

Clinical genetics and other aspects of neuropsychiatric disorders

Gholson Lyon, M.D. Ph.D.



STANLEY INSTITUTE FOR
COGNITIVE GENOMICS
COLD SPRING HARBOR LABORATORY

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Vignette #1: The genetic basis of a new syndrome with severe developmental delay and cardiac abnormalities.

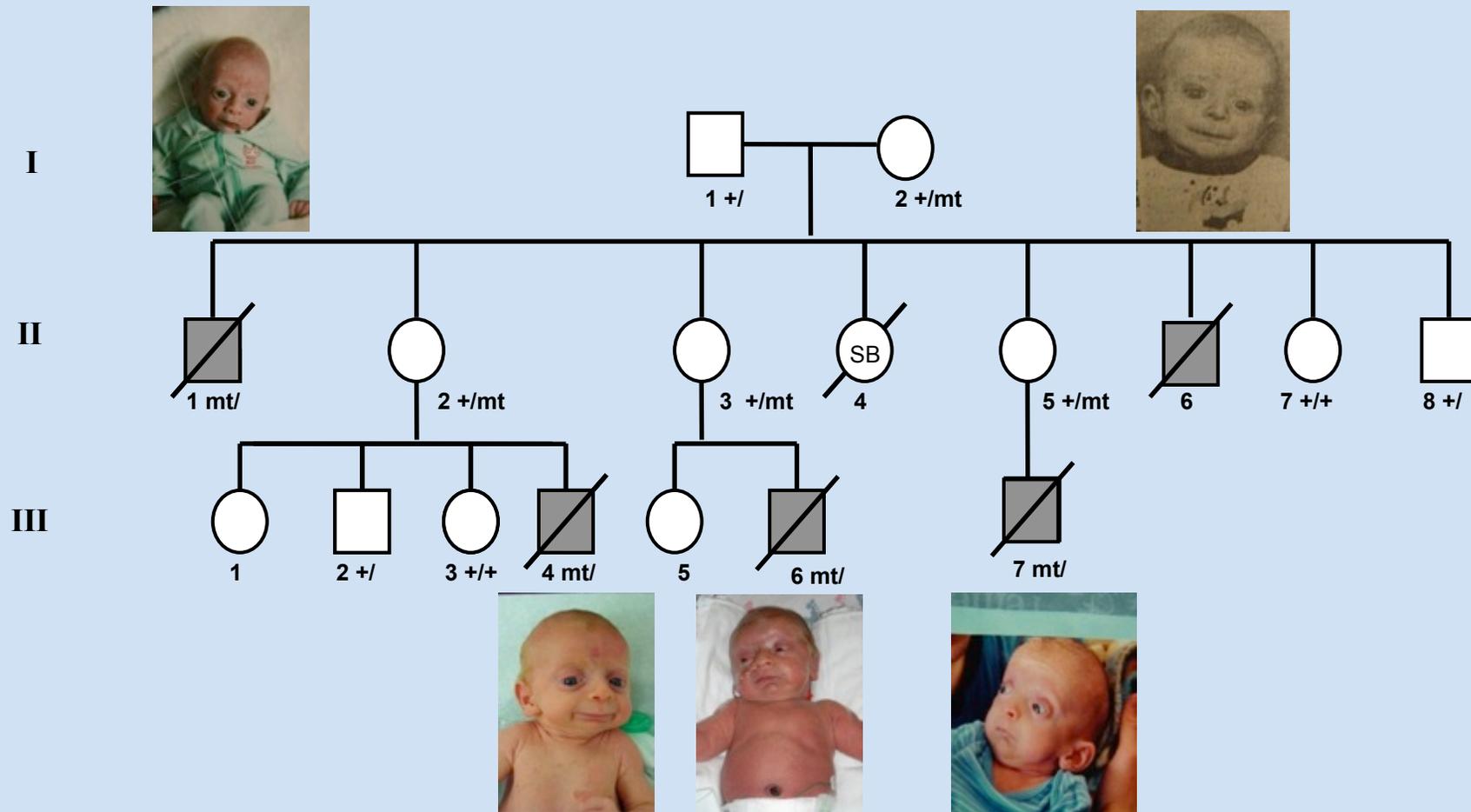
ARTICLE

Using VAAST to Identify an X-Linked Disorder Resulting in Lethality in Male Infants Due to N-Terminal Acetyltransferase Deficiency

Alan F. Rope,¹ Kai Wang,^{2,19} Rune Evjenth,³ Jinchuan Xing,⁴ Jennifer J. Johnston,⁵ Jeffrey J. Swensen,^{6,7} W. Evan Johnson,⁸ Barry Moore,⁴ Chad D. Huff,⁴ Lynne M. Bird,⁹ John C. Carey,¹ John M. Opitz,^{1,4,6,10,11} Cathy A. Stevens,¹² Tao Jiang,^{13,14} Christa Schank,⁸ Heidi Deborah Fain,¹⁵ Reid Robison,¹⁵ Brian Dalley,¹⁶ Steven Chin,⁶ Sarah T. South,^{1,7} Theodore J. Pysher,⁶ Lynn B. Jorde,⁴ Hakon Hakonarson,² Johan R. Lillehaug,³ Leslie G. Biesecker,⁵ Mark Yandell,⁴ Thomas Arnesen,^{3,17} and Gholson J. Lyon^{15,18,20,*}

The American Journal of Human Genetics 89, 1–16, July 15, 2011

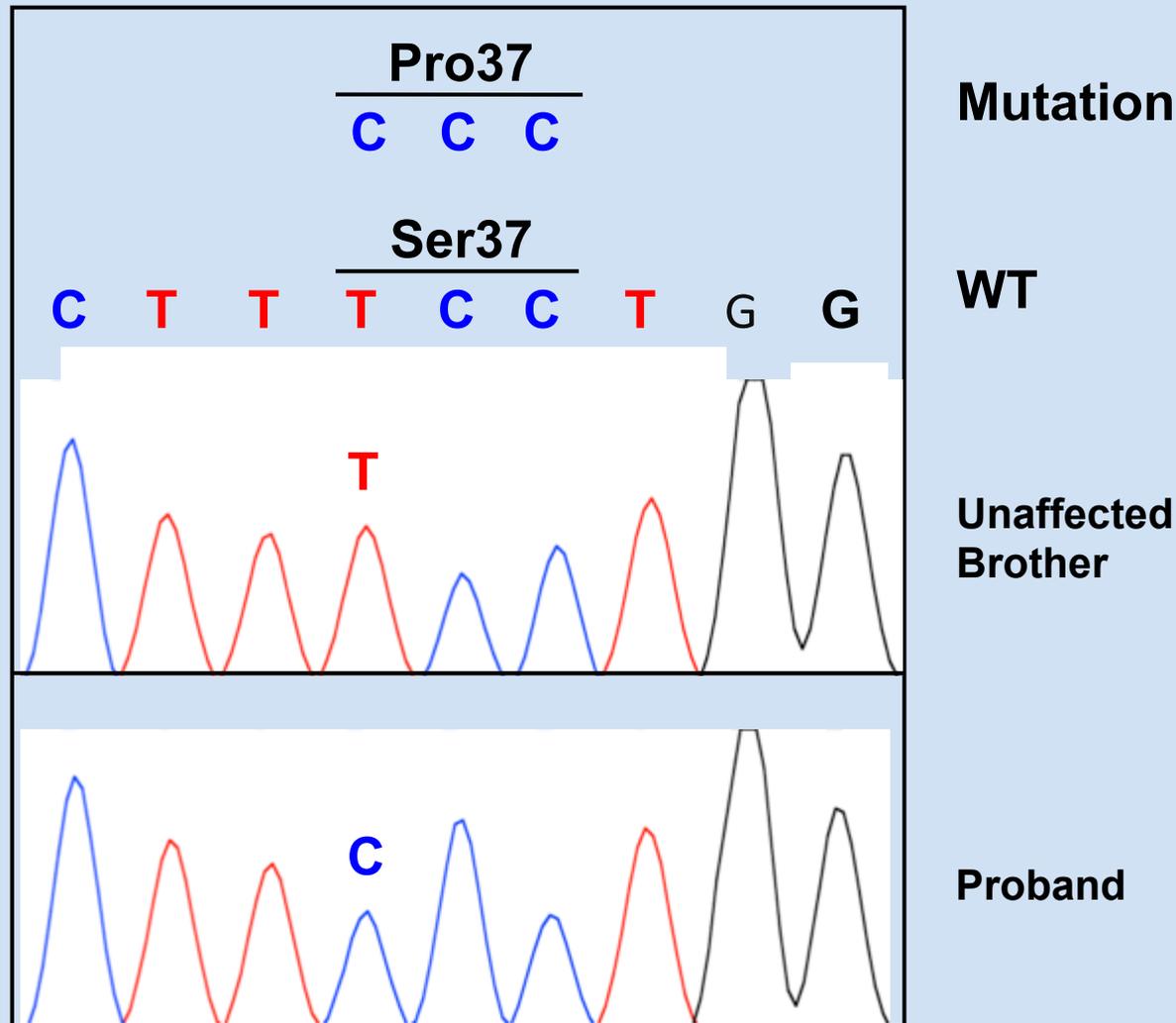
Family now in October 2011, with five mutation-positive boys dying from the disease.



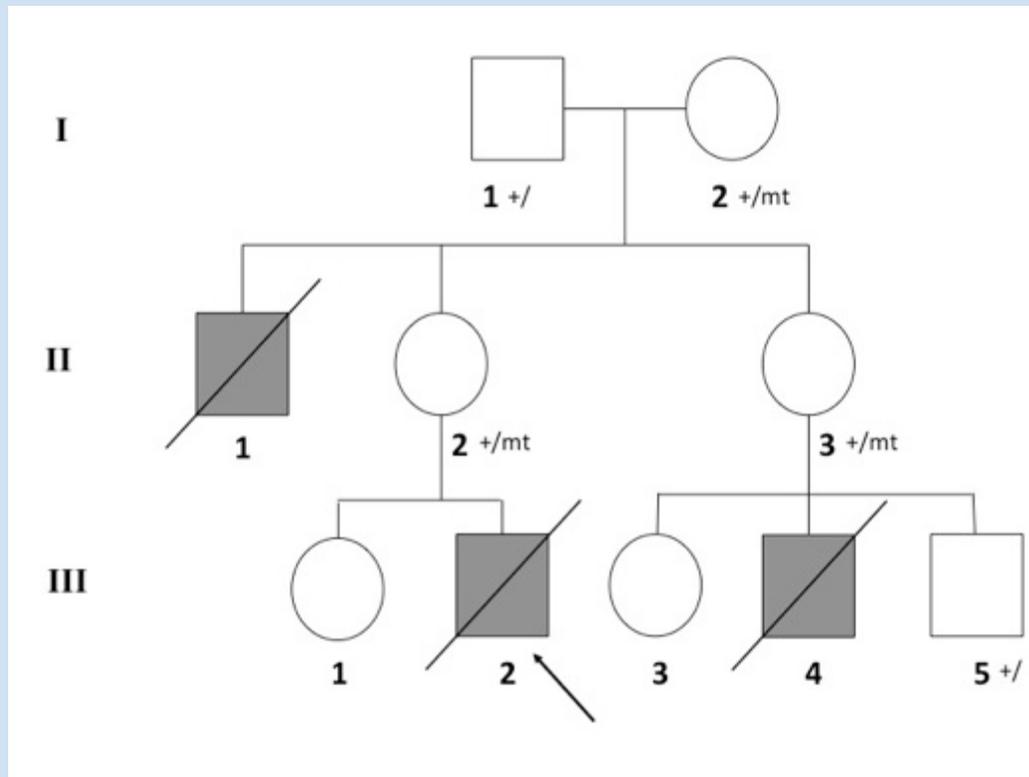
These are the Major Features of the Syndrome.

Table 1. Features of the syndrome	
Growth	post-natal growth failure
Development	global, severe delays
Facial	prominence of eyes, down-sloping palpebral fissures, thickened lids large ears beaking of nose, flared nares, hypoplastic alae, short columella protruding upper lip micro-retrognathia
Skeletal	delayed closure of fontanel broad great toes
Integument	redundancy / laxity of skin minimal subcutaneous fat cutaneous capillary malformations
Cardiac	structural anomalies (ventricular septal defect, atrial level defect, pulmonary artery stenoses) arrhythmias (Torsade de points, PVCs, PACs, SVtach, Vtach) death usually associated with cardiogenic shock preceded by arrhythmia.
Genital	inguinal hernia hypo- or cryptorchidism
Neurologic	hypotonia progressing to hypertonia cerebral atrophy neurogenic scoliosis
Shaded regions include features of the syndrome demonstrating variability. Though variable findings of the cardiac, genital and neurologic systems were observed, all affected individuals manifested some pathologic finding of each.	

This is the mutation we found... one nucleotide change out of 6 billion nucleotides in a diploid genome...



An unrelated second family was also identified, due to having the same mutation, but in a different genetic background.



II-1

III-2

Tentative name: Ogden Syndrome, in honor of where the first family lives, in Ogden, Utah



Bring clinical standards to human-genetics research

Study protocols need to be rigorous, because more than science is at stake. Sometimes participants' lives depend on the results, writes **Gholson J. Lyon**.

Nature. 2012 Feb 15;482(7385):300-1.



Guest post: Time to bring human genome sequencing into the clinic
02/16/2012



PRIVACY and PROGRESS in Whole Genome Sequencing

Presidential Commission
for the Study of Bioethical Issues

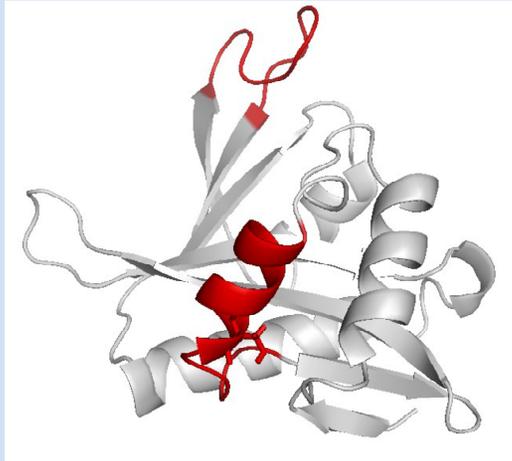
October 2012

Recommendation 4.1

Funders of whole genome sequencing research, relevant clinical entities, and the commercial sector should facilitate explicit exchange of information between genomic researchers and clinicians, while maintaining robust data protection safeguards, so that whole genome sequence and health data can be shared to advance genomic medicine.

Performing all whole genome sequencing in CLIA-approved laboratories would remove one of the barriers to data sharing. It would help ensure that whole genome sequencing generates high-quality data that clinicians and researchers can use to draw clinically relevant conclusions. It would also ensure that individuals who obtain their whole genome sequence data could share them more confidently in patient-driven research initiatives, producing more meaningful data. That said, current sequencing technologies and those in development are diverse and evolving, and standardization is a substantial challenge. Ongoing efforts, such as those by the Standardization of Clinical Testing working group are critical to achieving standards for ensuring the reliability of whole genome sequencing results, and facilitating the exchange and use of these data.²¹⁶

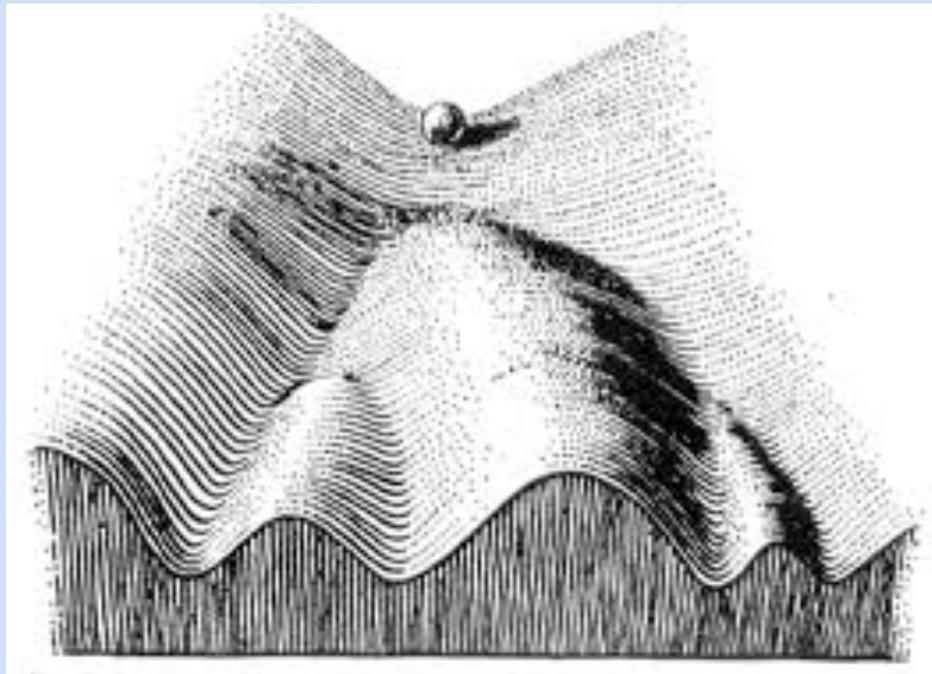
Big Question though:

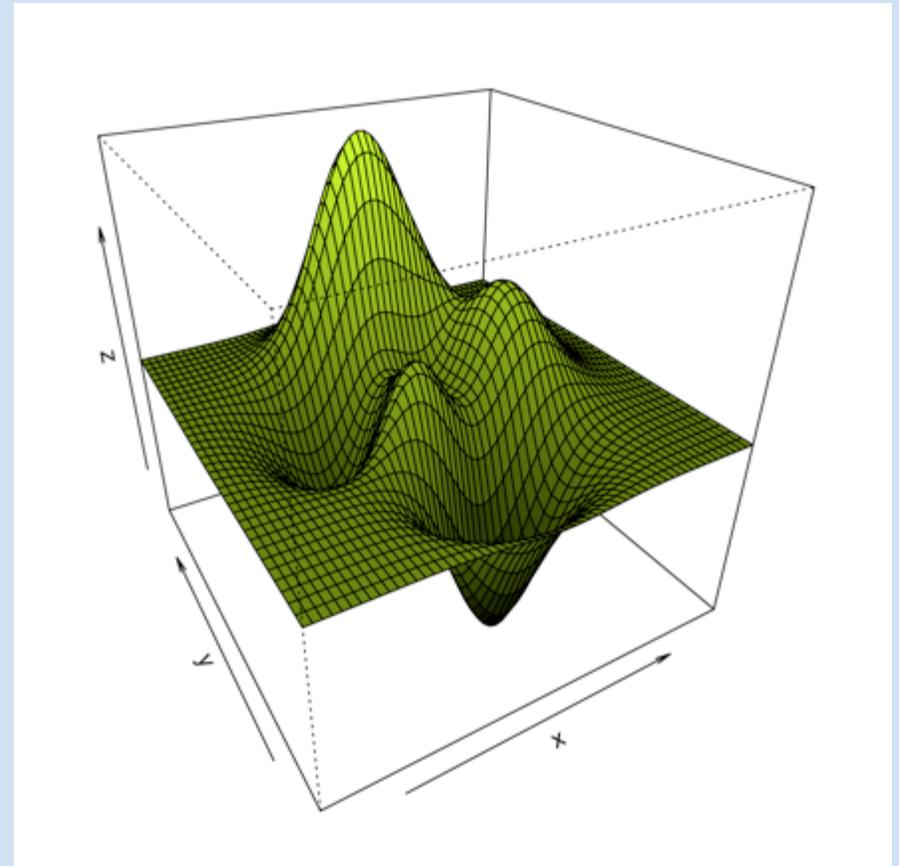
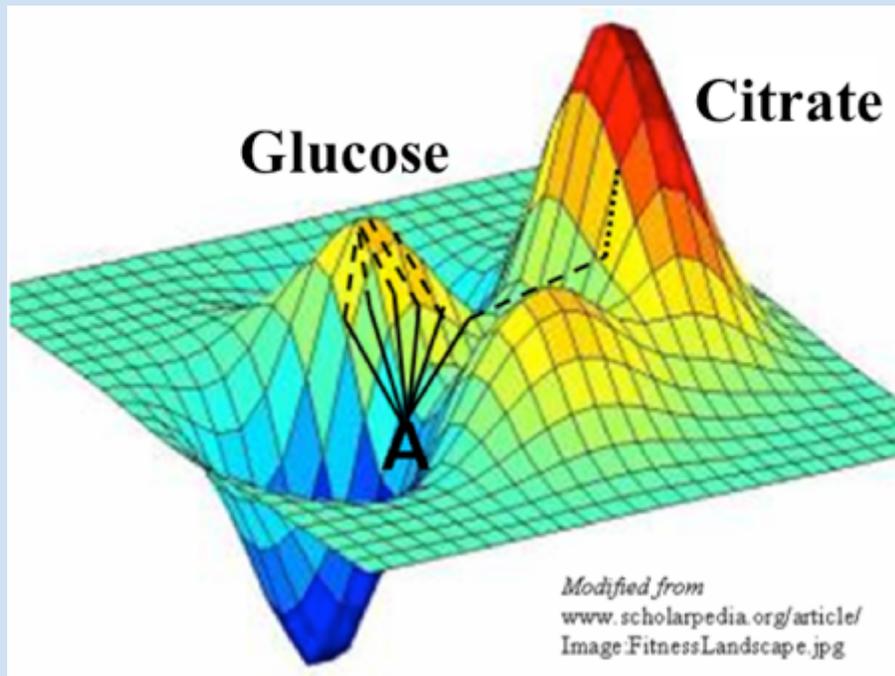


Simulated structure of S37P mutant



Waddington claimed that canals form in the epigenetic landscape during evolution, and that this heuristic is useful for understanding the unique qualities of biological robustness.





E. coli adapting to low glucose conditions, in the context of media containing citrate.
 – Richard Lenski experiment

"Finally, novel functions often emerge in rudimentary forms that must be refined to exploit the ecological opportunities. This three-step process — in which potentiation makes a trait possible, actualization makes the trait manifest, and refinement makes it effective — is probably typical of many new functions." - Lenski

The Biology of
MENTAL DEFECT

BY
LIONEL S. PENROSE, M.A., M.D.

WITH A PREFACE BY
PROFESSOR J. B. S. HALDANE, F.R.S.

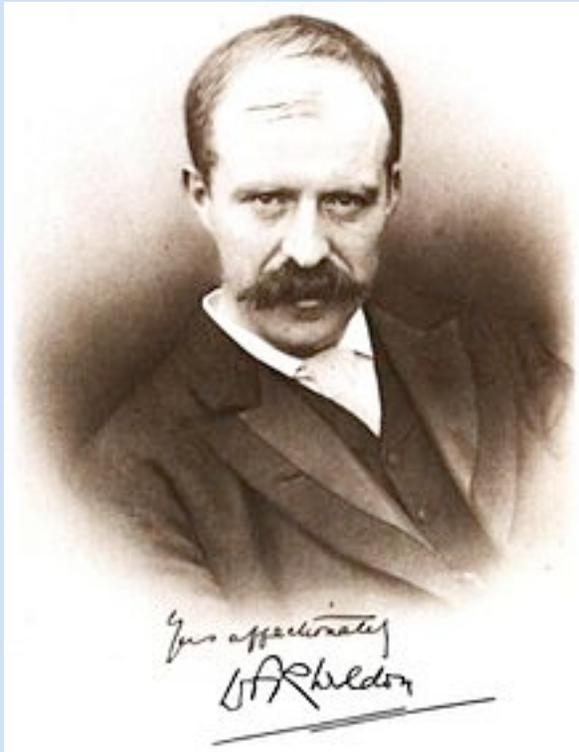


GRUNE & STRATTON
New York

1949

Beyond our Kuhnian inheritance

A recent lecture by Prof Greg Radick questions our scientific inheritance, through textbook histories of genetics and Thomas Kuhn's legacy
<http://www.guardian.co.uk/science/the-h-word/2012/aug/28/thomas-kuhn>



Walter Frank Raphael Weldon

Vs.



William Bateson

Forthcoming by Greg Radick. Scholarly edition of W. F. R. Weldon's Theory of Inheritance (1904-1905), coedited with Annie Jamieson.



Plate I.

Weldon, W. F. R. 1902. Mendel's laws of alternative inheritance in peas. *Biometrika*, 1:228-254.

Expression Issues

- We do not really know the expression of pretty much ALL mutations in **humans**, as we have not systematically sequenced or karyotyped any genetic alteration in **Thousands to Millions** of **randomly** selected people, nor categorized into ethnic classes, i.e. clans.
- There is a **MAJOR** clash of world-views, i.e. do single mutations drive outcome predominately, or are the results modified substantially by genetic background and/or environment? i.e. is there really such a thing as genetic determinism for **MANY** mutations?

Vignette #2: One person with very severe obsessive compulsive disorder, depression and intermittent psychoses

Individual Genome Sequencing Service

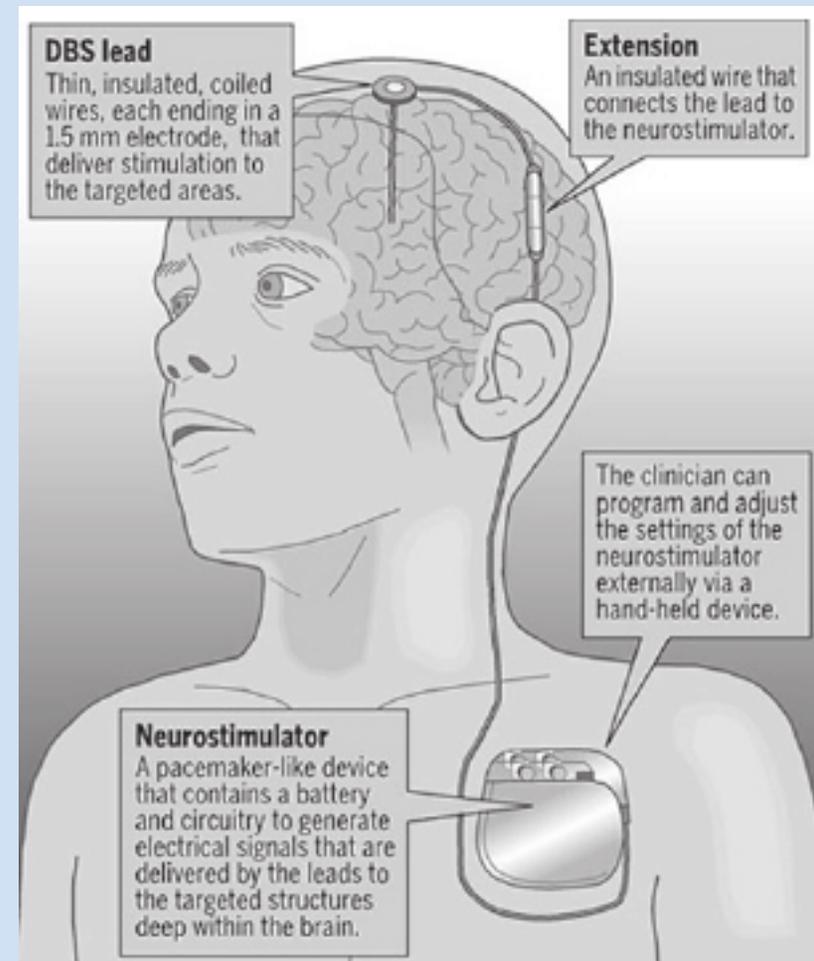
Available from Illumina's CLIA-certified laboratory.



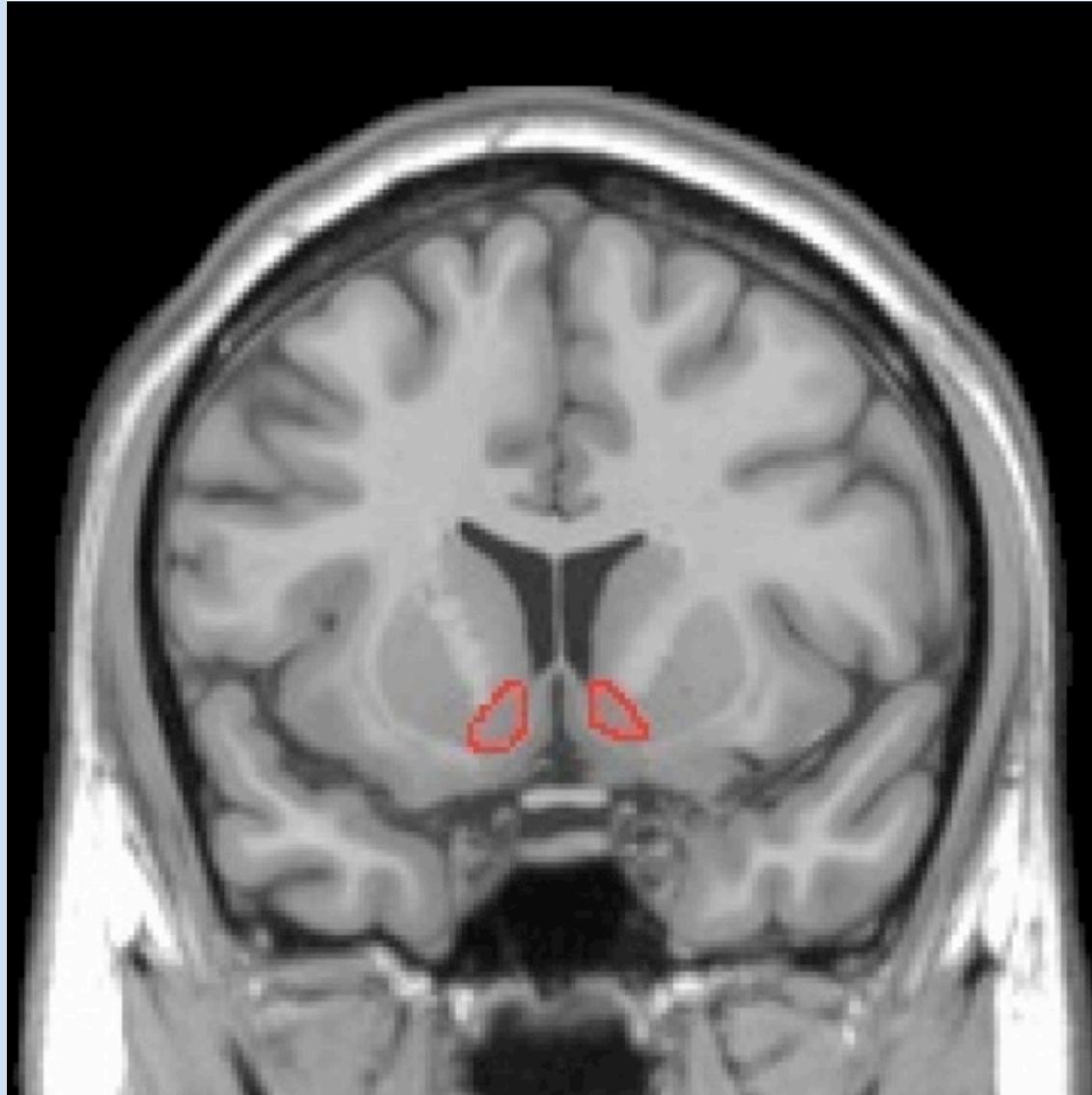
“This laboratory test was developed, and its performance characteristics were determined by the Illumina Clinical Services Laboratory (CLIA-certified, CAP-accredited). Consistent with laboratory-developed tests, it has not been cleared or approved by the U.S. Food and Drug Administration. If you have any questions or concerns about what you might learn through your genome sequence information, you should contact your doctor or a genetic counselor. Please note that Illumina does not accept orders for Individual Genome Sequencing services from Florida and New York.”

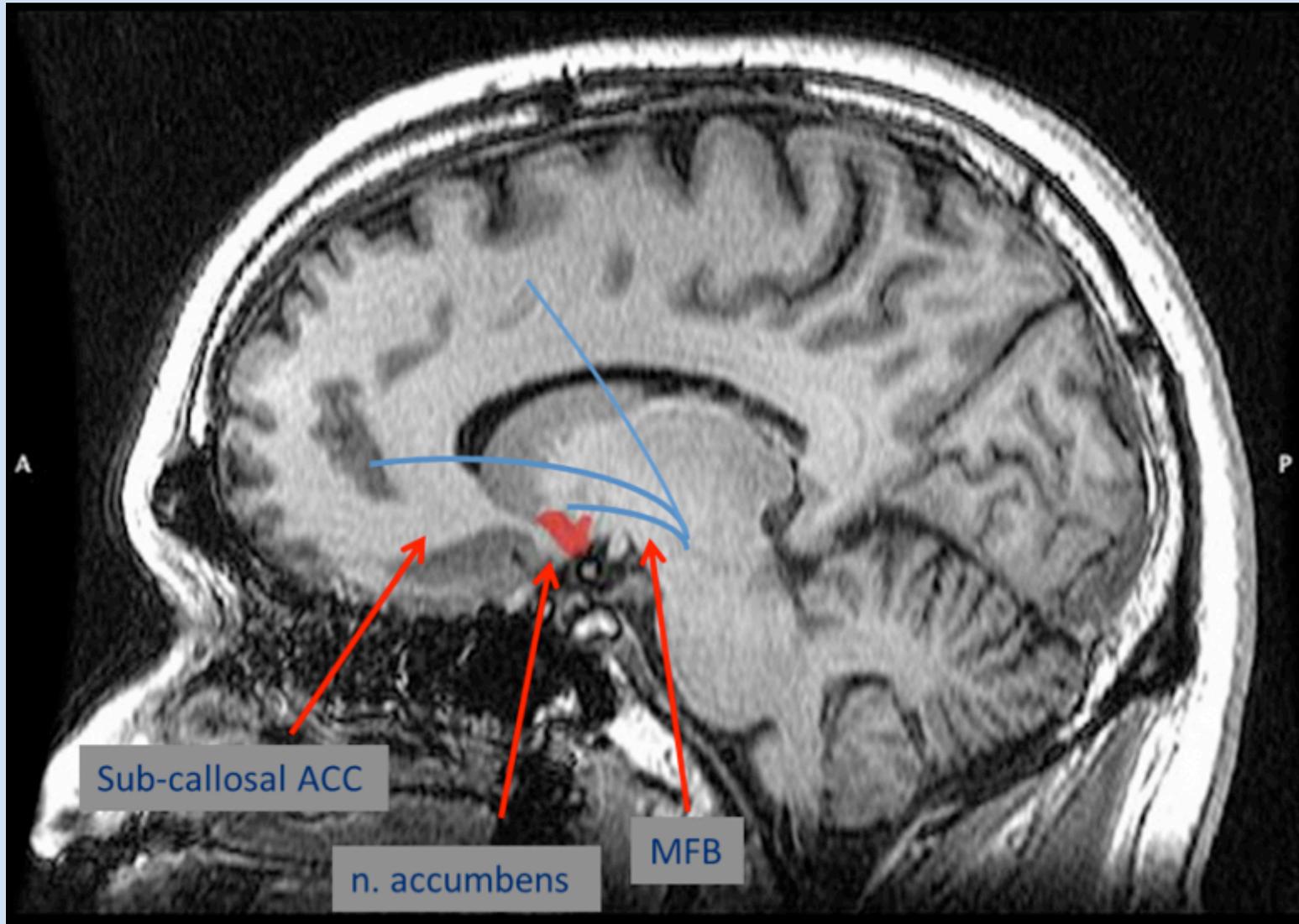
One person with very severe obsessive compulsive disorder, depression and intermittent psychoses

Gene name	Genomic coordinates	Amino acid change	Zygosity	Mutation type	Population Frequency	Clinical significance
MTHFR	chr1: 11854476	Glu>Ala	heterozygous	non-synon	T:77% G:23%	Susceptibility to psychoses, schizophrenia, occlusive vascular disease, neural tube defects, colon cancer, acute leukemia, and methylenetetra-hydrofolate reductase deficiency
BDNF	chr11: 27679916	Val>Met	heterozygous	non-synon	C:77% T:23%	Susceptibility to OCD, psychosis, and diminished response to exposure therapy
CHAT	chr10: 50824117	Asp>Asn	heterozygous	non-synon	G:85% A:15%	Susceptibility to schizophrenia and other psychopathological disorders.

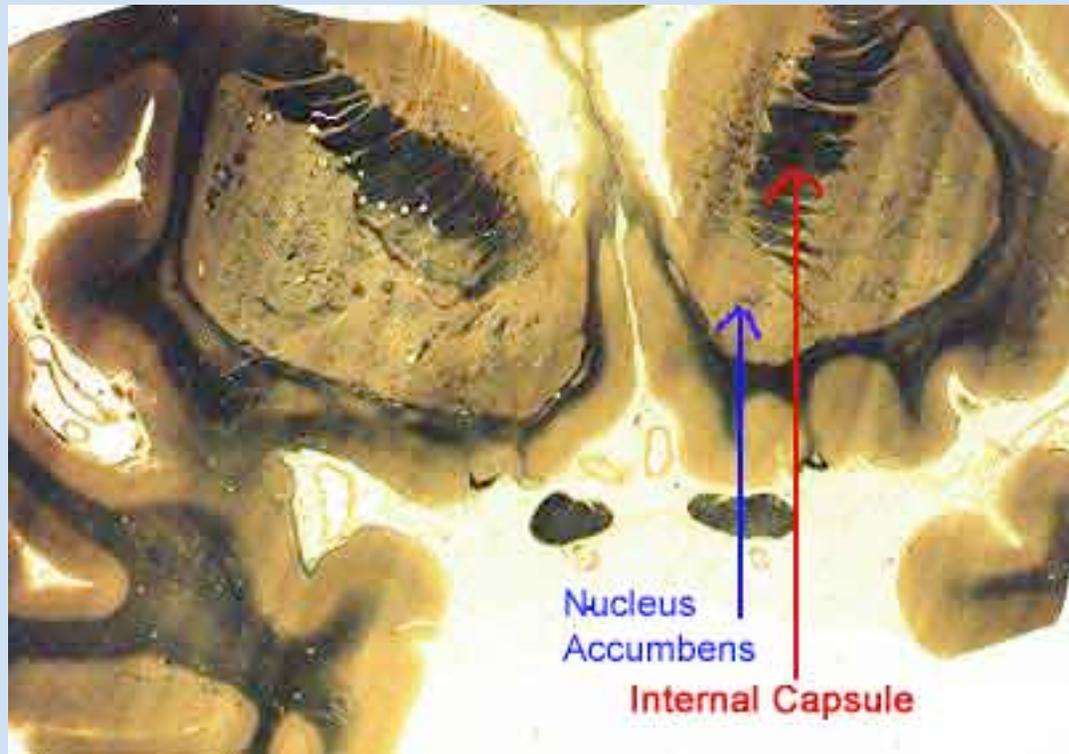


Nucleus accumbens





Approximate projections of the medial forebrain bundle to striatum, basal forebrain and prefrontal cortex (blue). Credit: Geoff B Hall, Via Wikimedia Commons (modified for current use)



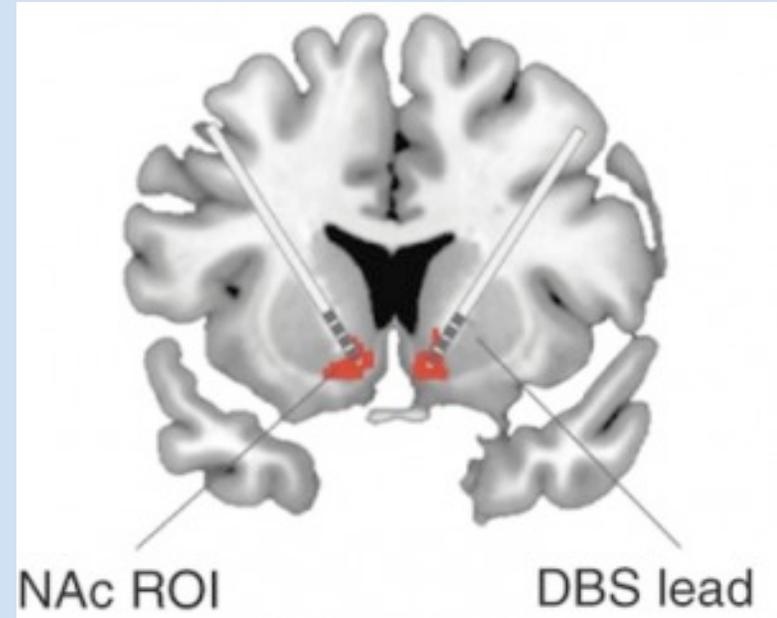
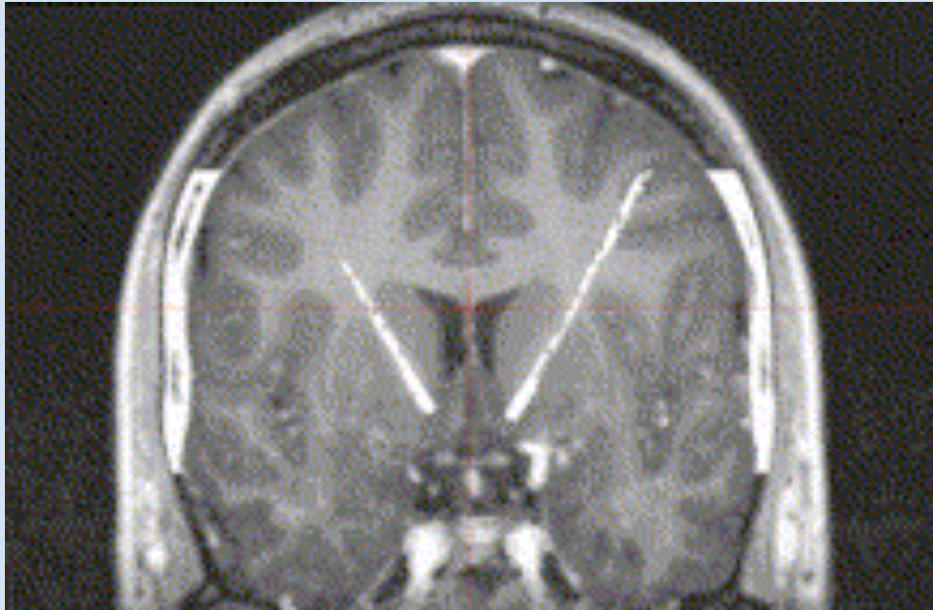
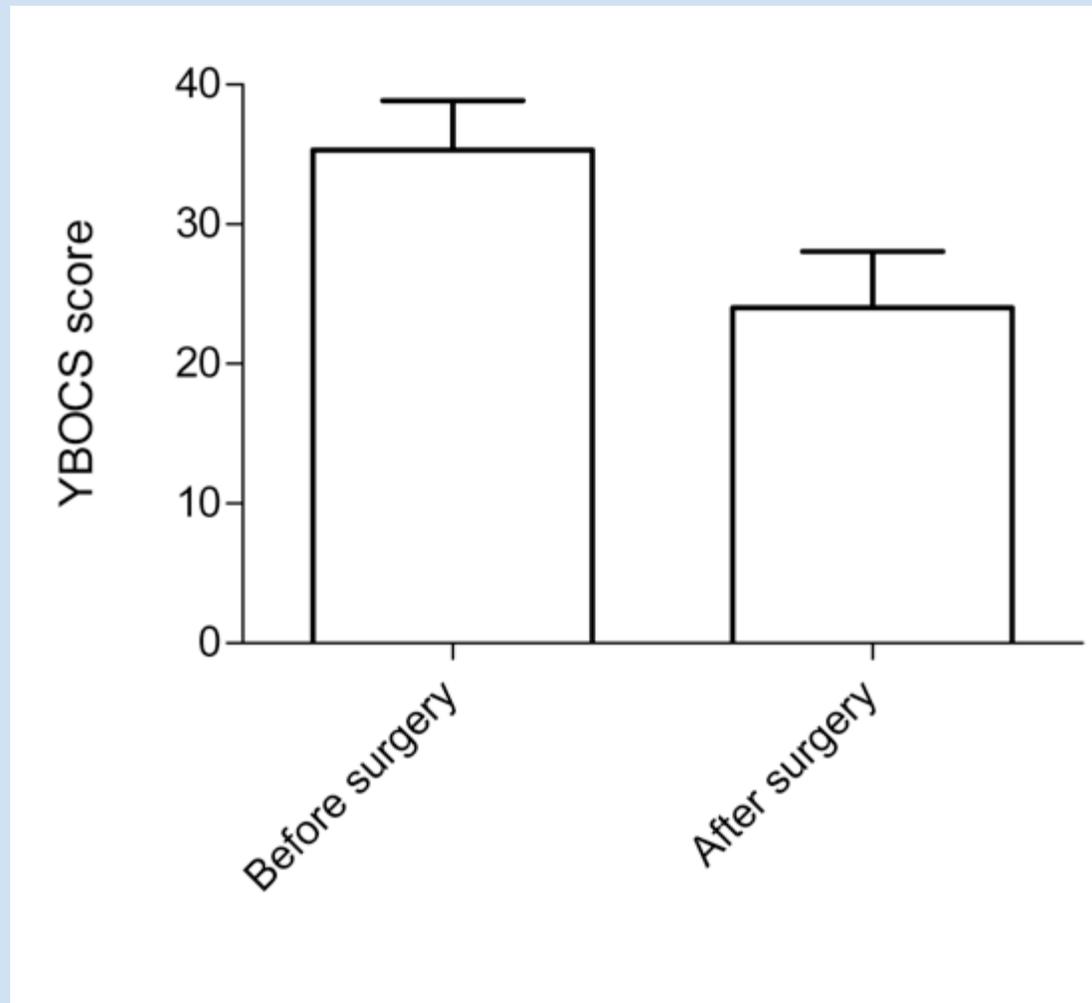


Fig. 1. Coronal section of the brain near the nucleus accumbens with the track of the electrodes on the left and right side.

Two year follow-up



Million Veteran Program: A Partnership with Veterans

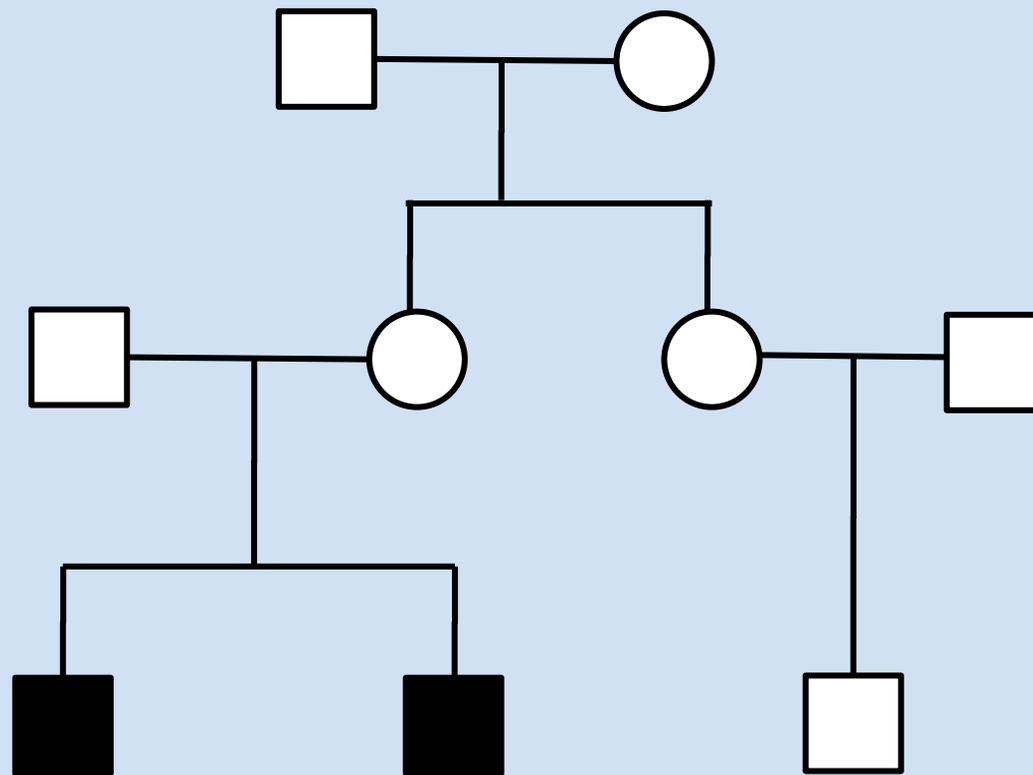


Genomics Advisory Panel

<http://lyonlab.cshl.edu/publications.html>

Preprints: **Lyon G.J.***, O'Rawe J, Robison R, Kiruluta E.S. , Higgins G, Reese M.G. Whole genome sequencing, clinical interpretation, and deep brain stimulation in a severely mentally ill person. 2013. *Corresponding author . [\(View PDF\)](#) [\(Download additional files\)](#)

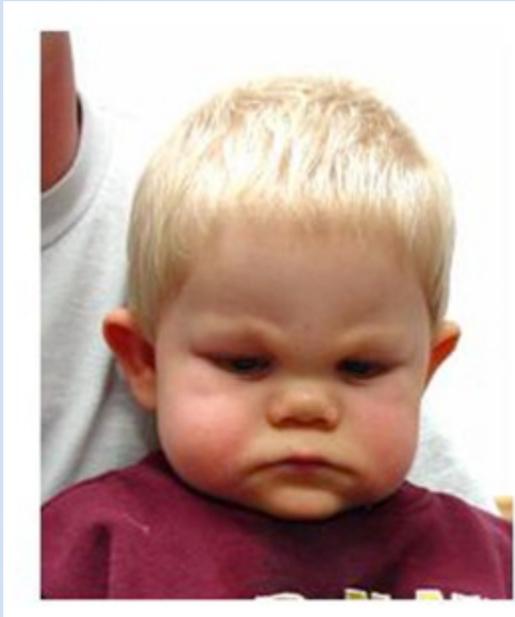
Vignette #3: New Syndrome with Mental Retardation, “Autism”, “ADHD”



Likely X-linked or Autosomal Recessive, with X-linked being supported by extreme X-skewing in the mother



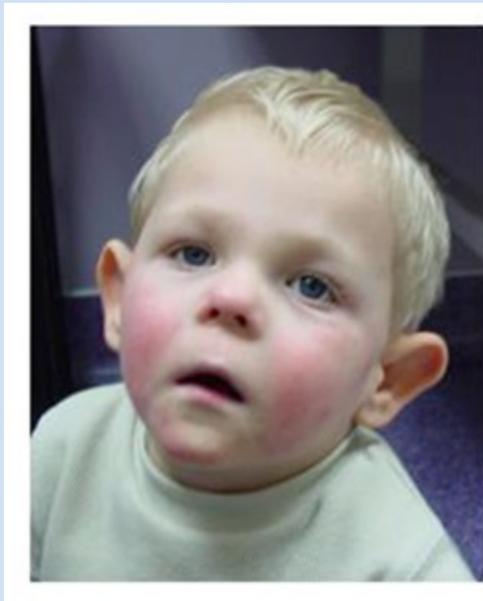
*Private Photographs – Do not further distribute.



1.5 years old



3.5 years old



3 years old



5 years old

Dysmorphic
Mental Retardation
“autism”
“ADHD”
Hearing difficulties

Workup Ongoing for past 10 years

- Numerous genetic tests negative, including negative for Fragile X and MANY candidate genes.
- Found one missense mutation in a known mental retardation gene, but the mutation is a very conservative nonsynonymous Asp to Glu. Is it relevant or not? What about the whole rest of the genome?

2 mutations present in mother and two boys, on X-chromosome, not in father, not in dbSNP135, not in 1000Genomes April 2012 release, and not in NHLBI 6500 Exomes

- Nonsyn SNV ZNF41 c.1191C>A p.Asp397Glu
- Nonsyn SNV TAF1 c.4010T>C p.Ile1337Thr

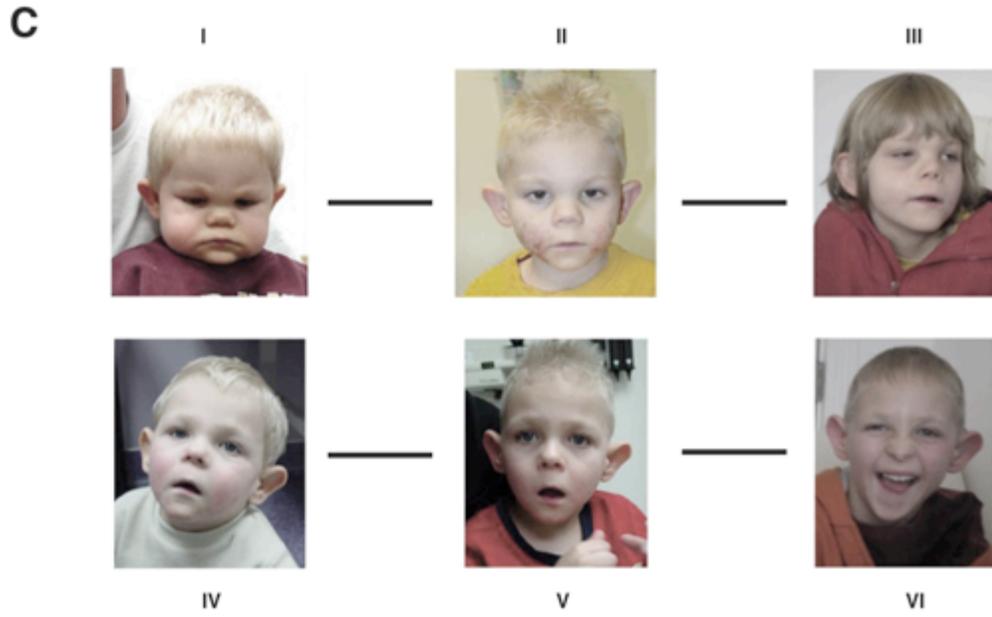
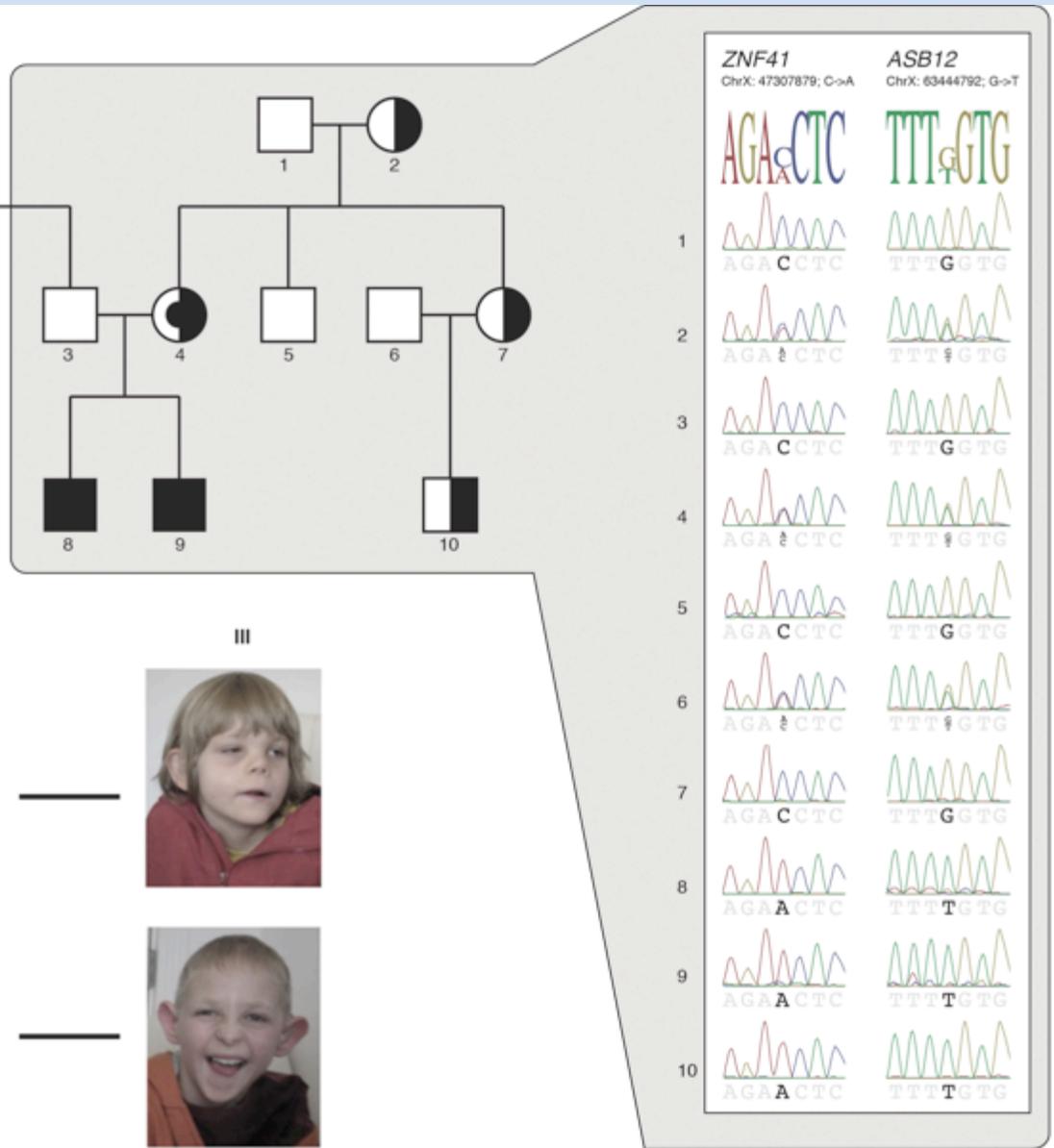
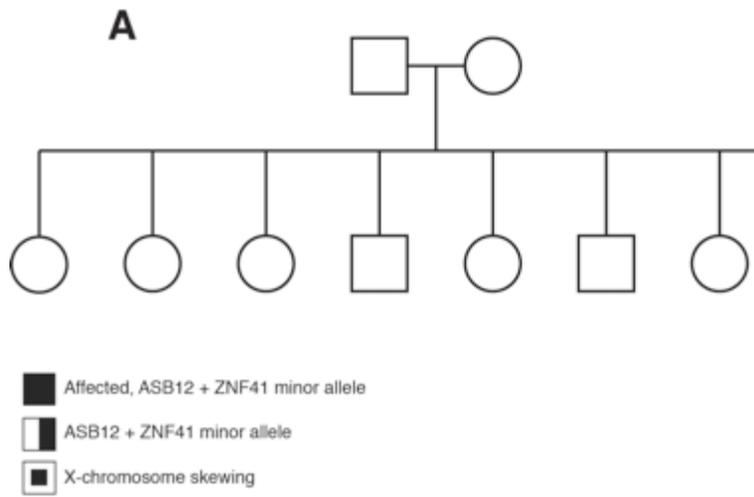
TAF1 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 250kDa

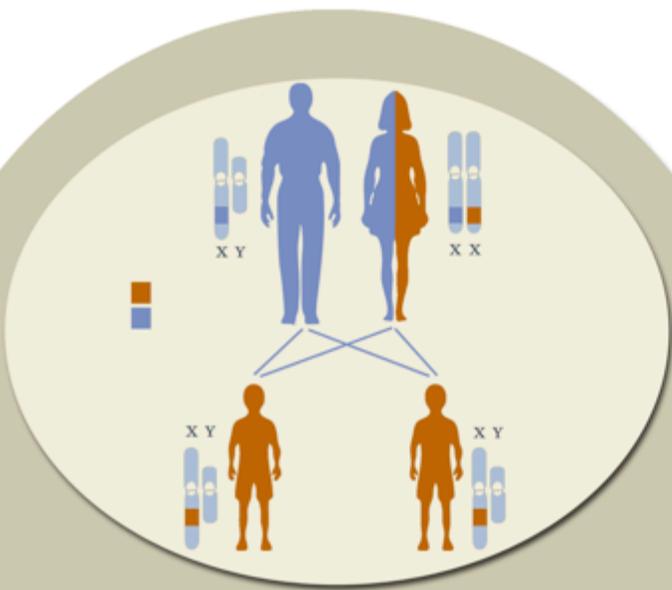
Mutations in the *ZNF41* Gene Are Associated with Cognitive Deficits: Identification of a New Candidate for X-Linked Mental Retardation

Sarah A. Shoichet,¹ Kirsten Hoffmann,¹ Corinna Menzel,¹ Udo Trautmann,² Bettina Moser,¹ Maria Hoeltzenbein,¹ Bernard Echenne,³ Michael Partington,⁴ Hans van Bokhoven,⁵ Claude Moraine,⁶ Jean-Pierre Fryns,⁷ Jamel Chelly,⁸ Hans-Dieter Rott,² Hans-Hilger Ropers,¹ and Vera M. Kalscheuer¹

¹Max-Planck-Institute for Molecular Genetics, Berlin; ²Institute of Human Genetics, University of Erlangen-Nuremberg, Erlangen-Nuremberg; ³Centre Hospitalier Universitaire de Montpellier, Hôpital Saint-Eloi, Montpellier, France, ⁴Hunter Genetics and University of Newcastle, Waratah, Australia; ⁵Department of Human Genetics, University Medical Centre, Nijmegen, The Netherlands; ⁶Services de Génétique-INSERM U316, CHU Bretonneau, Tours, France; ⁷Center for Human Genetics, Clinical Genetics Unit, Leuven, Belgium; and ⁸Institut Cochin de Génétique Moléculaire, Centre National de la Recherche Scientifique/INSERM, CHU Cochin, Paris

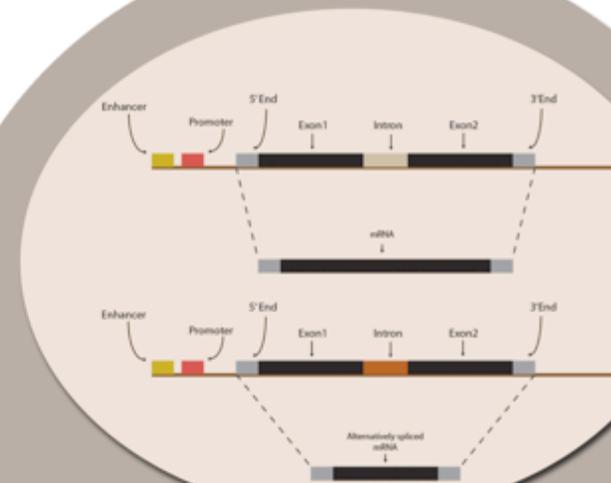
Am. J. Hum. Genet. 73:1341–1354, 2003





X-linked

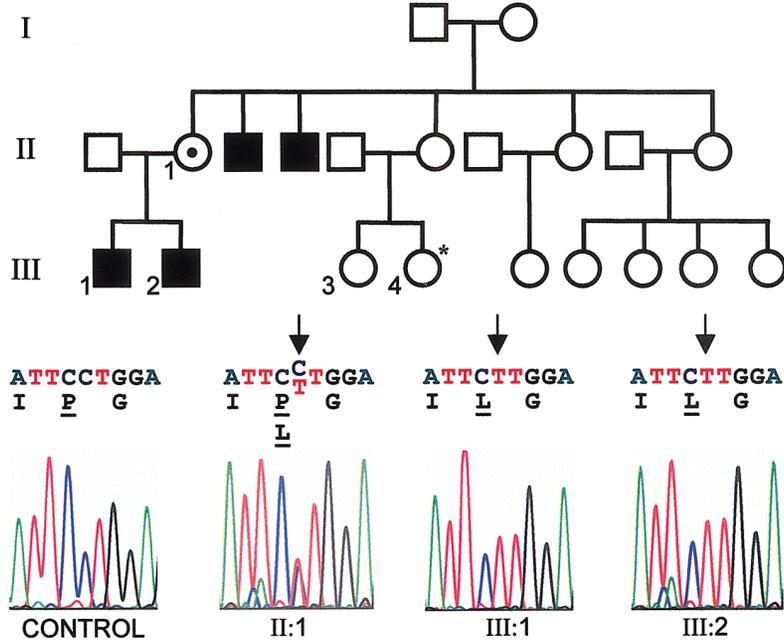
Gene	Locus	Exon	Protein
<i>ZNF41</i>	X:47307978	5	p.Asp397Glu
<i>ASB12</i>	X:63444792	2	p.Gly247Cys
<i>TAF1</i>	X:70621541	25	p.Ile1337Thr



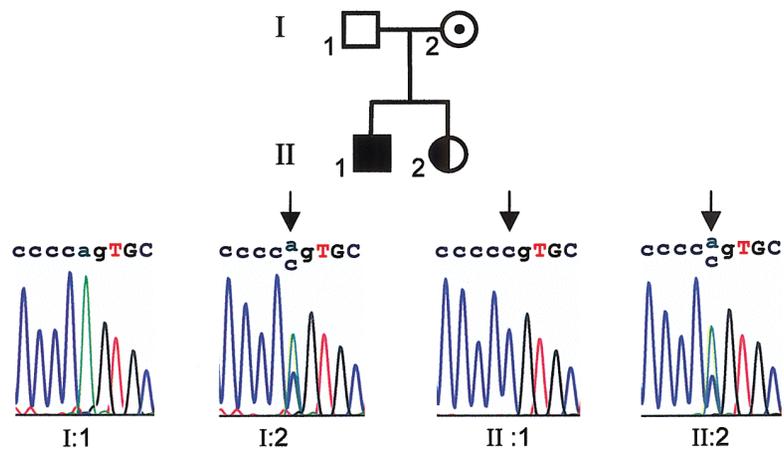
Non-coding

Gene	Locus	Exon	Protein
<i>UTR3 AR</i>	X:66945414	-----	-----
<i>FAM155B</i> (<i>dist=271971</i>)	X:68453113	-----	-----
<i>MIR221</i> (<i>dist=35606</i>)	X:45569979	-----	-----
<i>DMD-AS2</i> <i>intronic</i>	X:31284835	-----	-----
<i>MID1</i> (<i>dist=30252</i>)	X:10383096	-----	-----

A Family P13 with P111L mutation



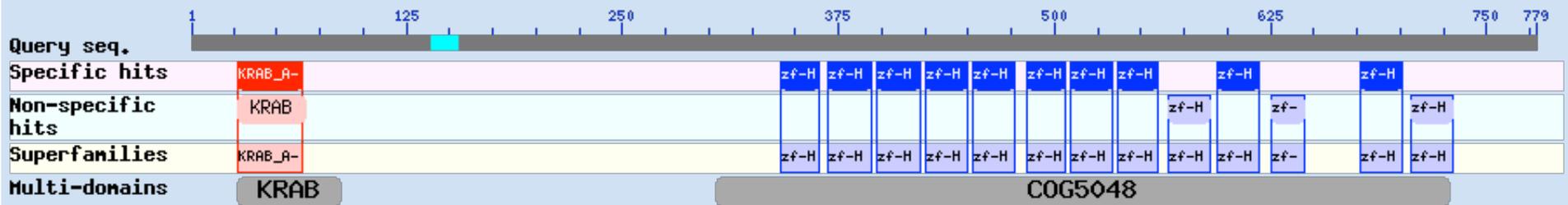
B Family P42 with 479-42A>C mutation



The two brothers with the P111L mutations reported in the prior paper do have mental deficiency, hyperkinesia, no motor or neurologic sign except for the delay, and slight dysmorphic facial anomalies: large low-set ears, thin upper lip, slight downward palpebral slants, but no upturned nose, and a short philtrum. The mother was normal in appearance.

- Previously reported P111L change in the ZNF41 protein has now also been found in two "male controls" (EVS server, ESP6500), and furthermore, there are two rare, likely heterozygous ZNF41 frameshift mutations and one heterozygous stop-gained mutation reported in control individuals (ESP6500) (personal communication from Dr. Vera Kalscheuer).

ZNF41



- KRAB (Kruppel-associated box) domain -A box.
- The KRAB domain is a transcription repression module, found in a subgroup of the zinc finger proteins (ZFPs) of the C2H2 family, KRAB-ZFPs. KRAB-ZFPs comprise the largest group of transcriptional regulators in mammals, and are only found in tetrapods.
- The KRAB domain is a protein-protein interaction module which represses transcription through recruiting corepressors. The KAP1/ KRAB-AFP complex in turn recruits the heterochromatin protein 1 (HP1) family, and other chromatin modulating proteins, leading to transcriptional repression through heterochromatin formation.

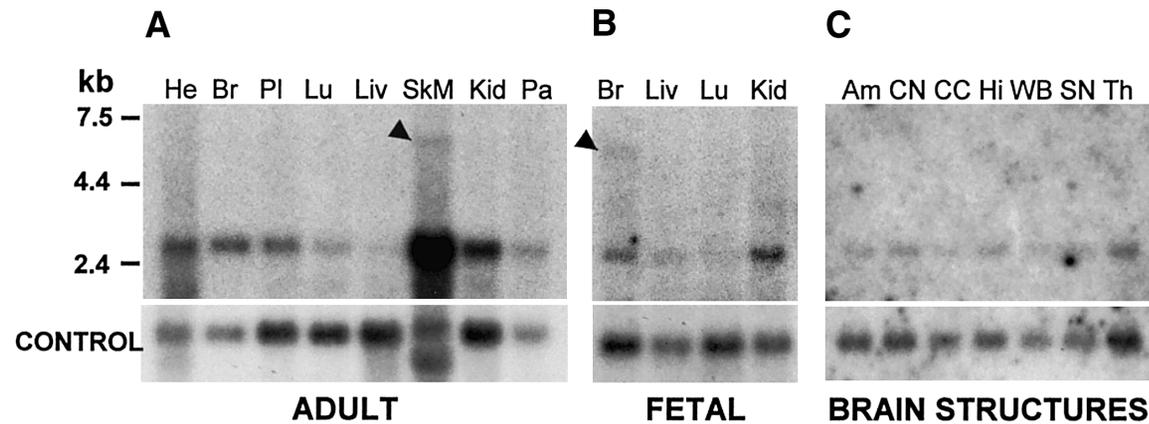


Figure 6 Northern blot hybridization of *ZNF41*, by use of a probe corresponding to nucleotides 621–1099 of *ZNF41* transcript variant 1. *A*, Adult tissues (left to right): heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas. *B*, Fetal tissues (left to right): brain, lung, liver, and kidney. *C*, Adult brain structures (left to right): amygdala, caudate nucleus, corpus callosum, hippocampus, whole brain, substantia nigra, and thalamus. Black arrowheads highlight the presence of a novel 6-kb transcript. *Actin* (*A* and *C*) or *GAPDH* (*B*) served as controls for RNA loading.

Proving Causality

- Will need to find a second, unrelated family with same exact mutation and similar phenotype.
- Can also perform in vitro/in vivo studies and structural modeling, and make knock-in mice and/or test in zebrafish, etc... for biological function.

Genotype First, Phenotype Second AND Longitudinally

Human Molecular Genetics, 2010, Vol. 19, Review Issue 2 R176–R187
doi:10.1093/hmg/ddq366
Advance Access published on August 31, 2010

Phenotypic variability and genetic susceptibility to genomic disorders

Santhosh Girirajan and Evan E. Eichler*

Department of Genome Sciences, Howard Hughes Medical Institute, University of Washington School of Medicine,
PO Box 355065, Foegen S413C, 3720 15th Avenue NE, Seattle, WA 98195, USA

Genome-Wide Association Study of Multiplex Schizophrenia Pedigrees

Am J Psychiatry Levinson et al.; *AiA*:1–11

“Rare CNVs were observed in regions with strong previously documented association with schizophrenia, but with variable patterns of segregation. This should serve as a reminder that we still know relatively little about the distribution of these CNVs in the entire population (e.g., in individuals with no or only mild cognitive problems) or about the reasons for the emergence of schizophrenia in only a minority of carriers, so great caution is required in genetic counseling and prediagnosis.”

Clinical Validity?

This is SO complex that the only solid way forward is with a “networking of science” model, i.e. online database with genotype and phenotype longitudinally tracked for thousands of volunteer families.



PatientsLikeMe



REVIEW

Identifying disease mutations in genomic medicine settings: current challenges and how to accelerate progress

Gholson J Lyon^{*1,2} and Kai Wang^{*2,3}



Contents lists available at [SciVerse ScienceDirect](#)

Applied & Translational Genomics

journal homepage: www.elsevier.com/locate/atg



Practical, ethical and regulatory considerations for the evolving medical and research genomics landscape

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^b *Utah Foundation for Biomedical Research, Salt Lake City, UT, United States*

^c *New York Genome Center, New York City, NY, United States*

The End

O'Rawe *et al. Genome Medicine* 2013, **5**:28
<http://genomemedicine.com/content/5/3/28>



RESEARCH

Open Access

Low concordance of multiple variant-calling pipelines: practical implications for exome and genome sequencing

Jason O'Rawe^{1,2}, Tao Jiang³, Guangqing Sun³, Yiyang Wu^{1,2}, Wei Wang⁴, Jingchu Hu³, Paul Bodily⁵, Lifeng Tian⁶, Hakon Hakonarson⁶, W Evan Johnson⁷, Zhi Wei⁴, Kai Wang^{8,9*} and Gholson J Lyon^{1,2,9*}

Major Conclusion: Clinical Validity?

This is SO complex that the only solid way forward is with a “networking of science” model, i.e. online database with genotype and phenotype longitudinally tracked for thousands of volunteer families.

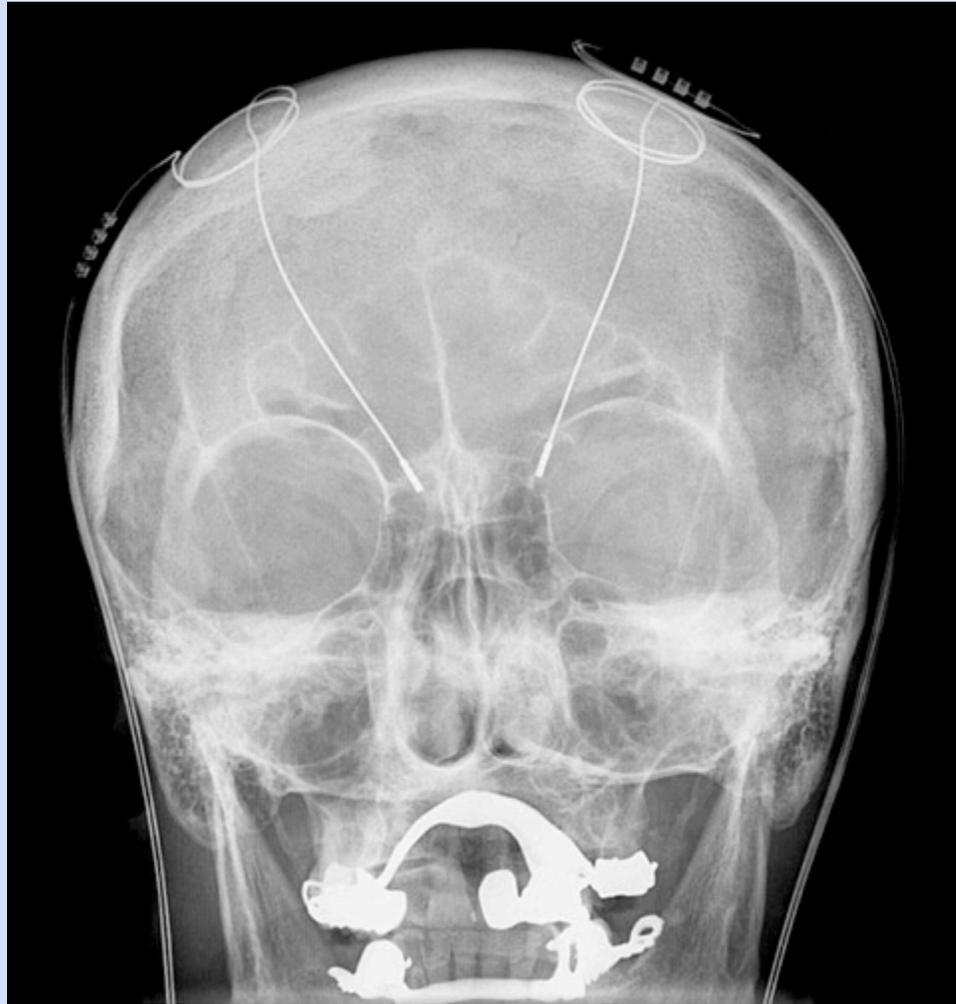


PatientsLikeMe



Sequenced whole genomes of Mother, Father and Two Boys, using Complete Genomics

- Sequenced “whole” genomes to obtain noncoding and other non-exonic regions.
- No obvious pathogenic CNVs – microarrays normal.
- ~6 million variants total in the 4 people different from Hg19 reference genome.
- No homozygous autosomal recessive mutations found.
- No Nonsense/Frameshift mutations in both boys.
- 2 mutations present in mother and two boys, on X-chromosome, not in father, not in dbSNP135, not in 1000Genomes April 2012 release, and not in NHLBI 6500 Exomes



DBS-probes shown in X-ray of the skull (white areas around [maxilla](#) and [mandible](#) represent metal [dentures](#) and are unrelated to DBS devices)

“Biological Indeterminacy”

- Bateson became famous as the outspoken [Mendelian](#) antagonist of [Walter Raphael Weldon](#), his former teacher, and [Karl Pearson](#) who led the [biometric](#) school of thinking. This concerned the debate over [saltationism](#) versus [gradualism](#) (Darwin had been a gradualist, but Bateson was a saltationist). Later, [Ronald Fisher](#) and [J.B.S. Haldane](#) showed that discrete mutations were compatible with gradual evolution: see the [modern evolutionary synthesis](#).

- Seguin E. 1866, - “our incomplete studies do not permit actual classification; but it is better to leave things by themselves rather than to force them into classes which have their foundation only on paper” - from Idiocy and its treatment by the physiological method.
- Walter Frank Raphael Weldon 1902 – “the accumulation of records, in which results are massed together in ill-defined categories of variable and uncertain extent, can only result in harm”.

Diagnostic Criteria for 299.00 Autistic Disorder

Diagnostic and Statistical Manual of Mental Disorders: DSM IV

(I) A total of six (or more) items from (A), (B), and (C), with at least two from (A), and one each from (B) and (C)

(A) qualitative impairment in social interaction, as manifested by at least two of the following:

1. marked impairments in the use of multiple nonverbal behaviors such as eye-to-eye gaze, facial expression, body posture, and gestures to regulate social interaction
2. failure to develop peer relationships appropriate to developmental level
3. a lack of spontaneous seeking to share enjoyment, interests, or achievements with other people, (e.g., by a lack of showing, bringing, or pointing out objects of interest to other people)
4. lack of social or emotional reciprocity (note: in the description, it gives the following as examples: not actively participating in simple social play or games, preferring solitary activities, or involving others in activities only as tools or "mechanical" aids)

(B) qualitative impairments in communication as manifested by at least one of the following:

1. delay in, or total lack of, the development of spoken language (not accompanied by an attempt to compensate through alternative modes of communication such as gesture or mime)
2. in individuals with adequate speech, marked impairment in the ability to initiate or sustain a conversation with others
3. stereotyped and repetitive use of language or idiosyncratic language
4. lack of varied, spontaneous make-believe play or social imitative play appropriate to developmental level

(C) restricted repetitive and stereotyped patterns of behavior, interests and activities, as manifested by at least two of the following:

1. encompassing preoccupation with one or more stereotyped and restricted patterns of interest that is abnormal either in intensity or focus
2. apparently inflexible adherence to specific, nonfunctional routines or rituals
3. stereotyped and repetitive motor mannerisms (e.g hand or finger flapping or twisting, or complex whole-body movements)
4. persistent preoccupation with parts of objects

(II) Delays or abnormal functioning in at least one of the following areas, with onset prior to age 3 years:

- (A) social interaction
- (B) language as used in social communication
- (C) symbolic or imaginative play

(III) The disturbance is not better accounted for by Rett's Disorder or Childhood Disintegrative Disorder

OBSERVATIONS ON AN ETHNIC CLASSIFICATION OF IDIOTS *

J. LANGDON H. DOWN M.D., *London*

London Hospital Clinical Lecture Report. 3, 259-262, 1866.

“Those who have given any attention to congenital mental lesions, must have been frequently puzzled how to arrange, in any satisfactory way, the different classes of this defect which may have come under their observation. Nor will the difficulty be lessened by an appeal to what has been written on the subject. The systems of classification are generally so vague and artificial, that, not only do they assist but feebly, in any mental arrangement of the phenomena represented, but they completely fail in exerting any practical influence on the subject.”