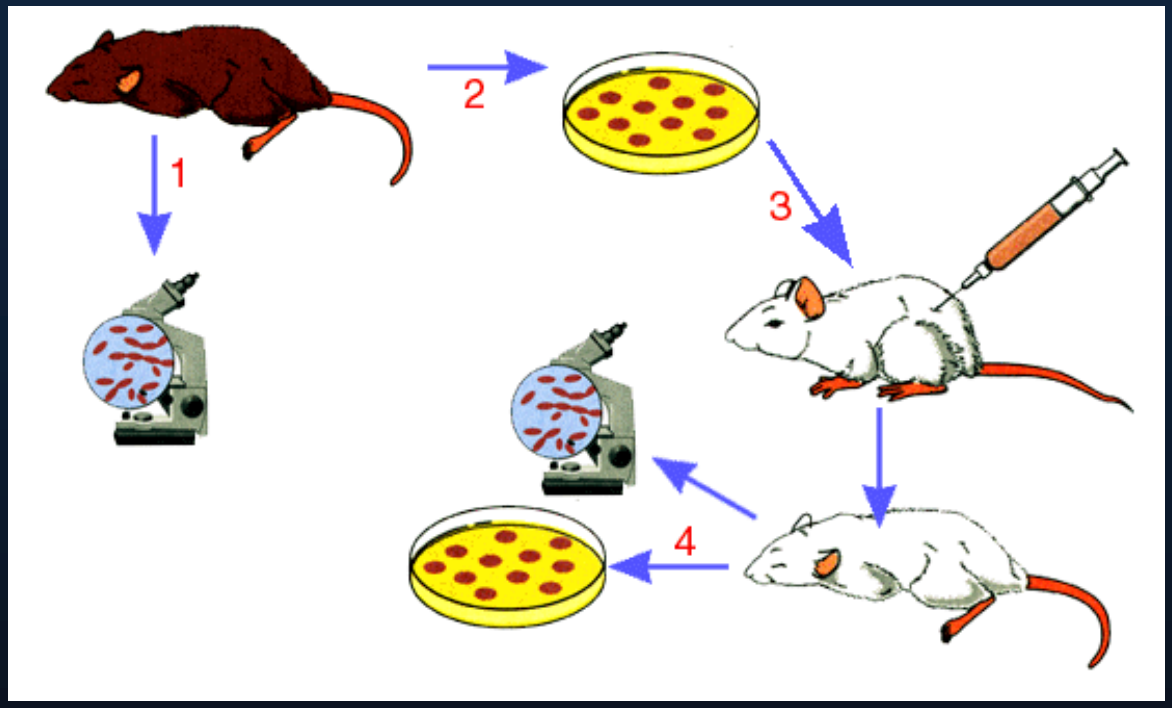
1. Trio-based recruitment
2. WES/WGS
3. Data filtering
4. Systematic functionalization of ALL candidate alleles

Heavily reliant on inferred data and predictions…



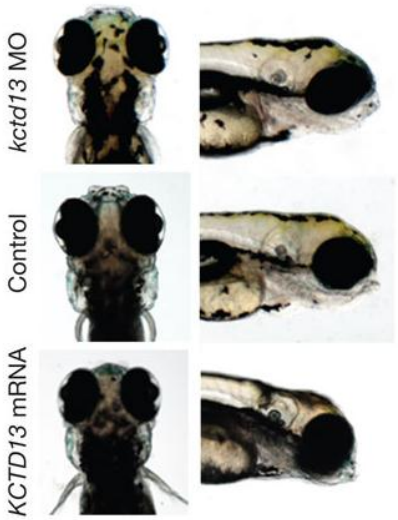
Interpretation





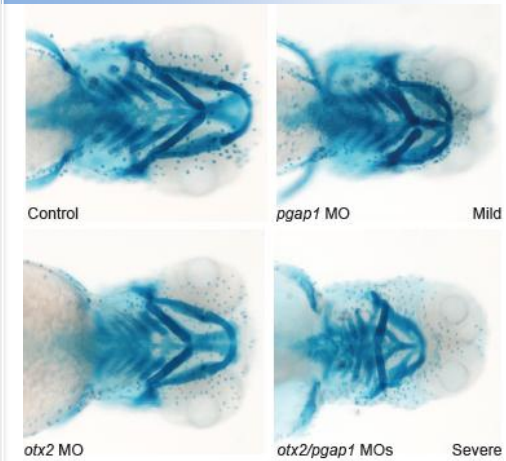
In vivo models in human disease modeling

Neuroanatomical



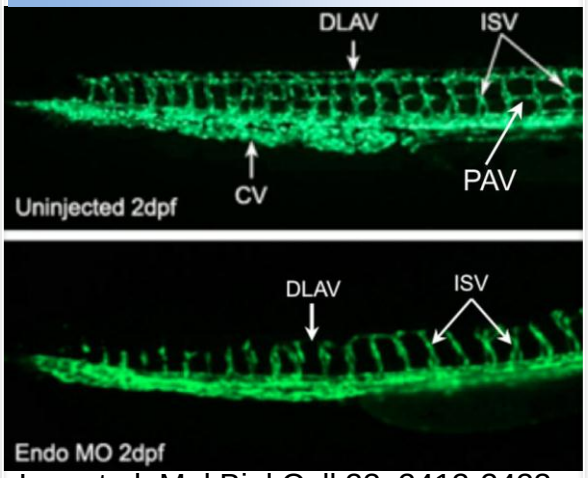
Golzio et al, Nature 485: 363-367 (2012)

Craniofacial anomalies



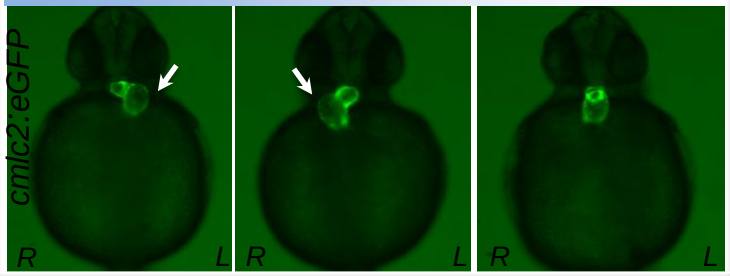
Chassaing et al, J Med Genet 49: 373-379 (2012)

Vascular integrity

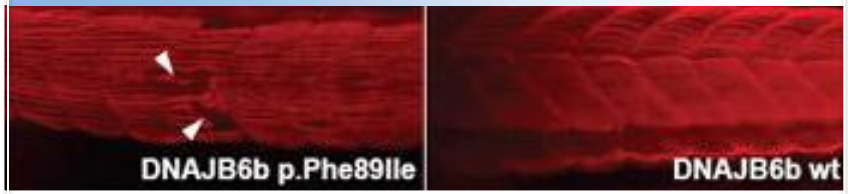


Lee et al, Mol Biol Cell 23: 2412-2423 (2012)

Cardiac malformations



Muscular dystrophy



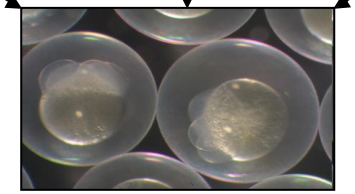
Sarparanta et al, Nat Genet 44: 450-455 (2012)

In vivo functional testing of missense variants

Loss of function

Dominant negative

Gain of function



Morpholino (MO) injection into 1-4 cell stage zebrafish embryos

Phenotype (1-7 days post-fertilization)

Functional null

Hypomorph

Benign

Dominant negative

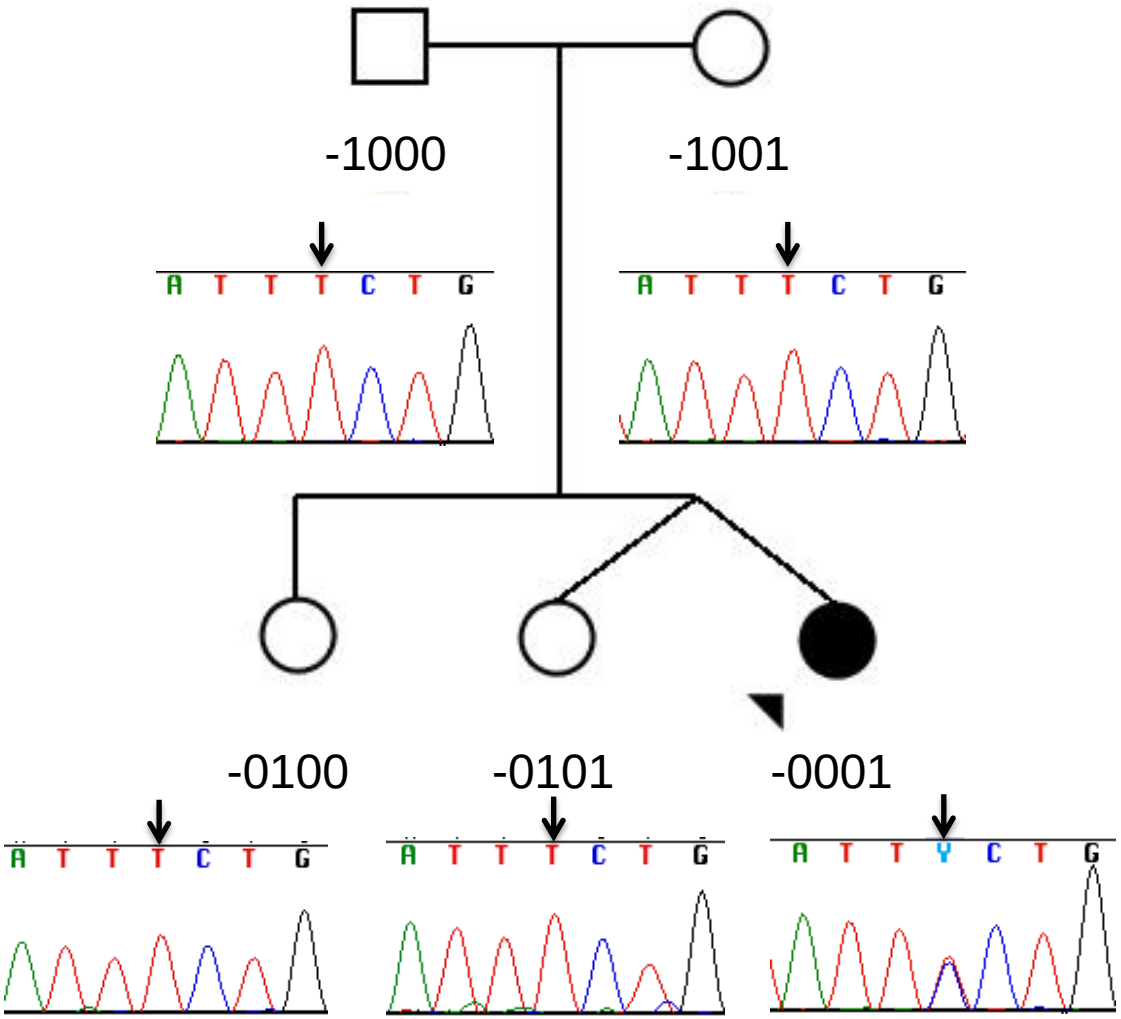
Gain of function

to date:
~350 genes modeled
~1,200 human non-synonymous variants

98% sensitivity
84% specificity

suite of models for testing
collapse into recurrent pathways
tools for lead compound discovery

Vignette1: An opportunity to learn new biology- *SCN2A* and congenital seizures



Clinical features

intractable seizures
severe developmental delay
irritability
cortical visual impairment
gastroesophageal reflux

Clinical testing

normal female karyotype
normal female microarray
metabolic testing – **negative**
genetic testing – **negative**

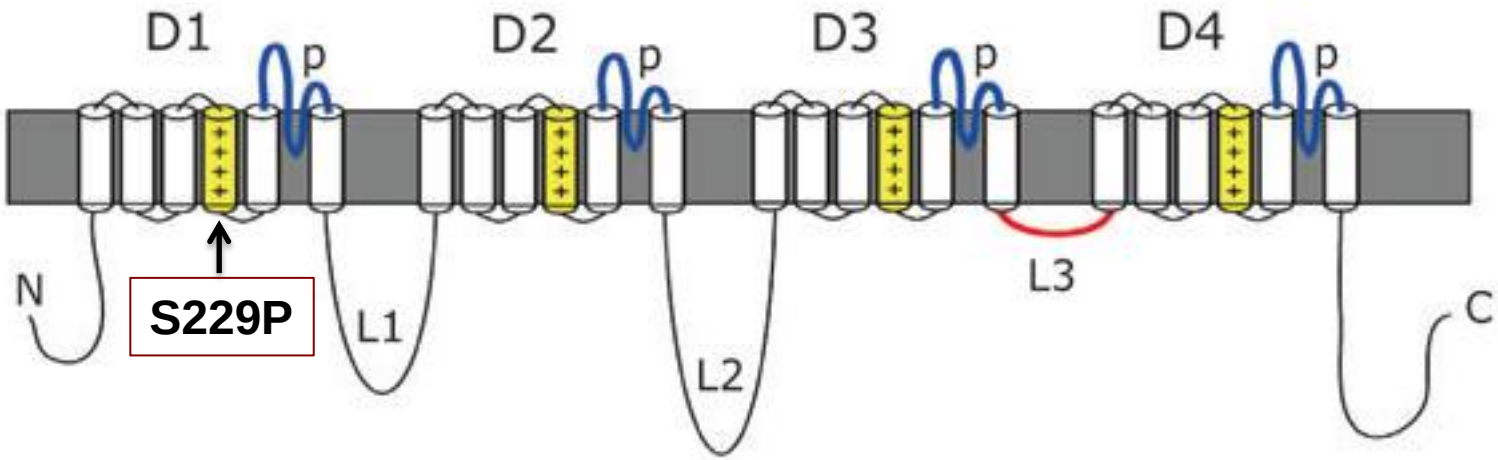
Referred for research studies

Marie McDonald (Med Genet)

Consented for research studies

proband age 21 months

The candidacy of *SCN2A* p.S229P



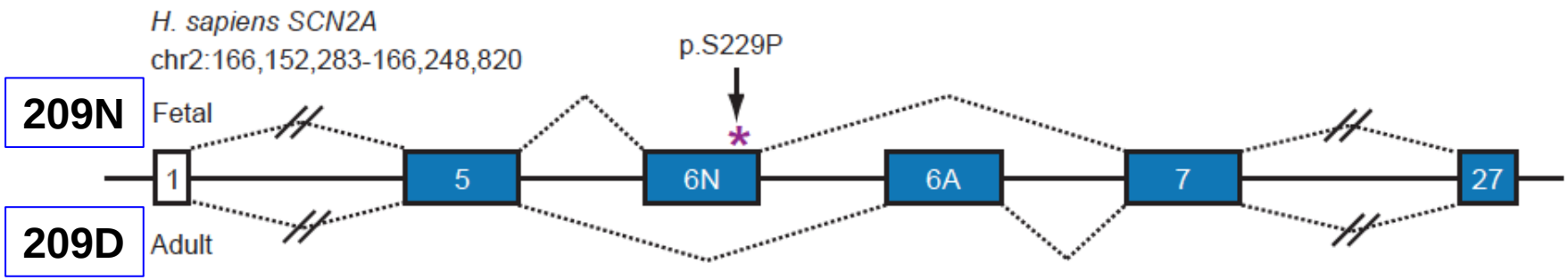
Arguments for causality:

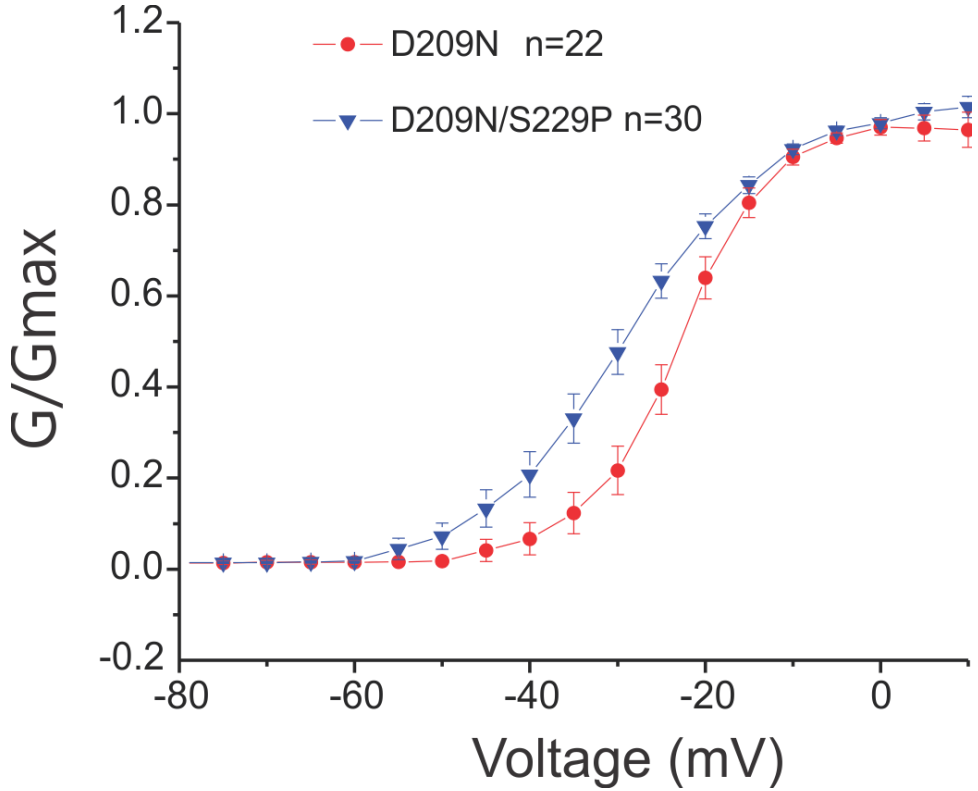
- Novel, *de novo,* protein-altering variant
- *SCN2A* is a known epilepsy gene

Arguments against causality:

- Patient phenotype more severe than most other documented *SCN2A* cases

SCN2A S229P: gain-of-function

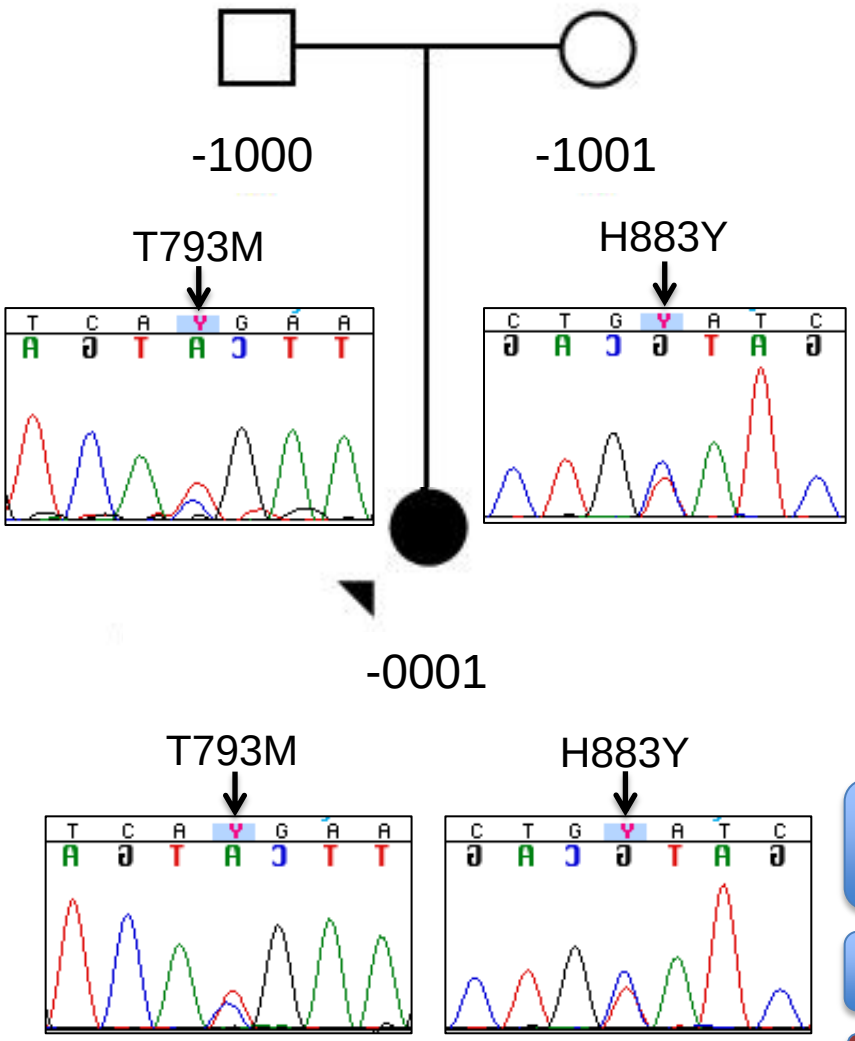




Haidun Yan
Geoff Pitt

hyper polarizing shift of voltage-dependent activation

Vignette 2: Surprising diagnoses



Clinical features

severe developmental delay
hypotonia
optic nerve disorder/nystagmus
auditory neuropathy spectrum disorder
demyelination on brain MRI

Clinical testing

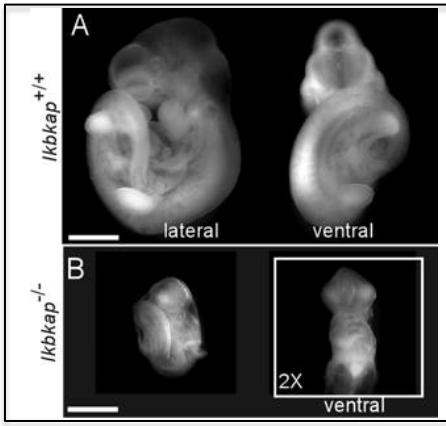
normal female microarray
metabolic testing – **negative**
extensive genetic testing – **negative**
- deafness panel
- mitochondrial disease panel
- *PLP1* and *GJC2* sequencing

***IKBKAP*:** Elongator protein complex 1. Causes familial dysautonomia

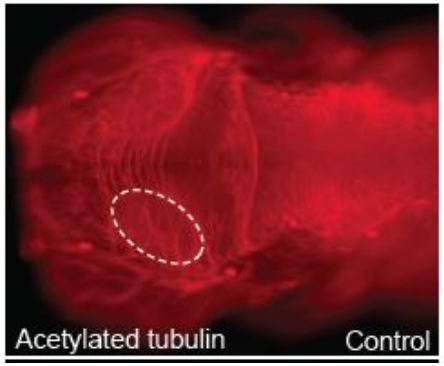
***MGAM*:** Maltase-glucoamylase

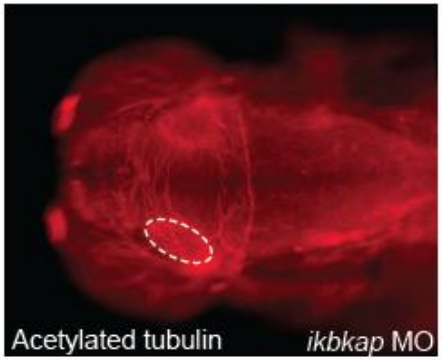
***RGS22*:** Regulator of G-protein signaling

A variant form of familial dysautonomia

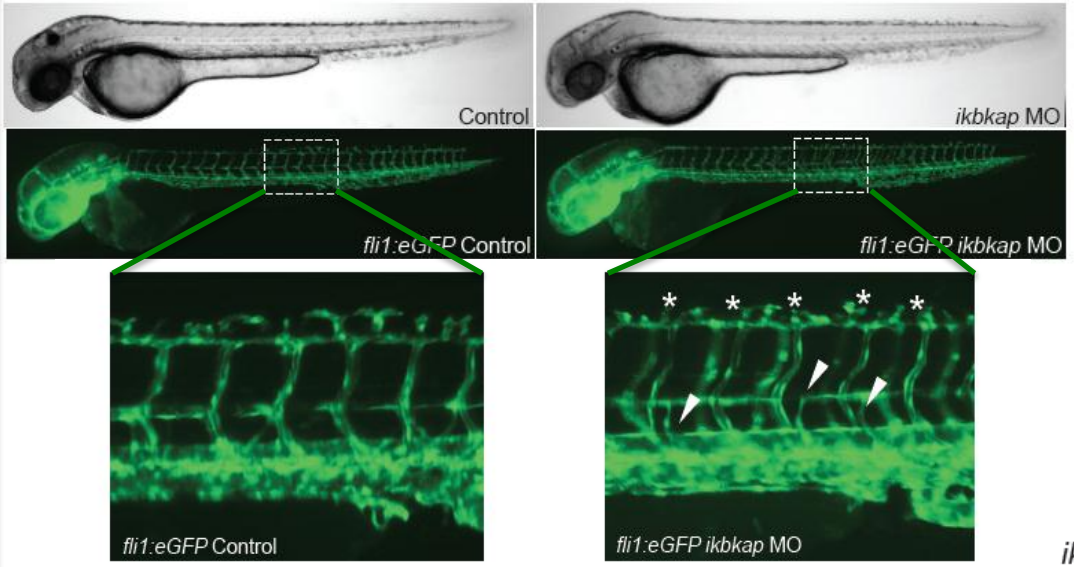


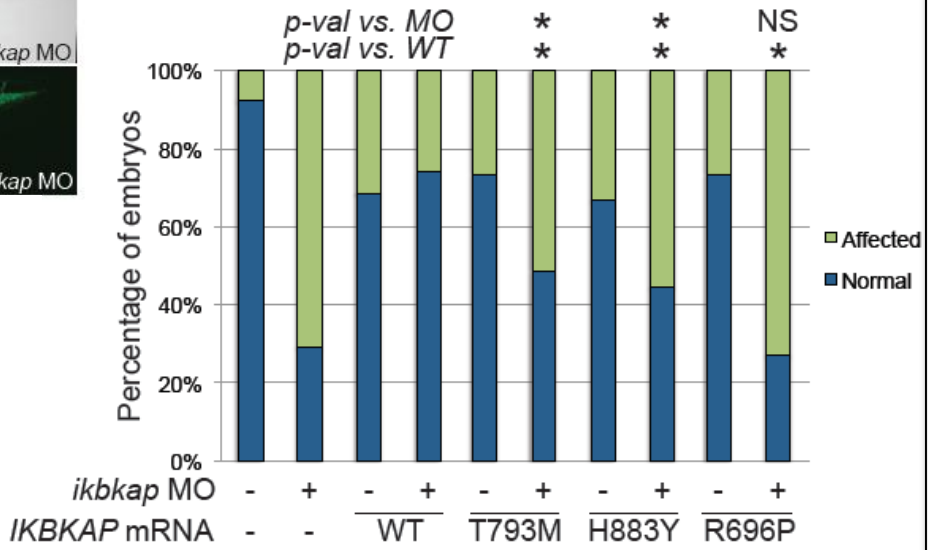
Reduced size of midbrain and cerebellar structures



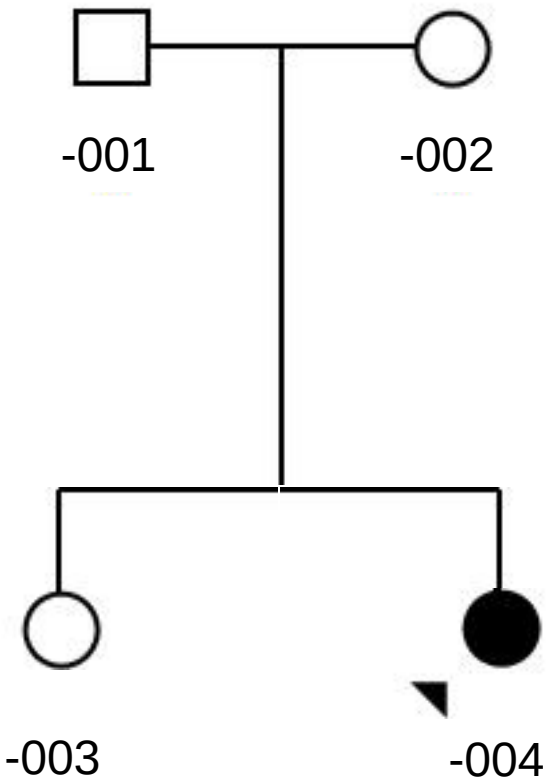


Disorganization of vascular networks rescuable by WT human mRNA





Vignette 3: Gene identification when genetic resolution reaches an impasse



Clinical features

Global developmental delay
microcephaly
feeding issues
failure to thrive
abnormal muscle tone
low immunoglobulins
frequent respiratory infections

Clinical testing

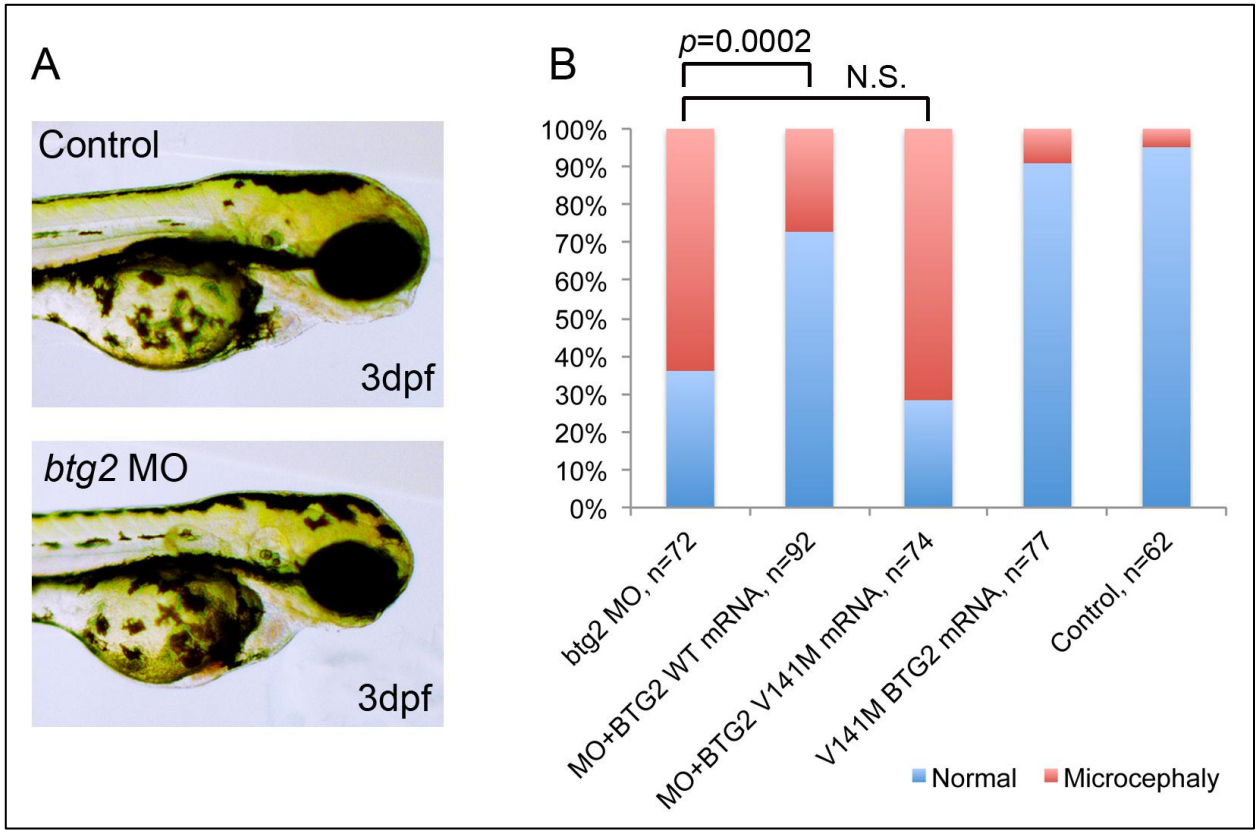
normal female microarray
metabolic testing – **negative**
extensive genetic testing – **negative**

***BTG2*:** Involved in the G1/S transition of the cell cycle

***NOS2*:** Nitric oxide synthase 2, inducible

***TTN*:** Titin

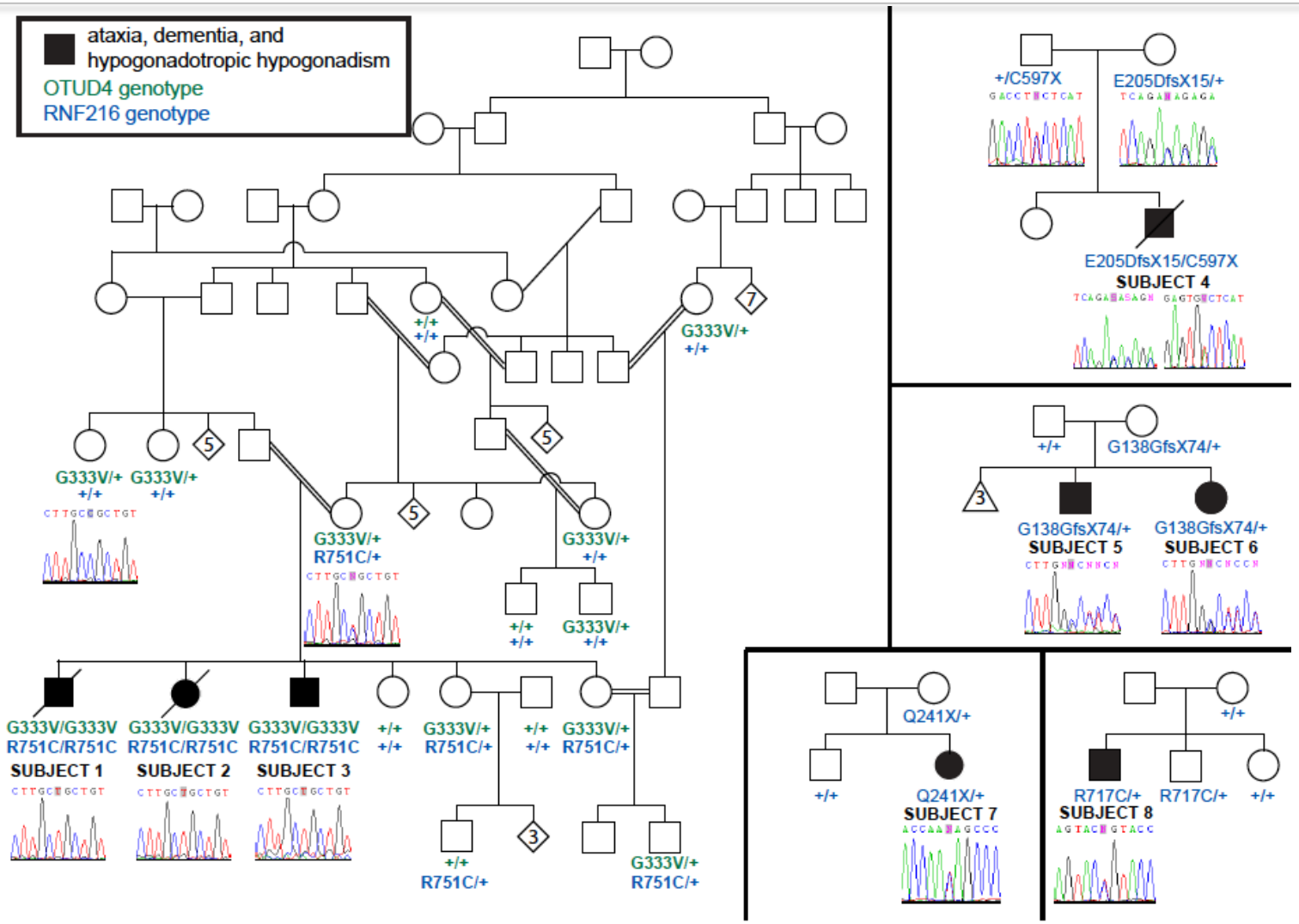
BTG2 is responsible for the microcephaly phenotype



BTG2: de novo

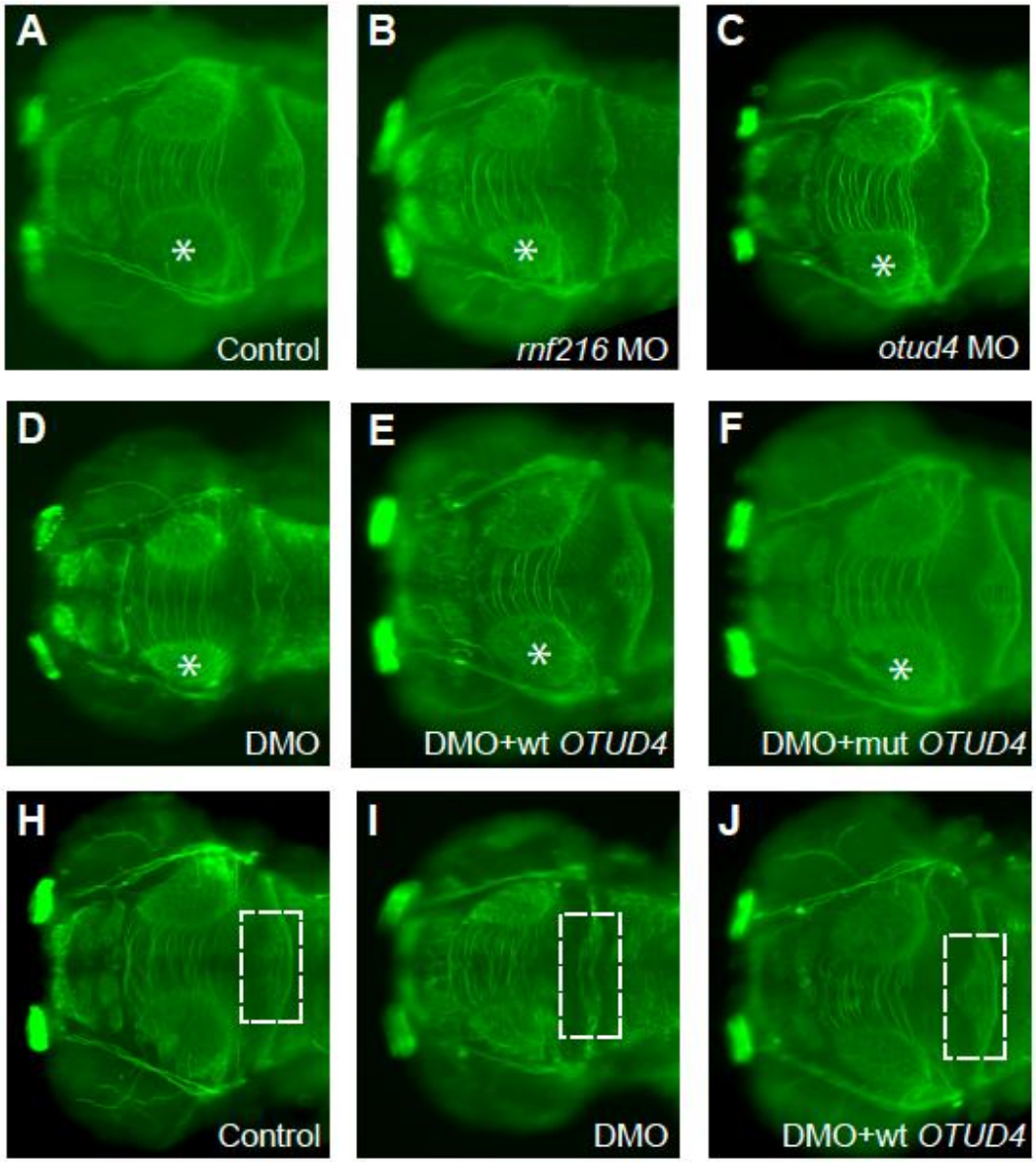
NOS2: de novo

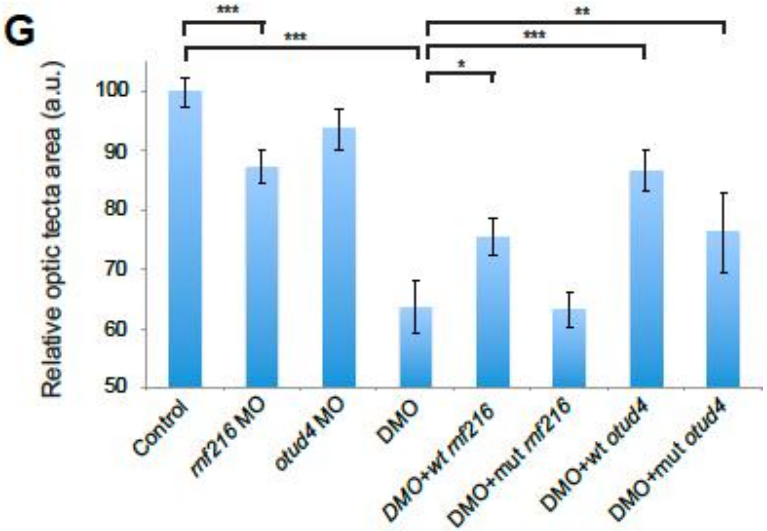
Vignette 4:The case for unbiased analysis of exomes

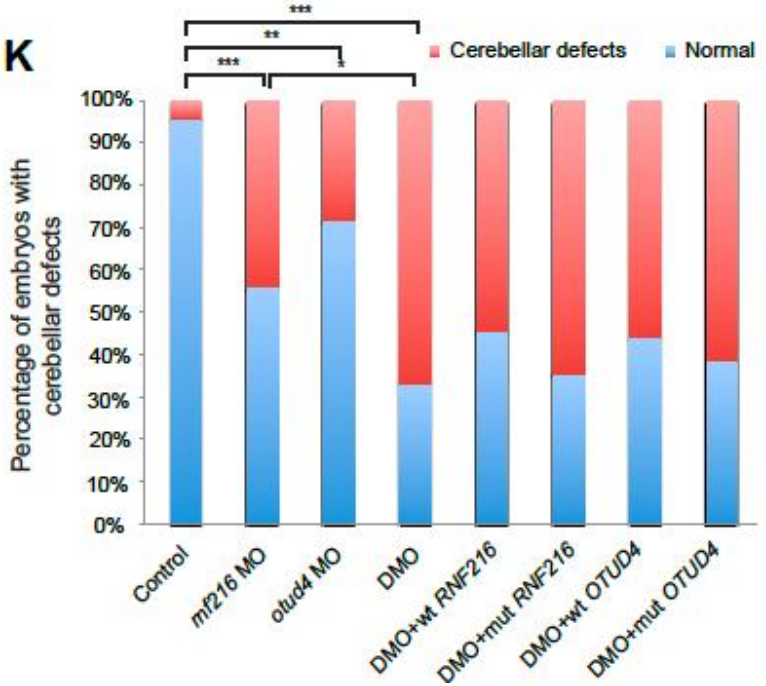


Chan et al, N Eng J Med in press

In vivo testing of epistasis







Chan et al, N Eng J Med in press

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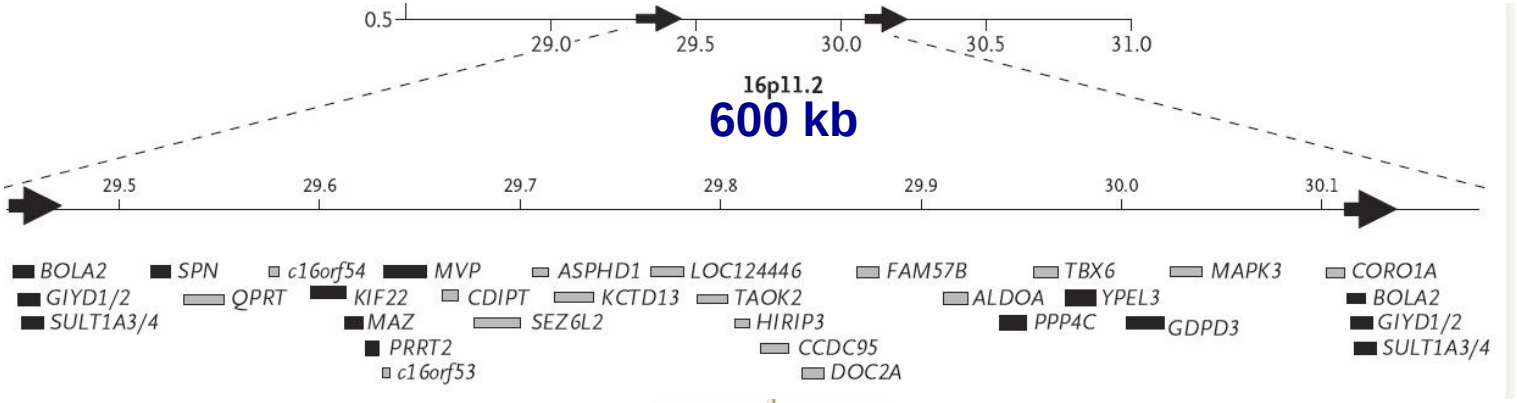
Geoffrey Pitt

U Lausanne:
Alex Raymond
Jacqui Beckmann

Mass General/Harvard:
Susan Slaugenhapt

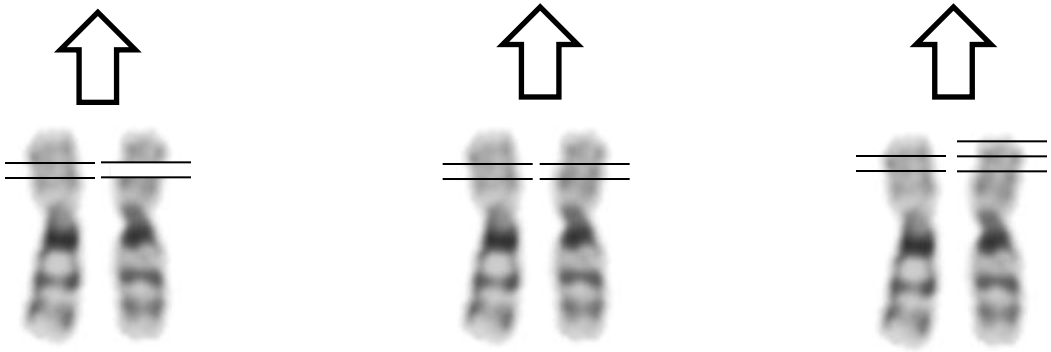
Can also model copy number variants

16p11.2 CNV:
Complex Mirrored Neurodevelopmental Phenotypes



AUTISM

HYPERPHAGIA
OBESITY
MACROCEPHALY
…



deletion

normal dose

duplication

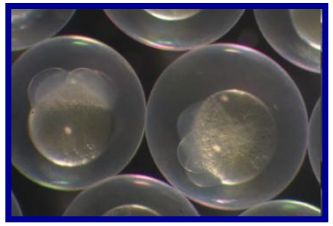
SCHIZOPHRENIA
AUTISM

ANOREXIA
UNDERWEIGHT
MICROCEPHALY
…

Strategy to Identify Which Gene(s) Cause(s) the Complex Mirror Phenotype at the 16p11.2 Locus

Overexpression human mRNAs

29 genes in the CNV
26 Zebrafish orthologs

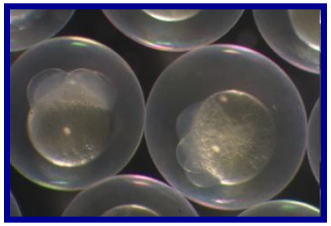


Microinjection mRNAs into Zebrafish embryos 1- to 2-cell stage

Screening for microcephaly at 5 dpf

Candidate gene(s) identified

Design Morpholino(s) against Zebrafish ortholog(s)

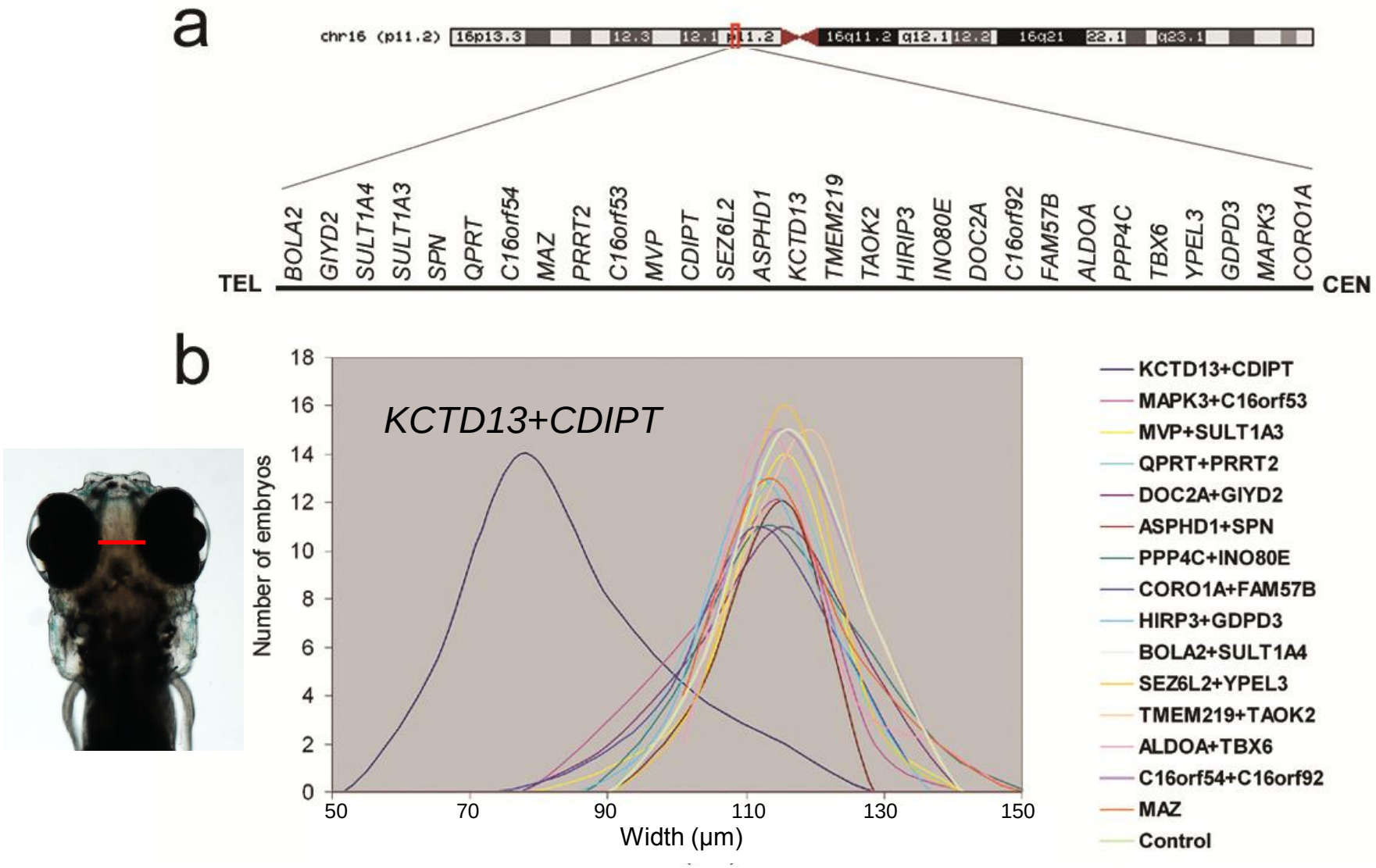


Microinjection morpholinos into Zebrafish embryos 1- to 2-cell stage

Screening for reciprocal phenotype: macrocephaly

Identification of the causal gene(s)

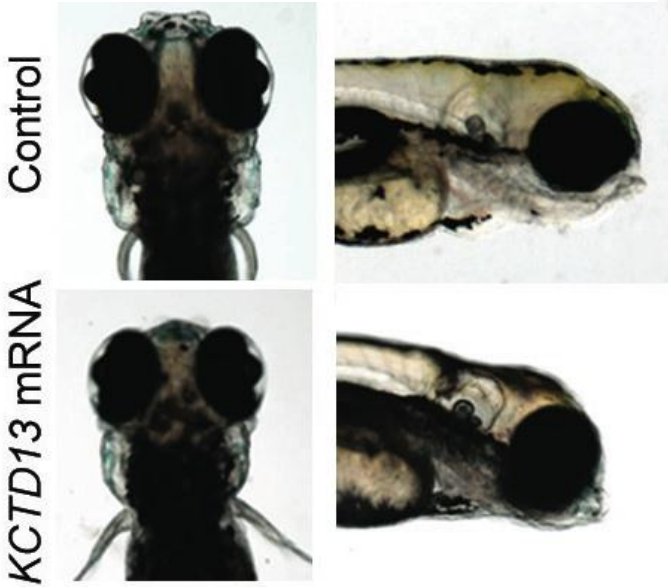
KCTD13+CDIPT Overexpression leads to Microcephaly

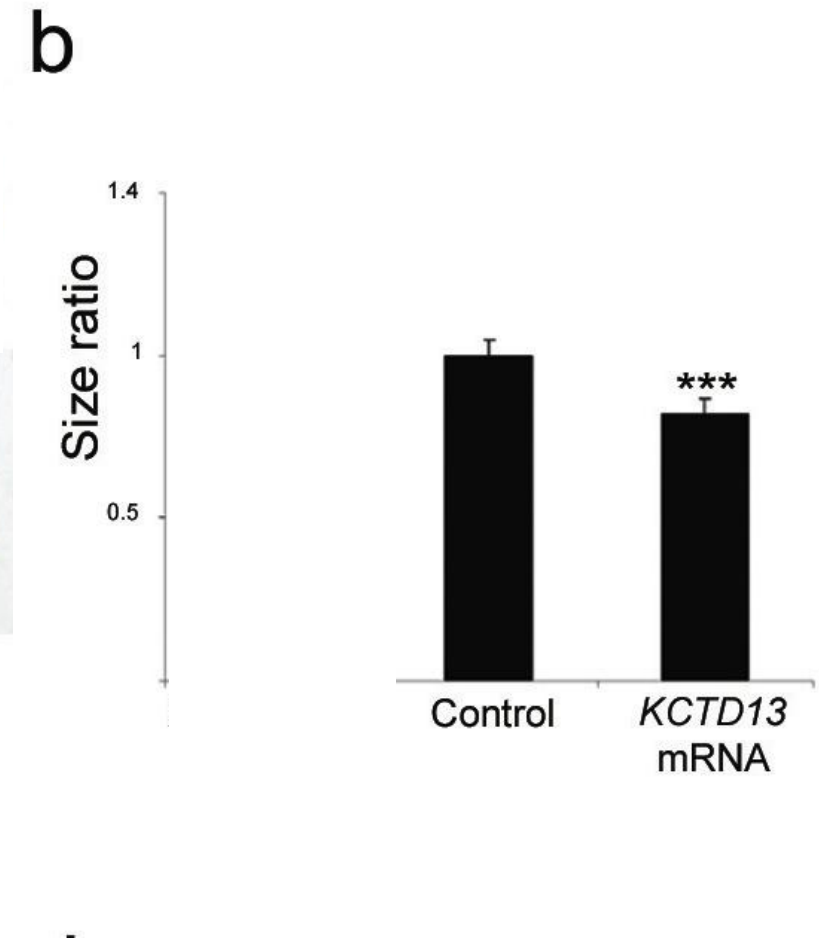


Golzio *et al*, Nature 2012

KCTD13 Dosage Changes lead to Head Size Defects

Microcephaly





Golzio *et al*, Nature 2012

Complex Mirror Phenotypes at the 16p11.2 CNV

Human Molecular Genetics, 2008, Vol. 17, No. 4 **628–638**
doi:10.1093/hmg/ddm376
Advance Access published on December 21, 2007

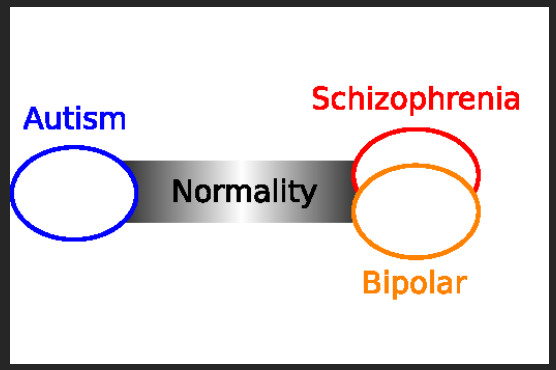
Recurrent 16p11.2 microdeletions in autism

Ravinesh A. Kumar[1], Samer KaraMohamed[1], Jyotsna Sudi[1], Donald F. Conrad[1], Camille Brune[5], Judith A. Badner[4], T. Conrad Gilliam[1], Norma J. Nowak[6], Edwin H. Cook Jr[5], William B. Dobyns[1,2,3] and Susan L. Christian[1,*]

[1]Department of Human Genetics, [2]Department of Neurology, [3]Department of Pediatrics and [4]Department of Psychiatry, University of Chicago, Chicago, IL 60637, USA, [5]Department of Psychiatry, University of Illinois at Chicago, Chicago, IL 60612, USA and [6]Department of Cancer Genetics, Roswell Park Cancer Institute, Buffalo, NY 14236, USA

Microduplications of 16p11.2 are associated with schizophrenia

Shane E McCarthy[*,1], Vladimir Makarov[1], George Kirov[2], Anjene M Addington[3], Jon McClellan[4], Seungtai Yoon[1], Diana O Perkins[5], Diane E Dickel[6], Mary Kusenda[1,7], Olga Krastoshevsky[8], Verena Krause[8], Ravinesh A Kumar[9], Detelina Grozeva[2], Dheeraj Malhotra[1], Tom Walsh[6], Elaine H Zackai[10], Paige Kaplan[11], Jaya Ganesh[11], Ian D Krantz[10], Nancy B Spinner[10], Patricia Roccanova[1], Abhishek Bhandari[1], Kevin Pavon[1], B Lakshmi[1,12], Anthony Leotta[1], Jude Kendall[1], Yoon-ha Lee[1], Vladimir Vacic[1], Sydney Gary[1], Lilia M Iakoucheva[13], Timothy J Crow[14], Susan L Christian[9], Jeffrey A Lieberman[15,16], T Scott Stroup[15], Terho Lehtimäki[17], Kaija Puura[18], Chad Haldeman-Englert[10], Justin Pearl[19], Meredith Goodell[20], Virginia L Willour[20], Pamela DeRosse[21], Jo Steele[19], Layla Kassem[19], Jessica Wolff[19], Nisha Chitkara[21], Francis J McMahon[19], Anil K Malhotra[21], James B Potash[20], Thomas G Schulze[19,22], Markus M Nöthen[23,24], Sven Cichon[23,24], Marcella Rietschel[22,25], Ellen Leibenluft[26], Vlad Kustanovich[27], Clara M Lajonchere[27], James S Sutcliffe[28], David Skuse[29], Michael Gill[30], Louise Gallagher[30], Nancy R Mendell[31], Wellcome Trust Case Control Consortium[32], Nick Craddock[2], Michael J Owen[2], Michael C O'Donovan[2], Tamim H Shaikh[10], Ezra Susser[15], Lynn E DeLisi[33,34], Patrick F Sullivan[35], Curtis K Deutsch[33,36], Judith Rapoport[3], Deborah L Levy[8,33], Mary-Claire King[6] & Jonathan Sebat[1]



Under-development of social brain

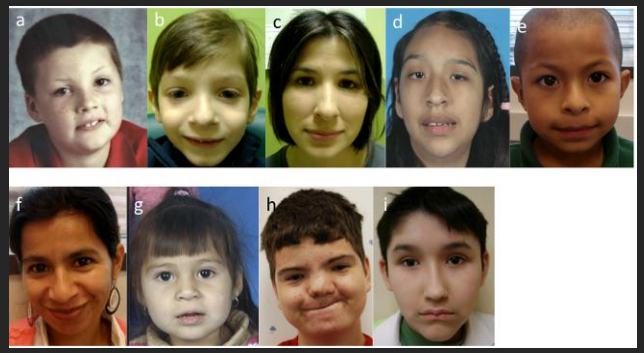
Dysregulated over-development of social brain

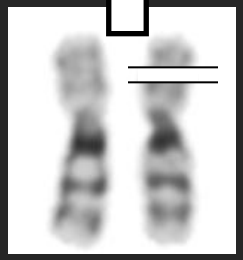
Crespi *et al.* (2010) *PNAS* *107* :1736-1741

Macrocephaly

Normal

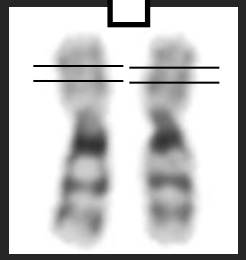
Microcephaly





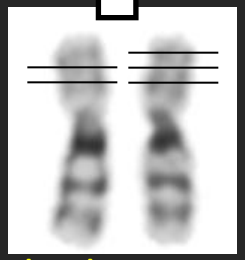
deletion

Autism



normal dose

Sociability



duplication

Schizophenia

Shinawi M et al, JMG, 2010

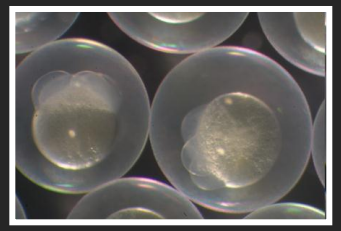
Etiology: Gene(s) Within the 16p11.2 CNV but Which One(s)?

Gene name	CDS start	CDS end	Strand	Change in Expr[a]	Protein function
BOLA2 *BOLA2B*	29365833 30111796	29373786 30112615	- -	0.6 (0.8)	**Possibly involved in cell proliferation or cell-cycle regulation**
GIYD1 *GIYD2*	29373376 30112906	29377041 30116288	+ +	-	GIY-YIG domain containing
SULT1A4 *SULT1A3*	29373902 30119550	29383801 30122742	+ +	1.0 (1.5)	**Induced in response to fasting or as a result of a defect in leptin signalling** *Catalyzes the sulfate conjugation of phenolic monoamine neurotransmitters*
SPN	29582550	29583753	+	1.1 (1.1)	**Sialophorin, CD43. Activator of JNK1 and MAPK3 signalling**
QPRT	29598019	29616233	+	1.2 (1.3)	*Catabolism of quinolinate, a neural excitotoxin and NMDA receptor agonist*
C16orf54	29663098	29663773	-	0.5 (0.8)	
MAZ	29725523	29728564	+	0.7 (0.8)	***Interacts with SP1 in regulating transcription of serotonin receptor gene HTR1A***
PRRT2	29731876	29733460	+		Proline-rich transmembrane protein
C16orf53	29735347	29738576	+	0.8 (0.8)	
MVP	29749371	29766811	+	0.5 (0.8)	**Regulates cytoplasmic localisation of PTEN**
CDIPT	29778010	29781679	-	0.4 (0.5)	Phosphatidylinositol synthesis
SEZ6L2	29790520	29817841	-	0.9 (0.9)	*Seizure-related. May contribute to specialized ER function in neurons*
ASPHD1	29819793	29824719	+	1.0 (1.1)	Aspartate beta-hydroxylase domain containing
KCTD13	29825693	29844855	-	0.6 (0.6)	**Similar to TNFAIP1, a mediator of insulin resistance in rodent obesity models**
TMEM219	29881965	29890367	+	0.6 (0.7)	Transmembrane protein
TAOK2	29896594	29906802	+	0.8 (0.8)	**Activates JNK1 and MAPK3 pathways via the upstream MKK3 and MKK6 kinases**
HIRIP3	29912028	29914427	-	0.6 (0.5)	Possibly functions in some aspects of chromatin and histone metabolism
INO80E	29915132	29924264	+	0.5 (0.5)	INO80 complex subunit E
DOC2A	29925007	29929044	-	1.0 (1.0)	*Possibly involved in Ca^{2+}-dependent neurotransmitter release*
C16orf92	29942176	29943049	+	1.1 (1.1)	
FAM57B	29944004	29949349	-	0.9 (0.8)	
ALDOA	29986076	29989034	+	0.5 (0.6)	Fructose-bisphosphate aldolase A
PPP4C	29995199	30003884	+	0.7 (0.8)	**Regulates JNK1 signalling**
TBX6	30005046	30010015	-	1.0 (1.0)	Transcription factor involved in regulation of early developmental processes
YPEL3	30011531	30014190	-	0.6 (0.6)	Possibly involved in proliferation and apoptosis in myeloid precursor cells
GDPD3	30023693	30032300	-	0.8 (0.9)	**Glycerophosphodiesterase domain**
MAPK3	30035658	30042031	-	0.7 (0.7)	**ERK1. Multiple roles in proliferation and differentiation of preadipocytes**
CORO1A	30104031	30107786	+	0.3 (0.5)	Coronin. Actin binding protein

Strategy to Identify Which Gene(s) Cause(s) the Complex Mirror Phenotype at the 16p11.2 Locus

Overexpression human mRNAs

29 genes in the region
26 Zebrafish orthologs

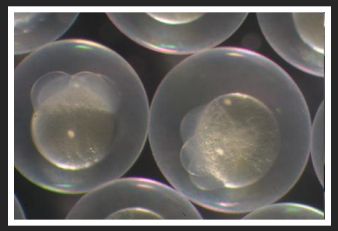


Microinjection mRNAs into Zebrafish embryos 1- to 4-cell stage

Screening for microcephaly

Candidate gene(s) identified

Design Morpholino(s) against Zebrafish ortholog(s)

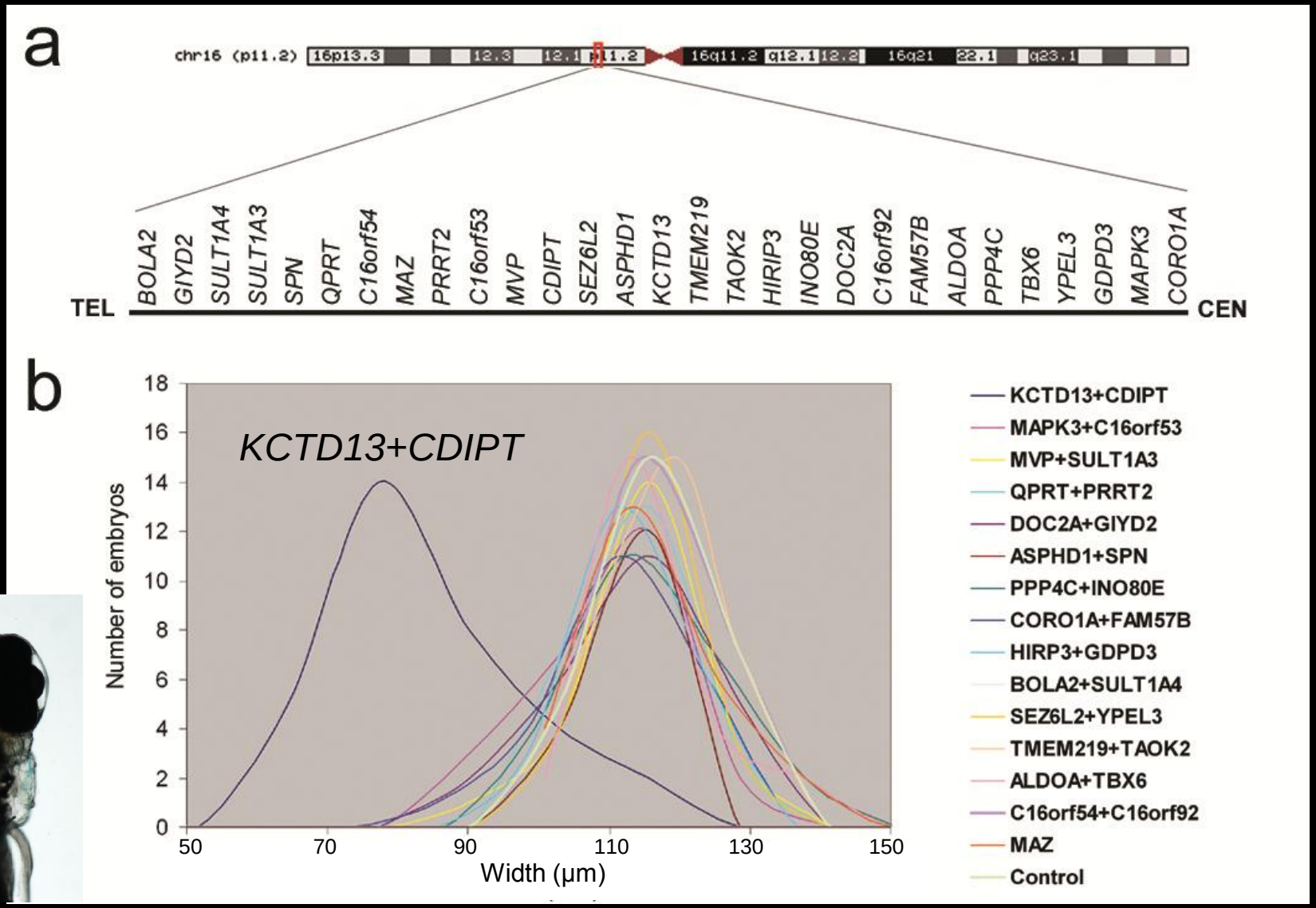


Microinjection morpholinos into Zebrafish embryos 1- to 4-cell stage

Screening for reciprocal phenotype: macrocephaly

Identification of c ontributory gene(s)

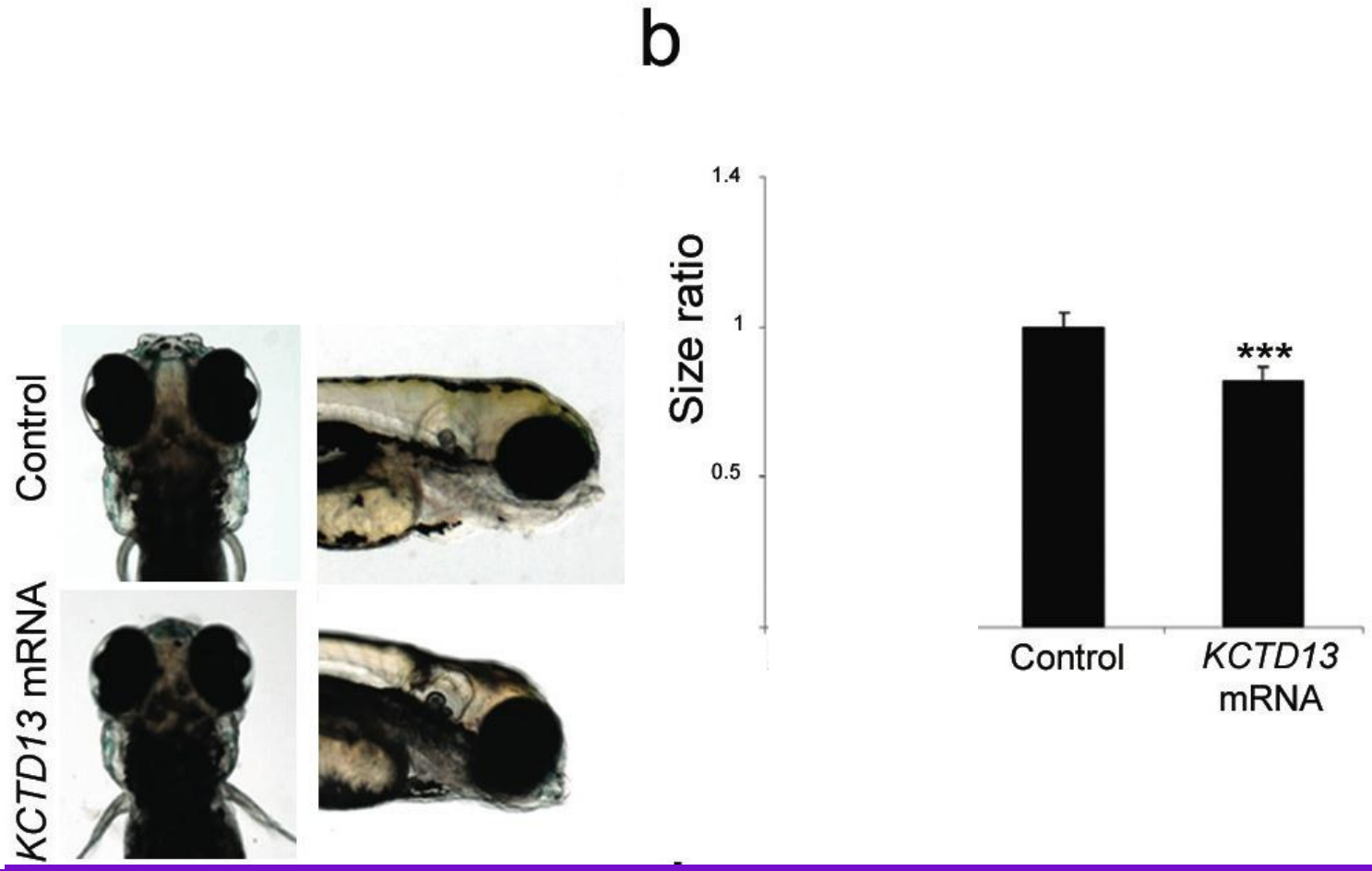
KCTD13+CDIPT Overexpression leads to Microcephaly



Golzio *et al*, Nature 2012

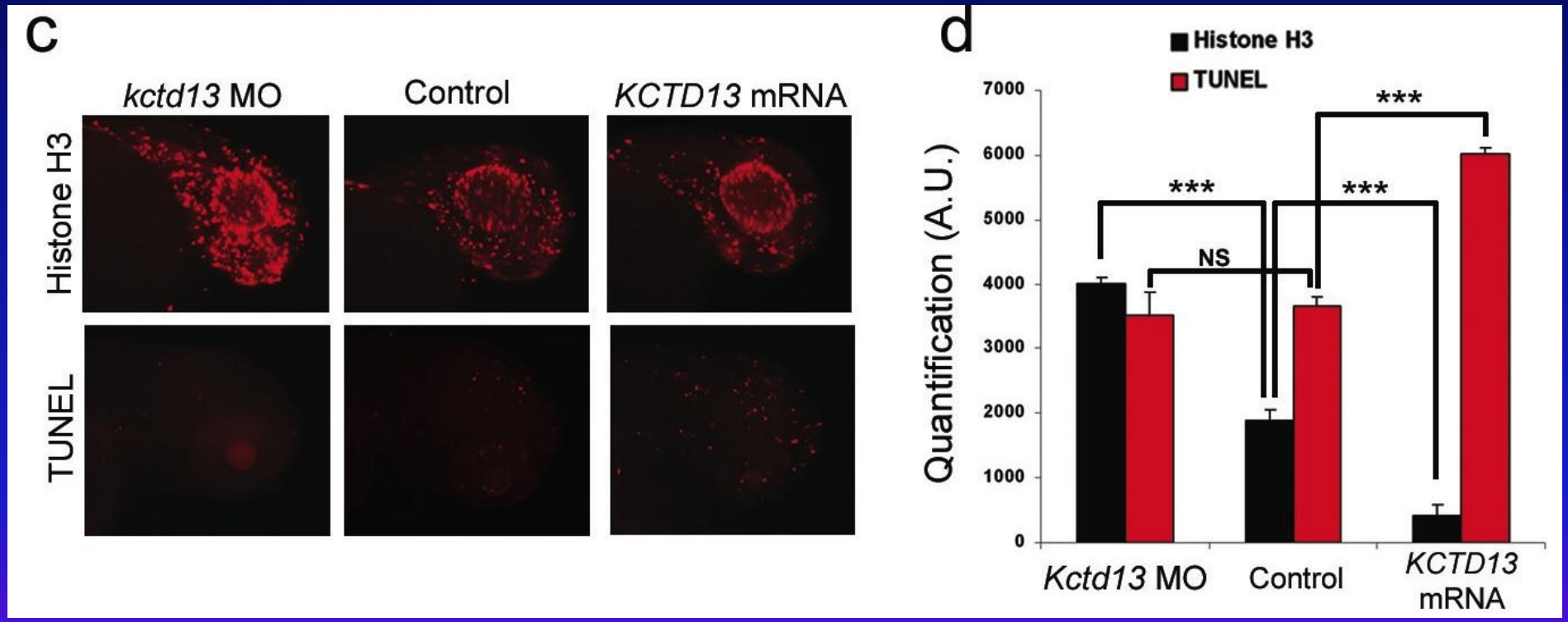
KCTD13 Dosage Changes lead to Head Size Defects

Microcephaly



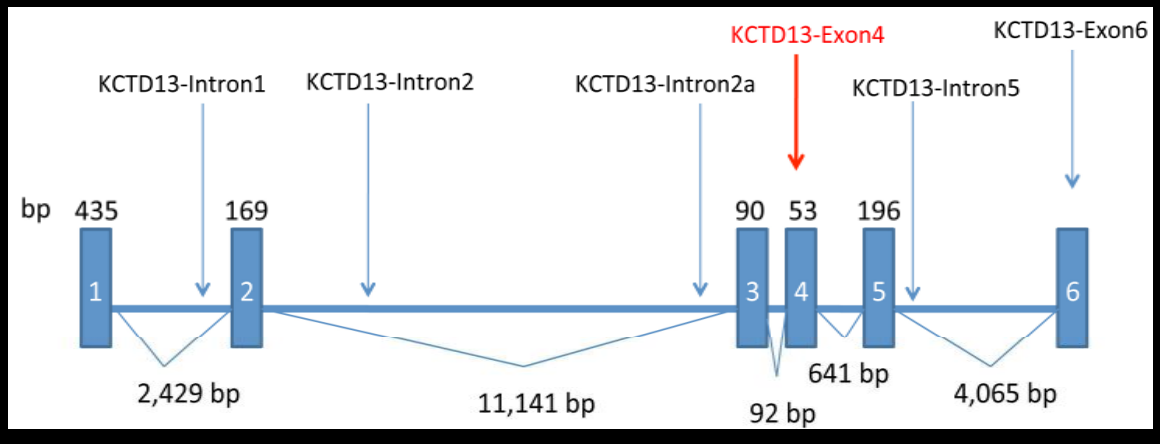
Golzio *et al*, Nature 2012

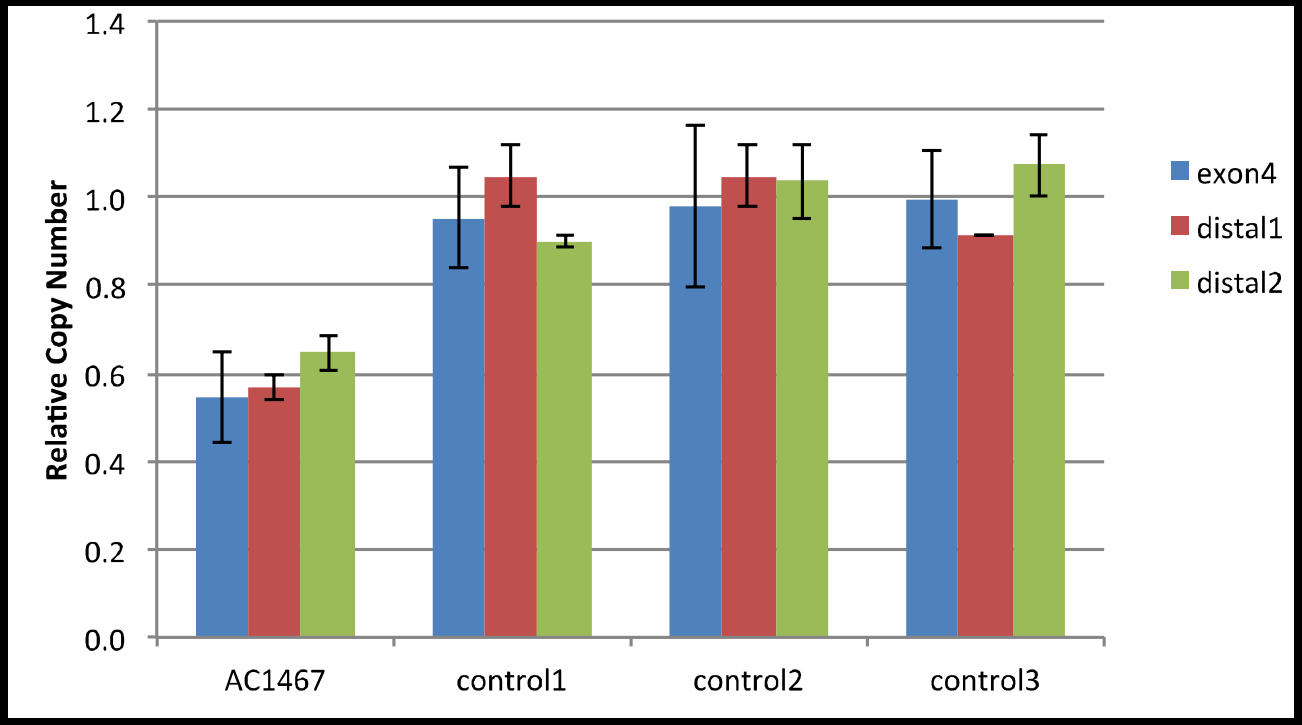
KCTD13 Dosage Changes lead to Proliferation and Apoptosis Defects at 2-3 dpf



Golzio *et al*, Nature 2012

De Novo deletion of exon 4 of *KCTD13* in a Patient with Autism





Golzio *et al*, Nature 2012

Summary

- ✓ Accelerated molecular diagnosis, early diagnosis
- ✓ Complementation of WES data with functional assays aid interpretation significantly
- ✓ Identify genetic phenomena such as synergistic effects, modifiers *in trans*, etc
- ✓ can be used to model CNVs with anatomical surrogates