Clinical genetics and other aspects of neuropsychiatric disorders

Gholson Lyon, M.D. Ph.D.

Acknowledgments

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our study families

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

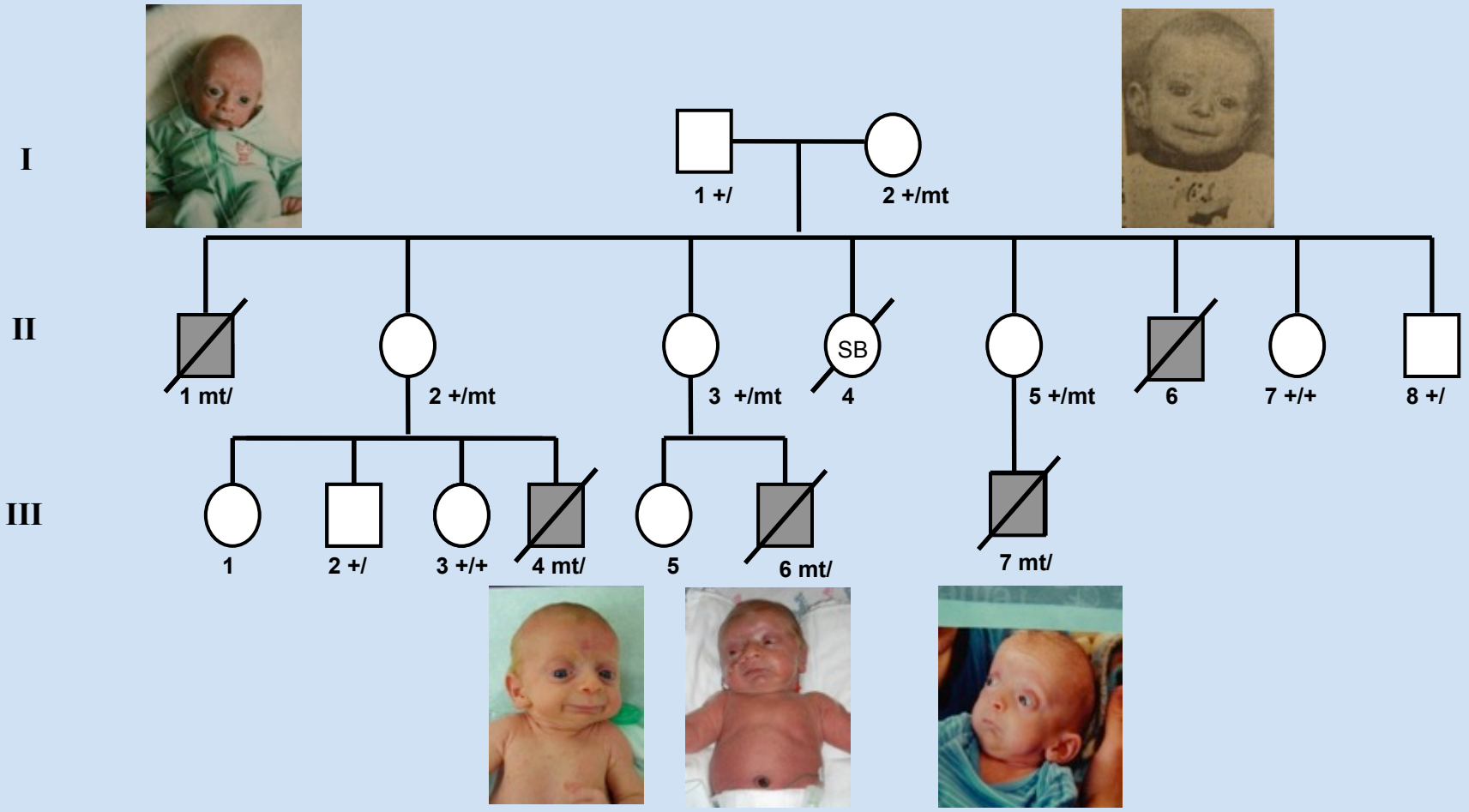
ARTICLE

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

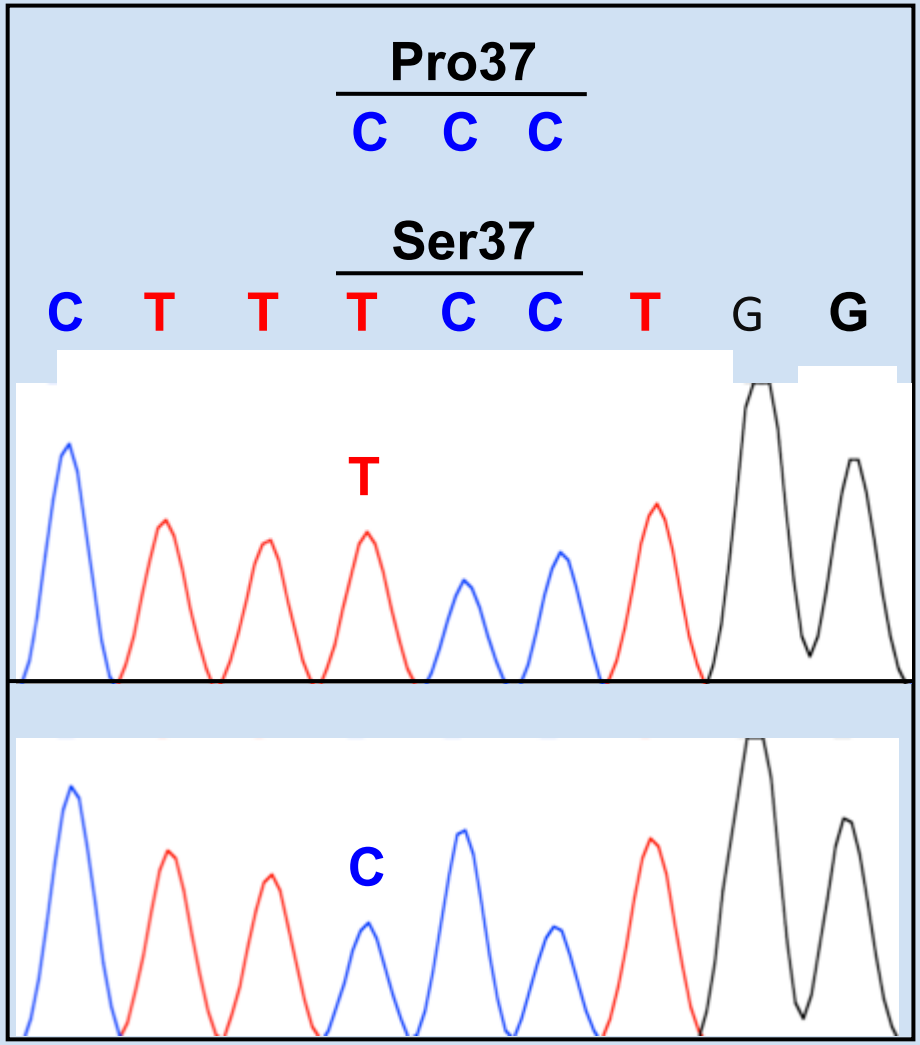
Family now in October 2011, with five mutation-positive boys dying from the disease.



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 fontanels 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 arrythmia.
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…



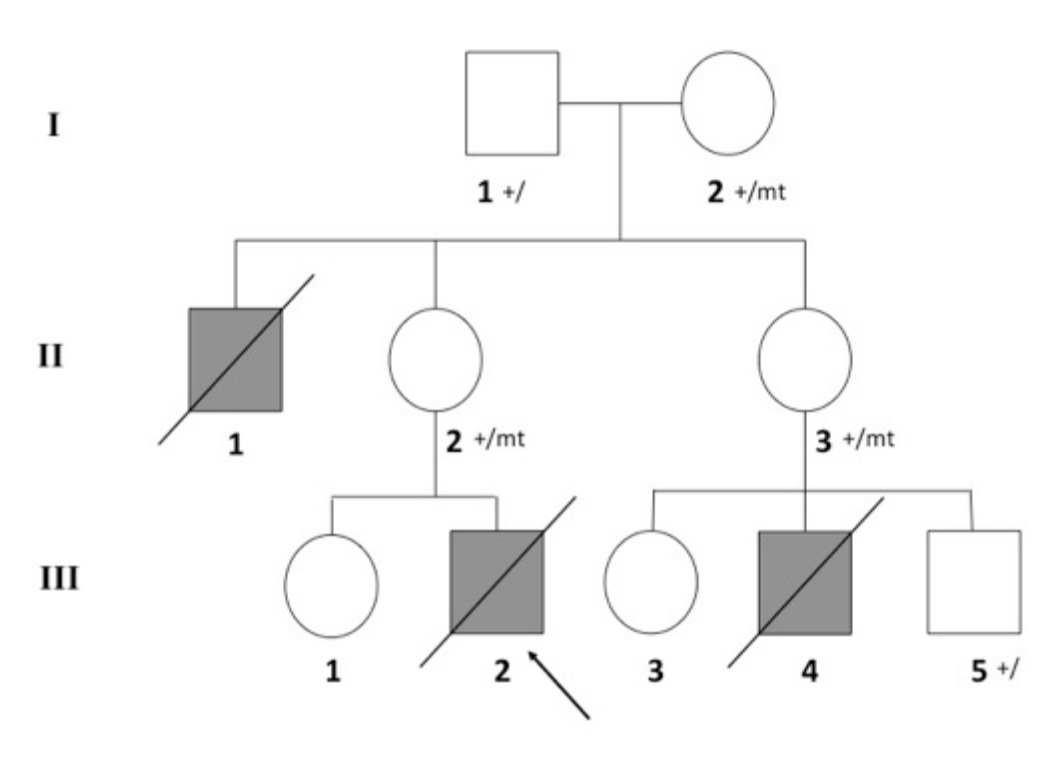
Mutation

WT

Unaffected Brother

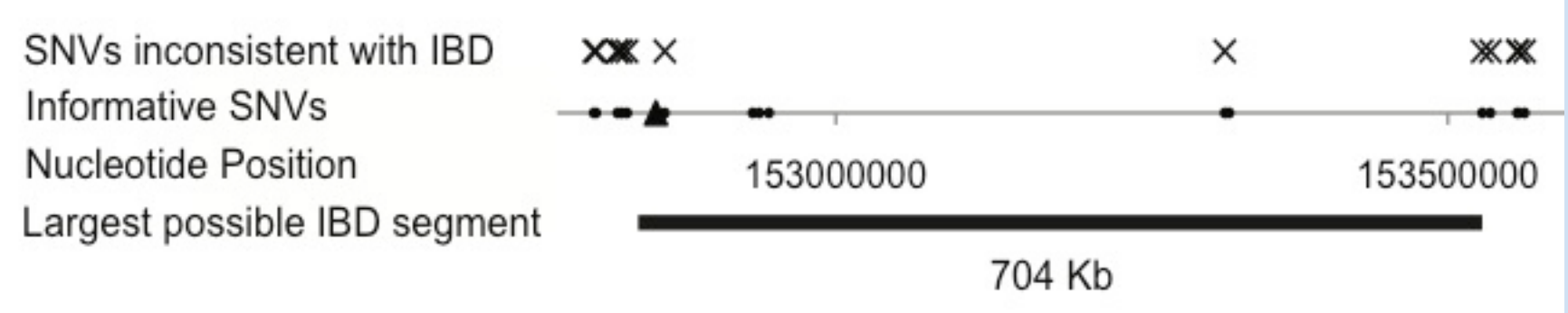
Proband

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



II-1 III-2

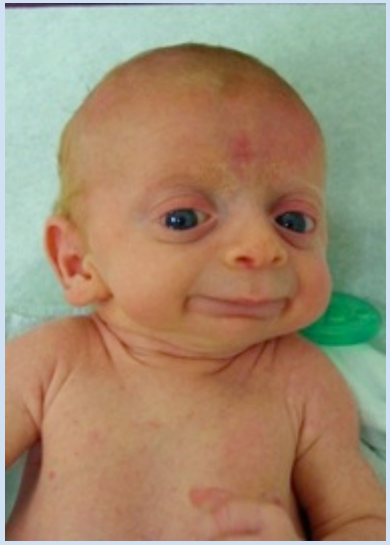
These two families are UNRELATED, i.e. no common founder.

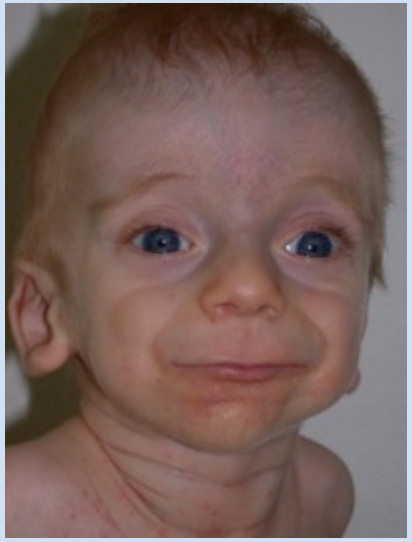


Courtesy of Chad Huff and Lynn Jorde

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



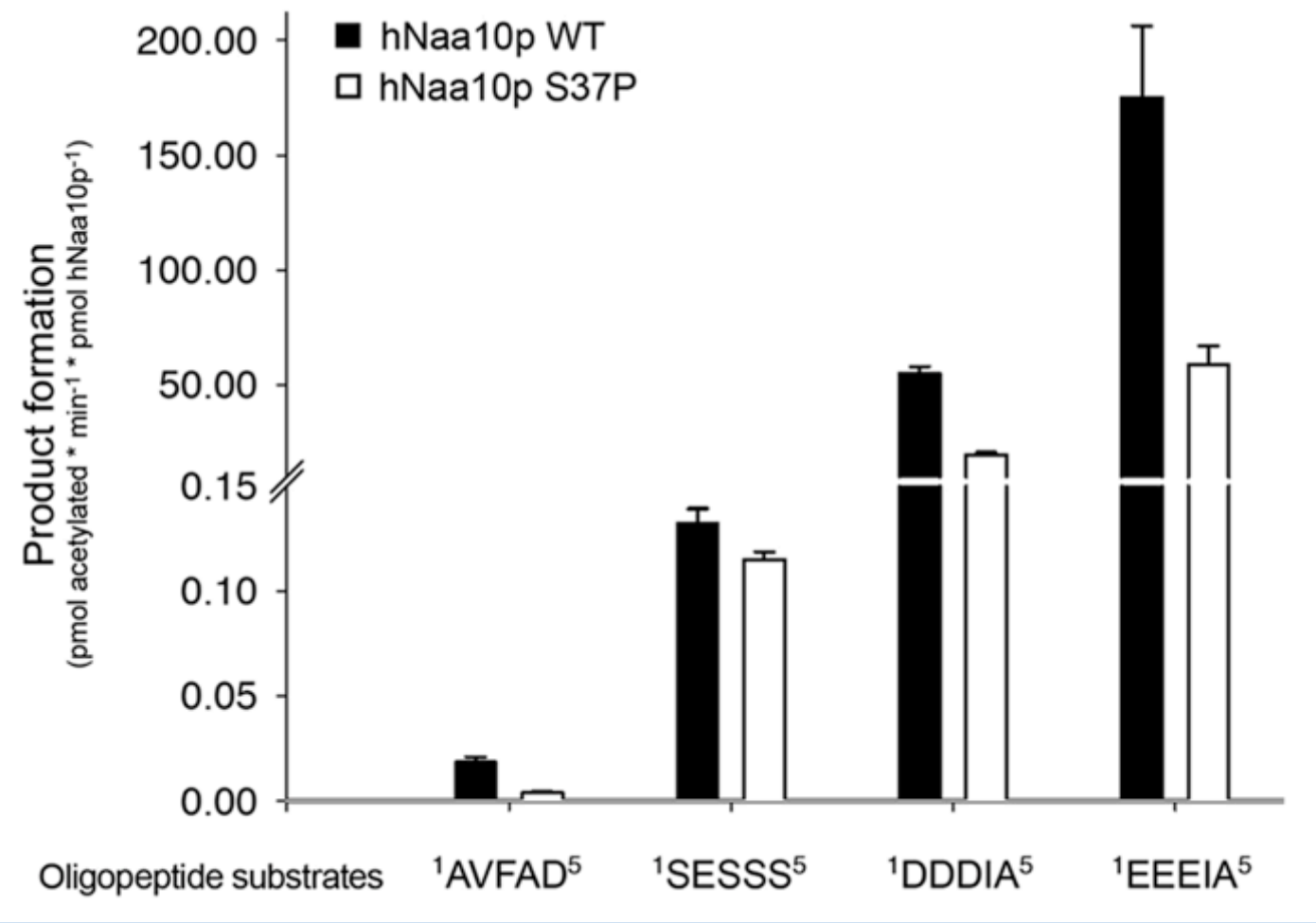



Proving the mutation likely plays some role

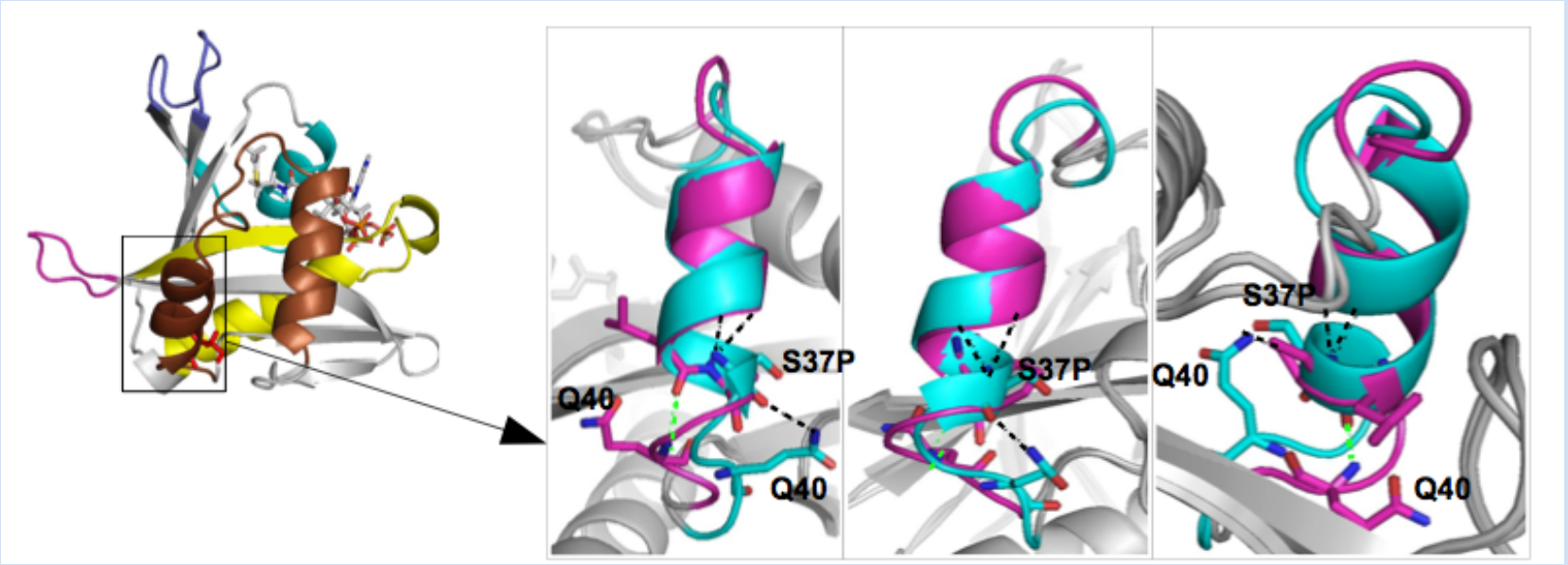
- Present in two unrelated families with very similar phenotype of affected boys.
- Blinded Sanger sequencing showed perfect segregation of the mutation with the disease. Mutation present in Proband, Carrier Mother, Carrier Grandmother and other carrier mothers. Absent in unaffected brother and unaffected uncle.
- Also present in DNA from formalin-fixed paraffin-embedded tissue from two other deceased affected boys, found in pathology department, saved in one case for 30 years.
- Mutation NOT present in ~6000 exomes or genomes sequenced at BGI, CHOP and Utah for other projects.

NAT activity of recombinant hNaa10p WT or p.Ser37Pro towards synthetic N-terminal peptides

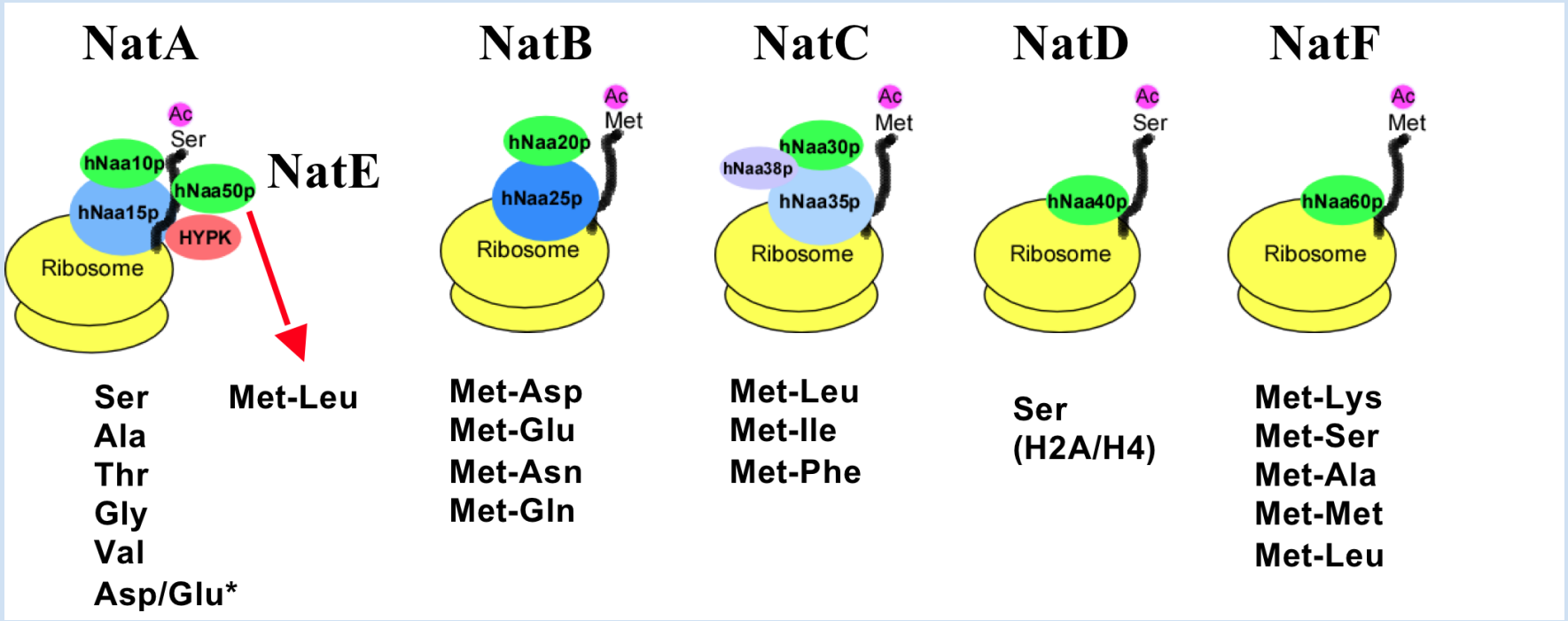


The mutation is a missense resulting in Serine to Proline change in Naa10p

- Ser 37 is conserved from yeast to human
- Ser37Pro is predicted to affect functionality (SIFT and other prediction programs)
- Structural modelling of hNaa10p wt (cyan) and S37P (pink)



The mutation disrupts the N-terminal acetylation machinery (NatA) in human cells.



Slide courtesy of Thomas Arnesen

Open question:
Function of N-terminal acetylation?

Protein stability? Protein secretion?

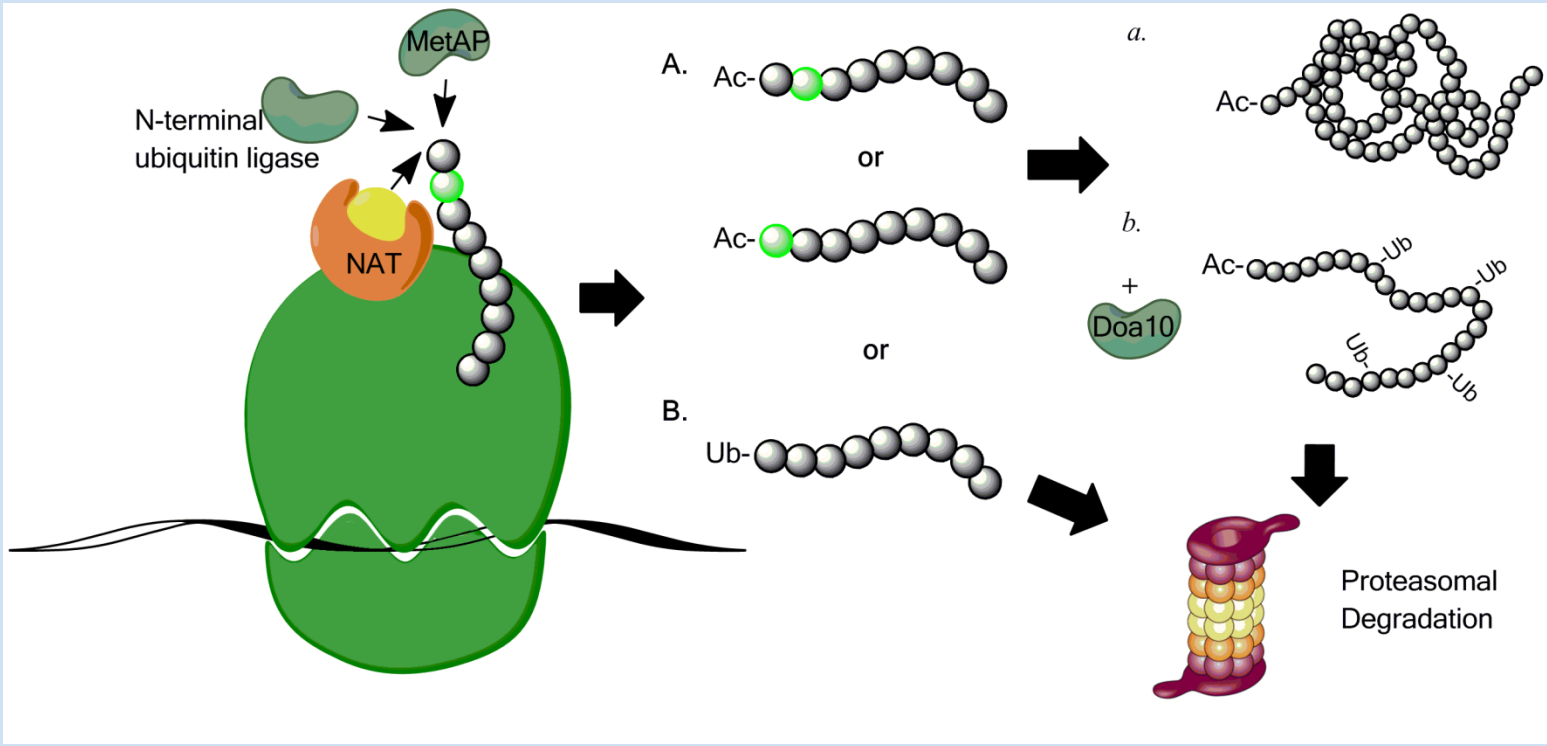
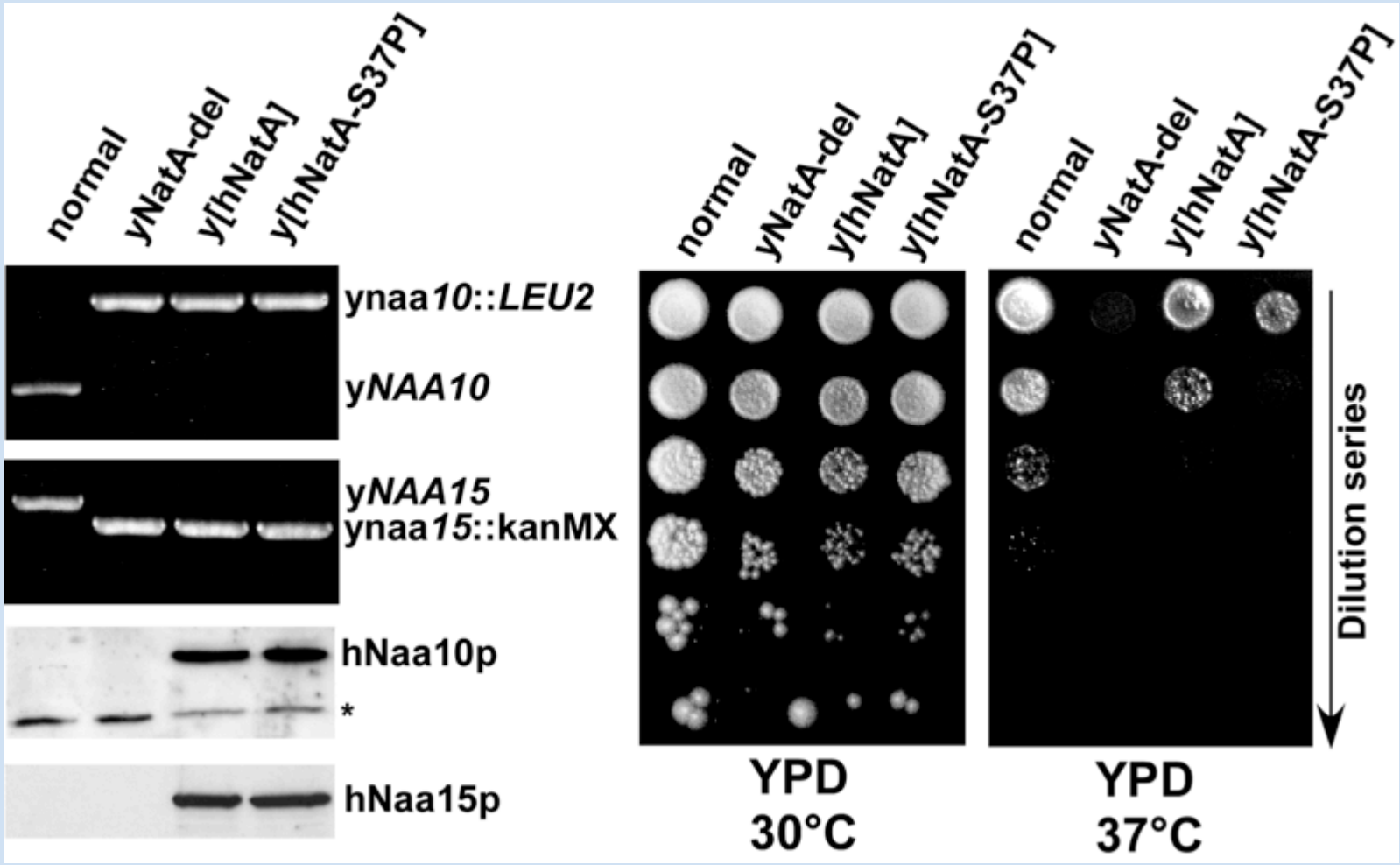


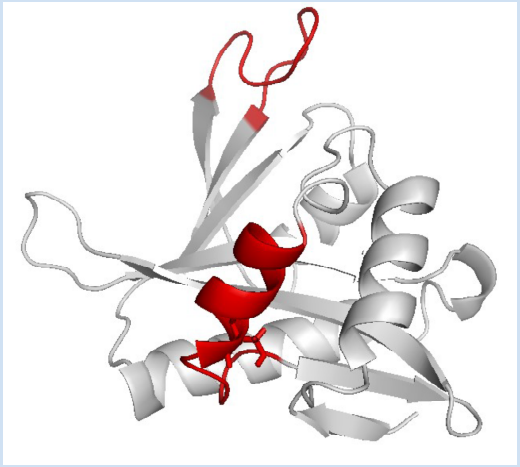
Figure courtesy of Kris Gevaert

hNaa10p-S37P is functionally impaired *in vivo* using a yeast model.

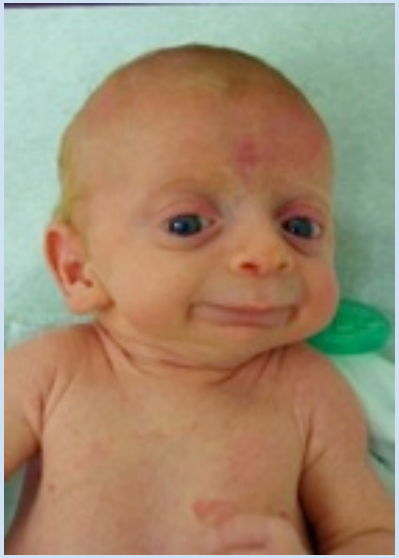


Unpublished data, do not further distribute.

Big Question though:

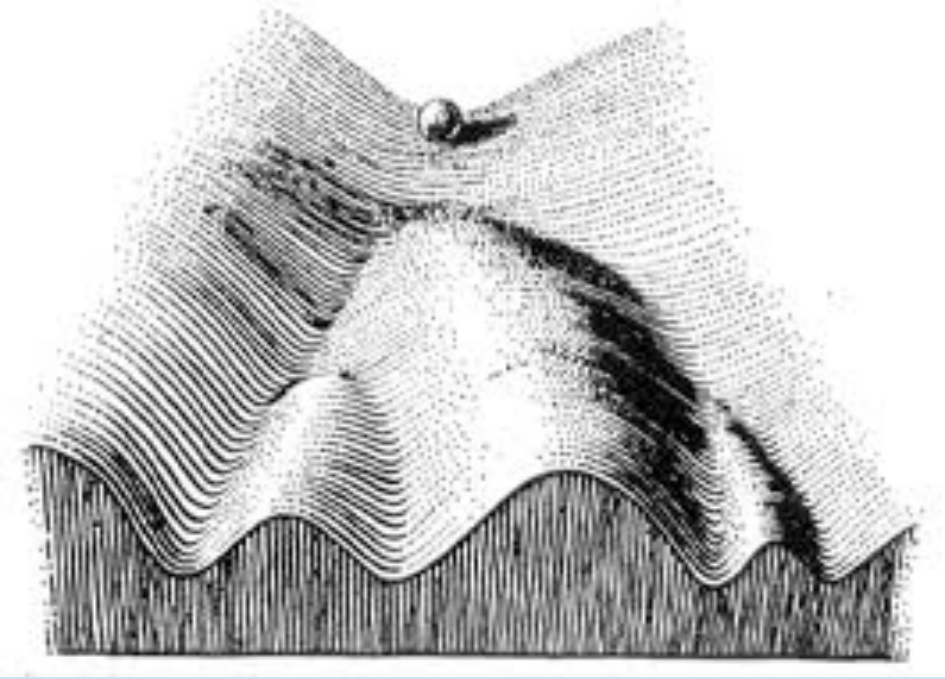


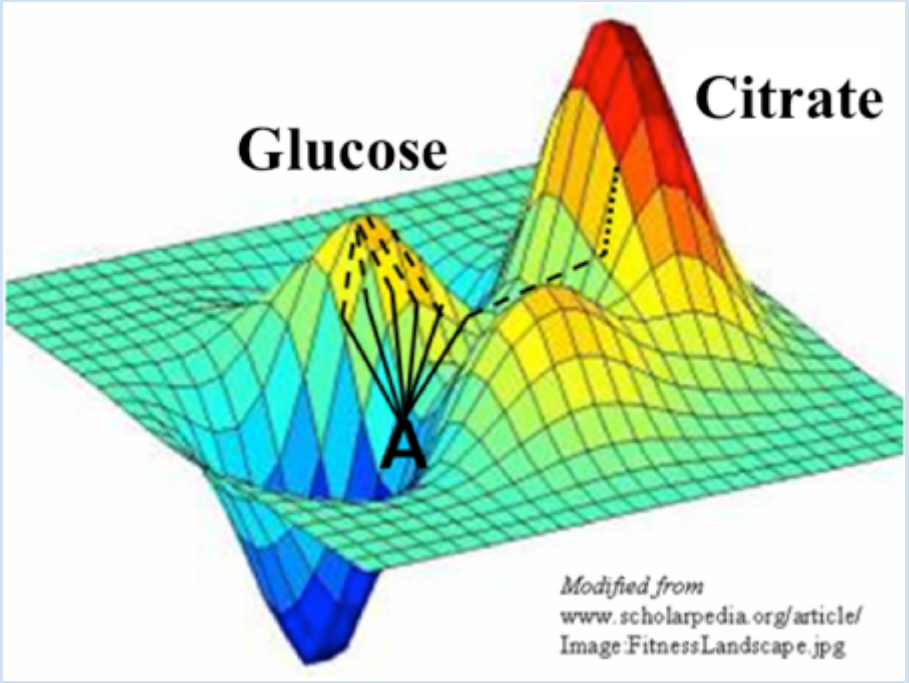
?

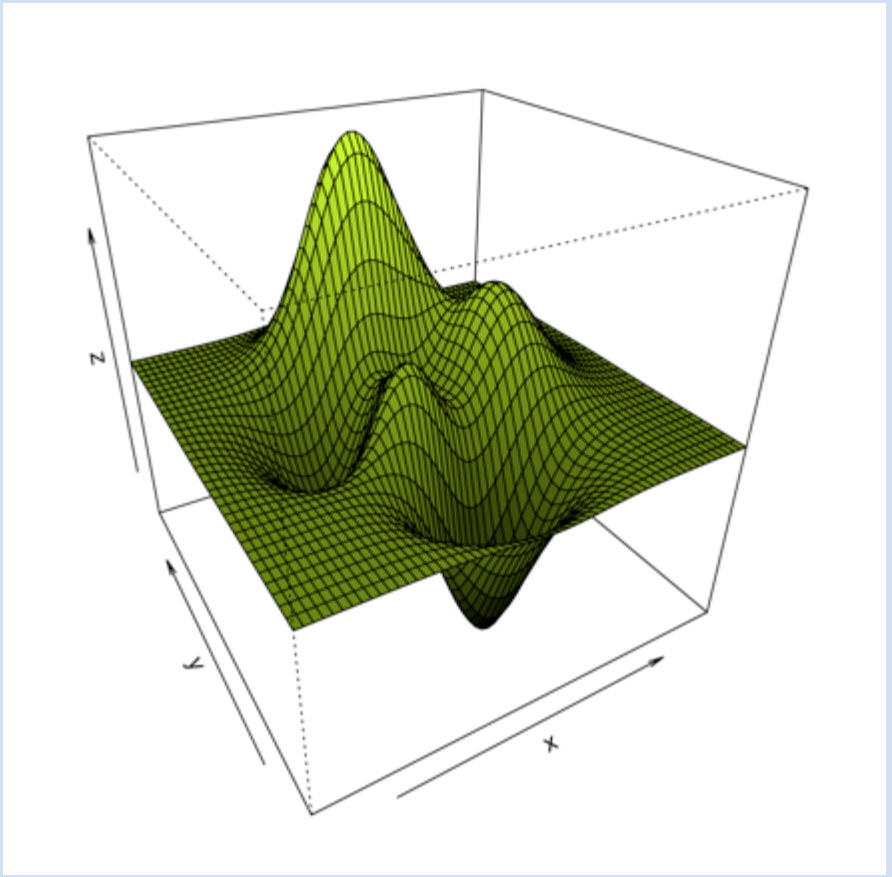


Simulated structure of S37P mutant

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 Lemski 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." - Lemski

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

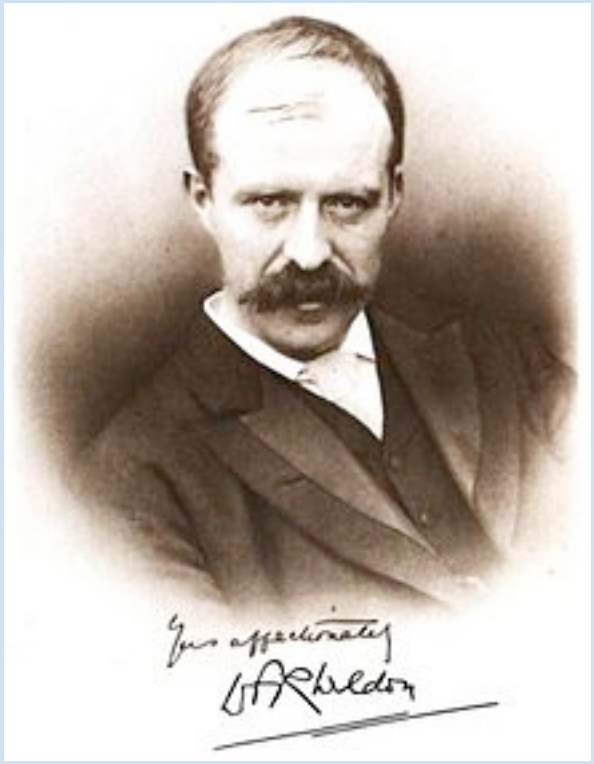
New York

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.

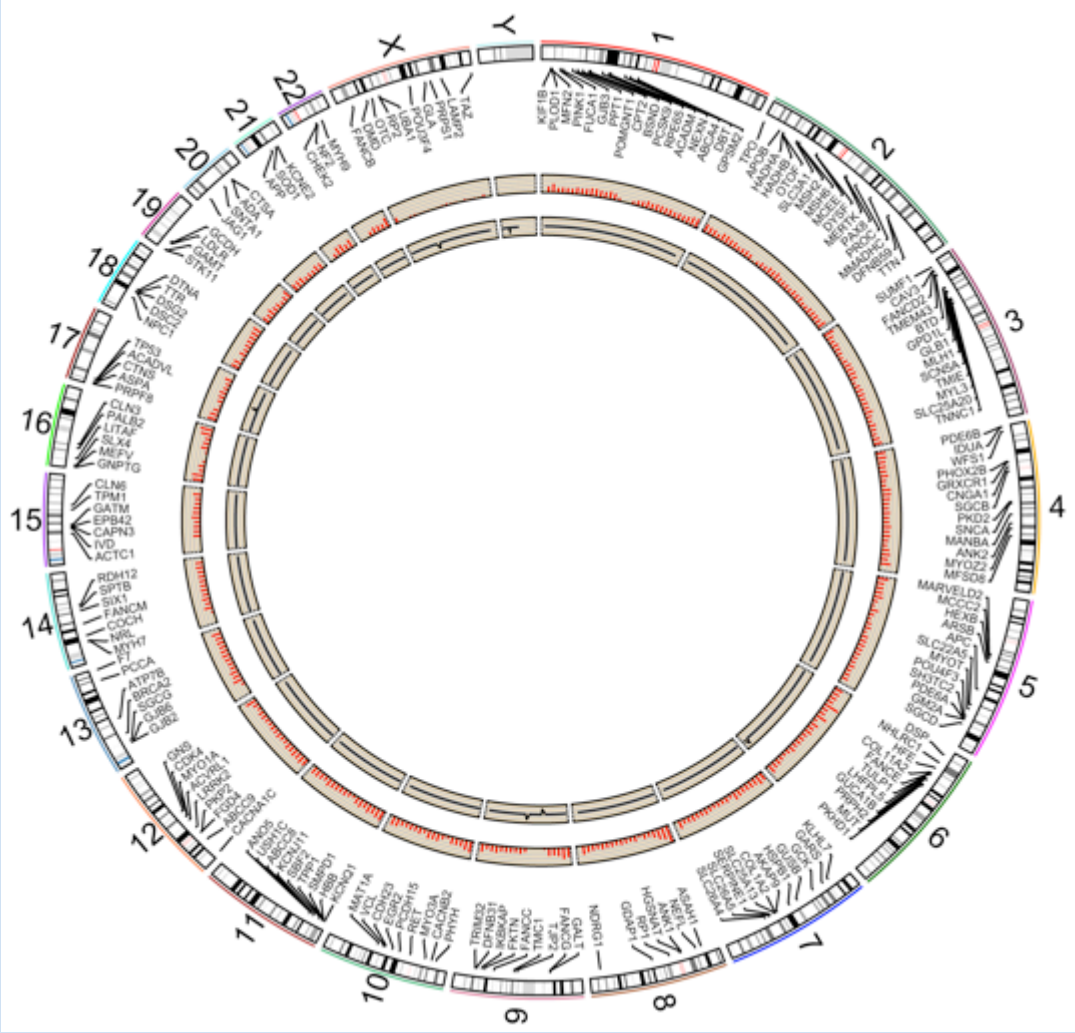
Weldon, W. F. R. 1902. Mendel's laws of alternative inheritance in peas. *Biometrika*, 1:228-254.

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.

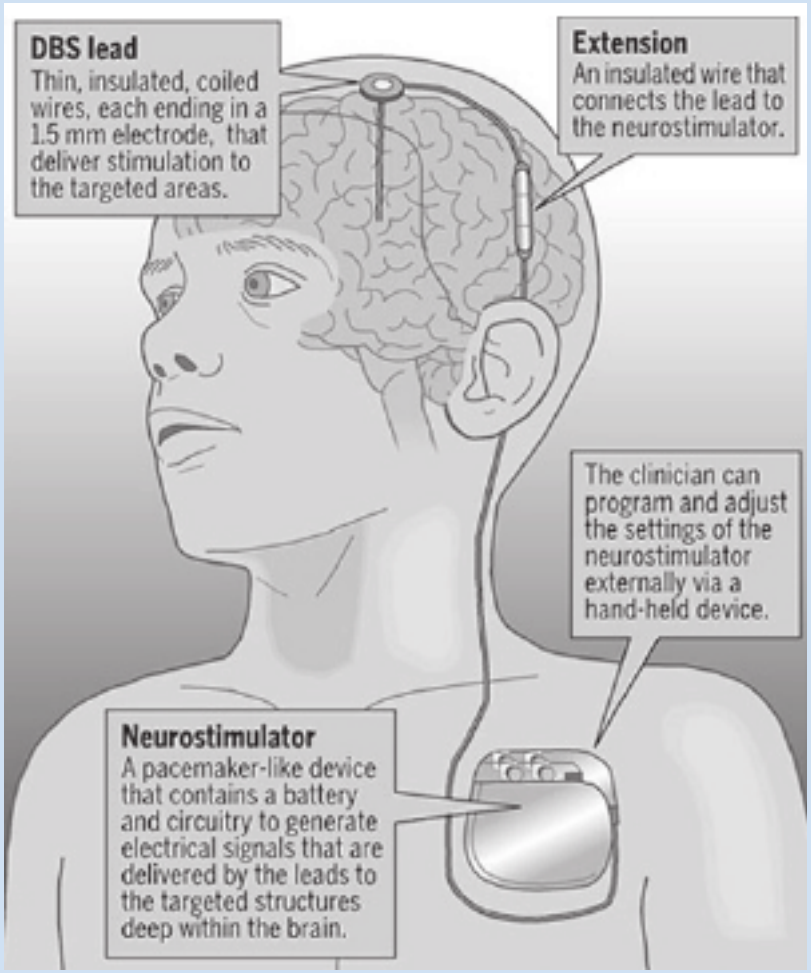
- There is a **MAJOR** clash of world-views, i.e. do single mutations drive outcome predominately, or are the results modified substantially by genetic background and/or environment? i.e. is there really such a thing as genetic determinism for **MANY** mutations?

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

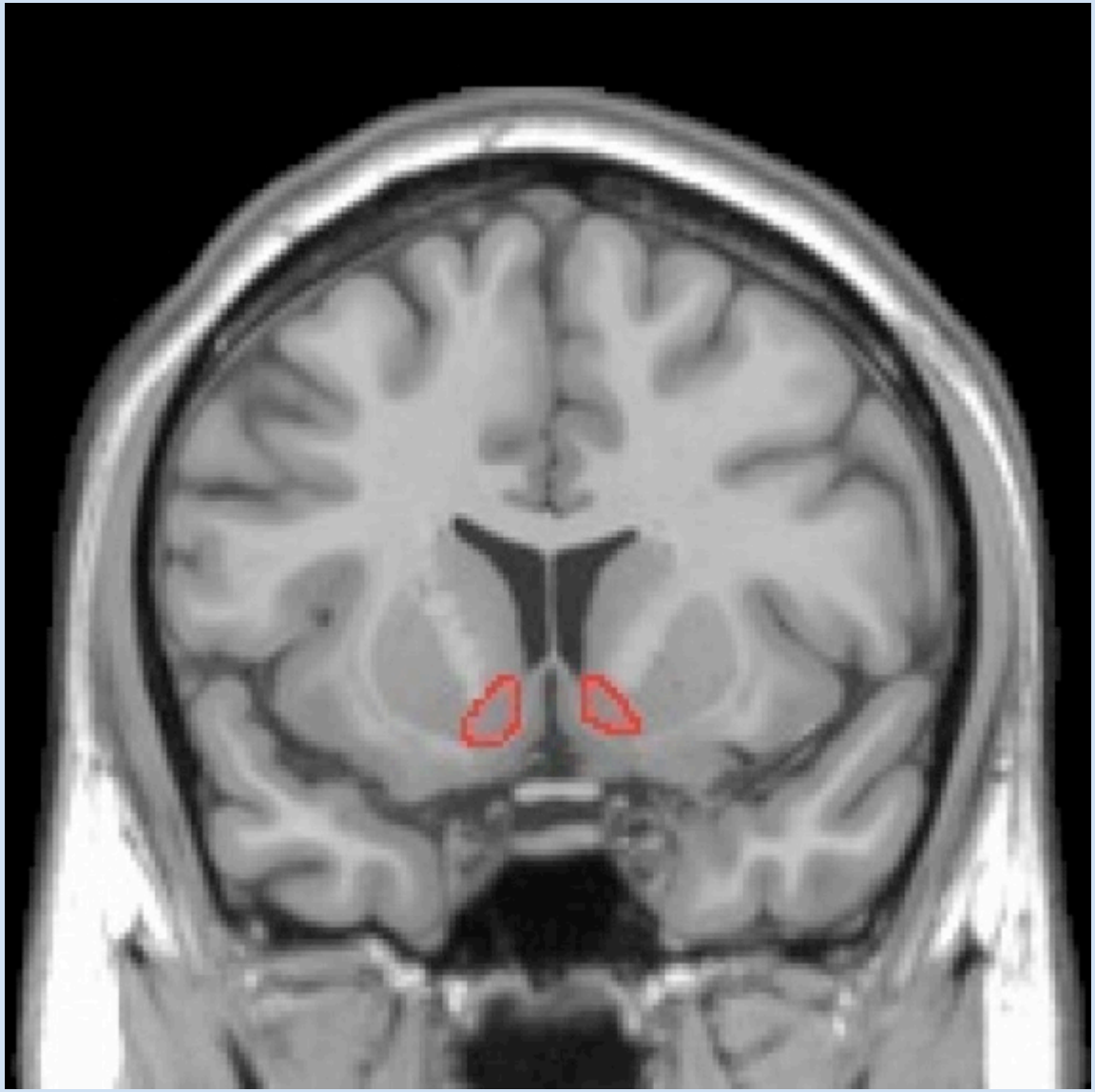


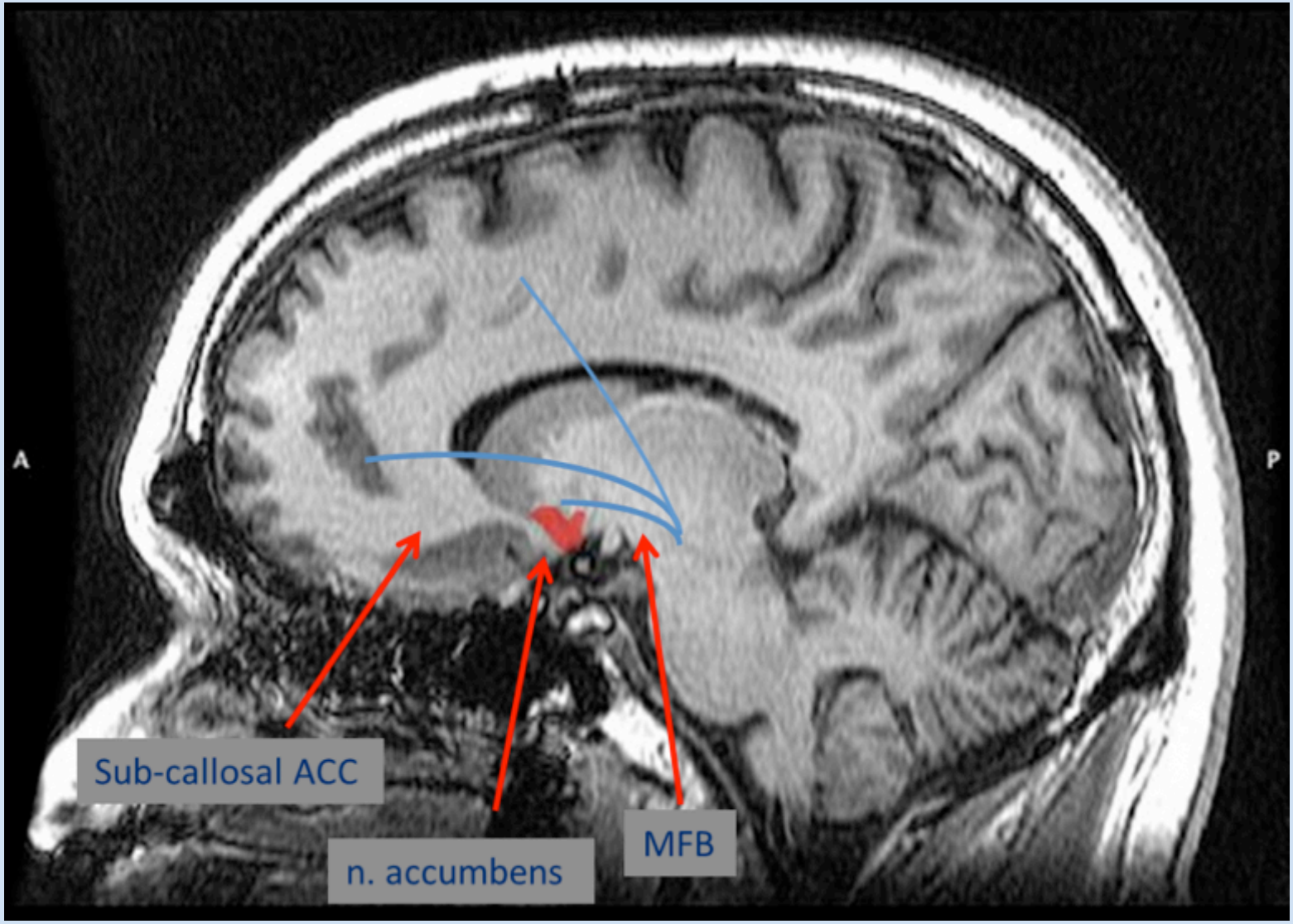
One person with very severe obsessive compulsive disordere, depression and intermittent psychoses

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.

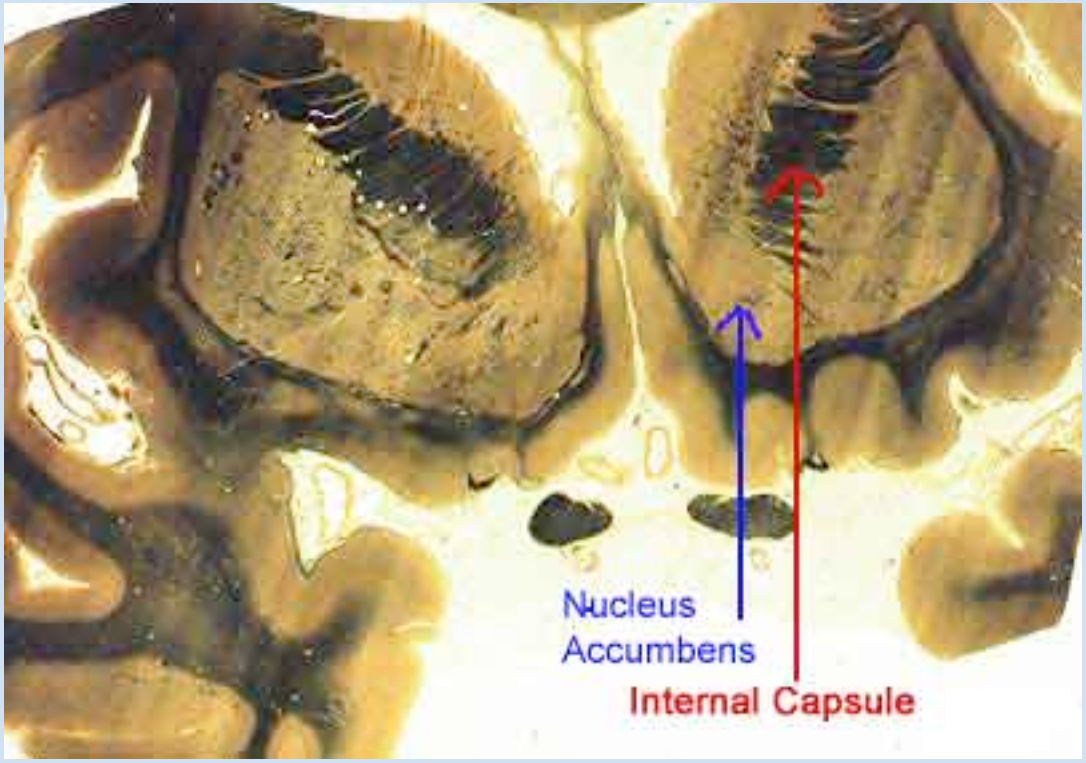

DBS lead
Thin, insulated, coiled wires, each ending in a 1.5 mm electrode, that deliver stimulation to the targeted areas.
Extension
An insulated wire that connects the lead to the neurostimulator.
The clinician can program and adjust the settings of the neurostimulator externally via a hand-held device.
Neurostimulator
A pacemaker-like device that contains a battery and circuitry to generate electrical signals that are delivered by the leads to the targeted structures deep within the brain.

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)


Nucleus
Accumbens
Internal Capsule

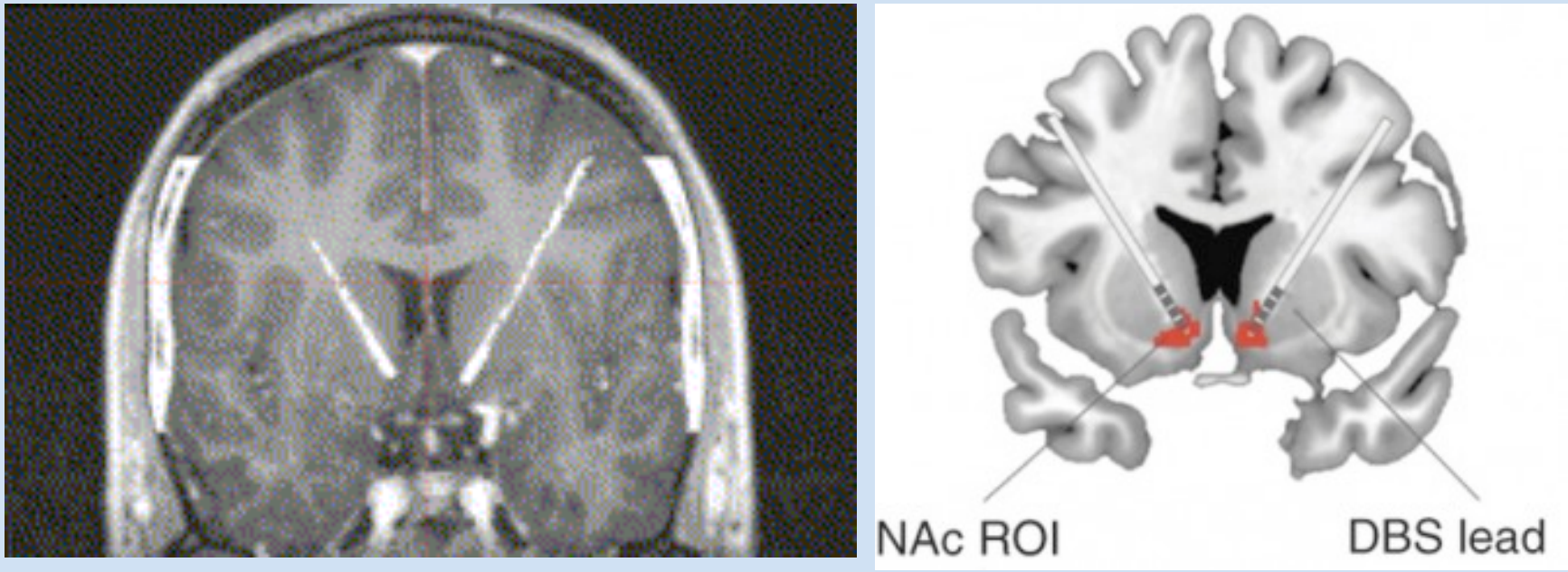
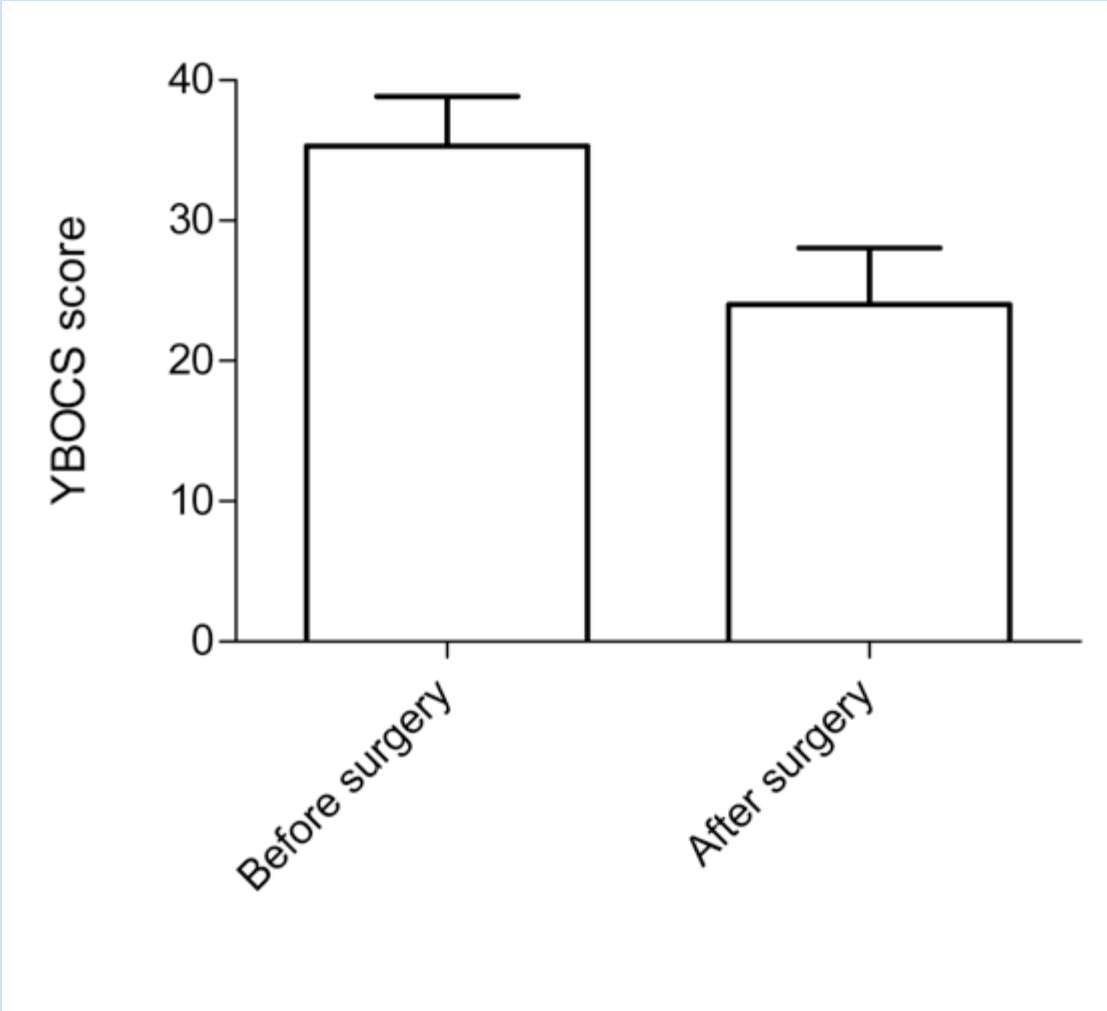
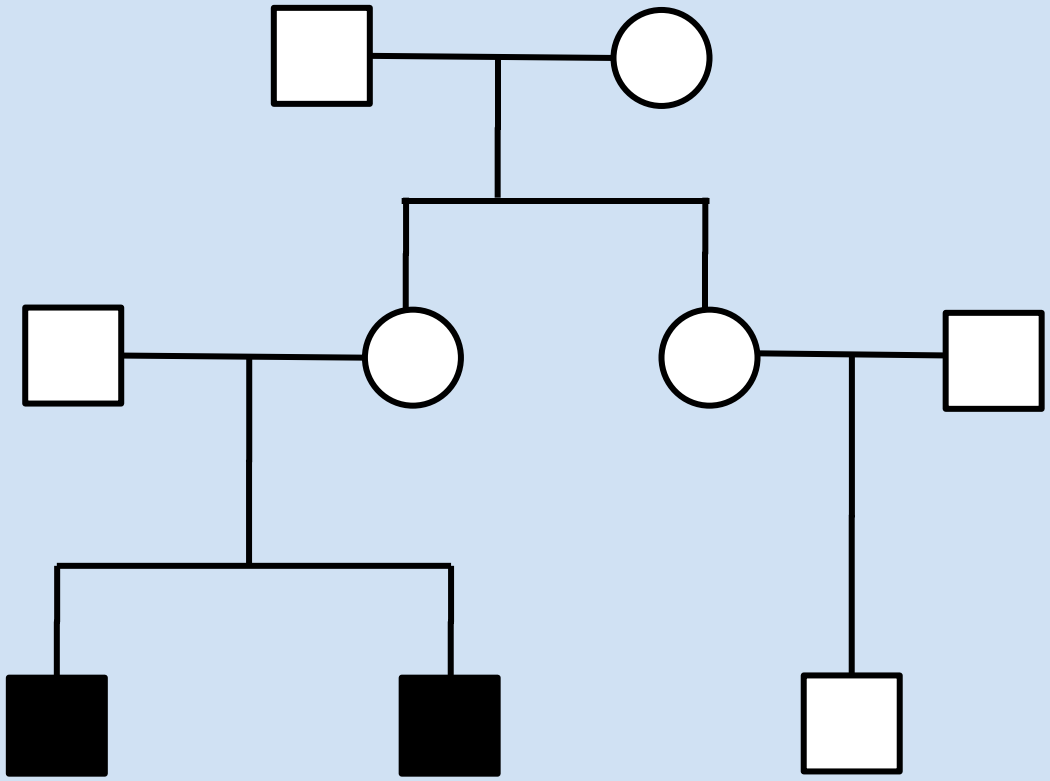


Fig. 1. Coronal section of the brain near the nucleus accumbens with the track of the electrodes on the left and right side.

Two year follow-up



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

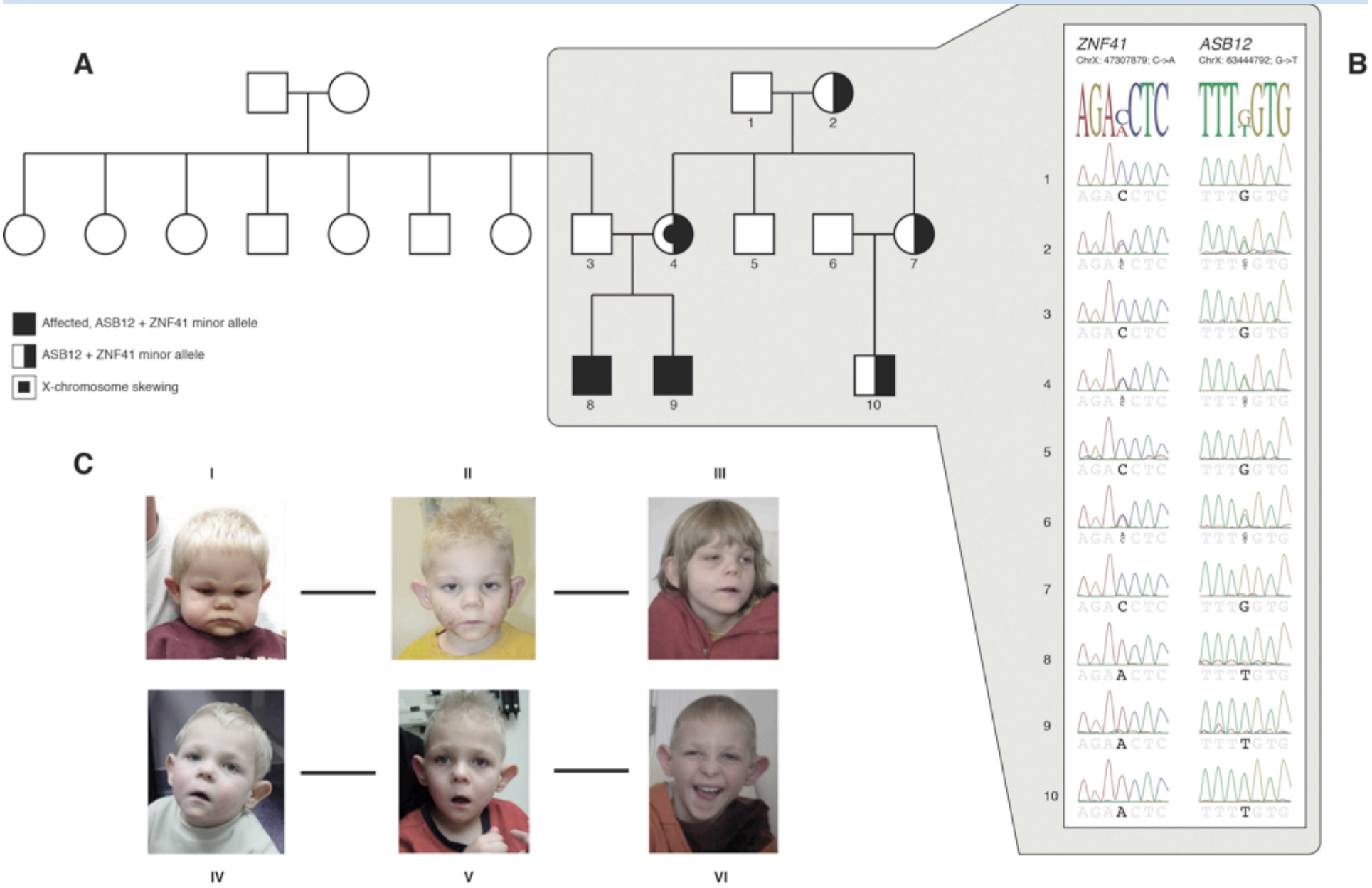
TAF1 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 250kDa

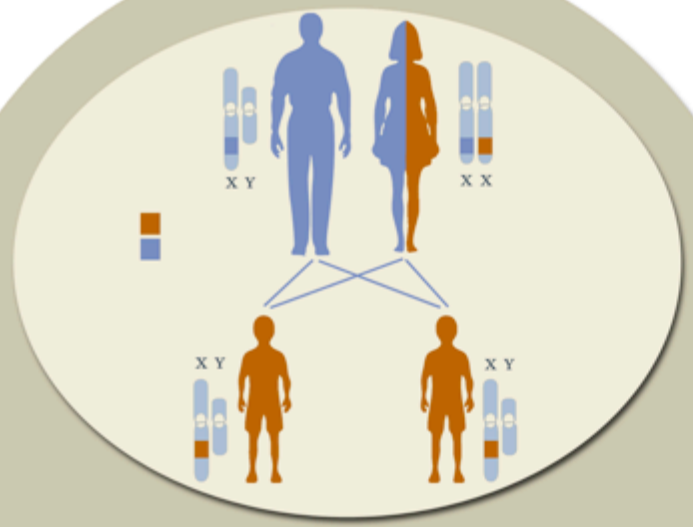
Mutations in the *ZNF41* Gene Are Associated with Cognitive Deficits: Identification of a New Candidate for X-Linked Mental Retardation

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. Kalscheuer[1]

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

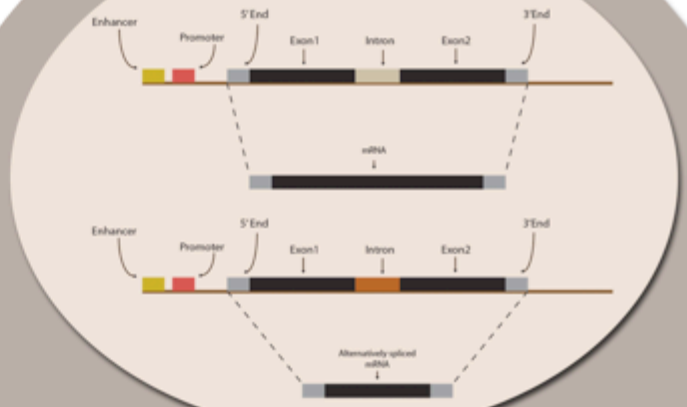
Am. J. Hum. Genet. 73:1341–1354, 2003


A
1
2
3
4
5
6
7
8
9
10
Affected, ASB12 + ZNF41 minor allele
ASB12 + ZNF41 minor allele
X-chromosome skewing
B
ZNF41
ChrX: 47307879; C->A
ASB12
ChrX: 63444792; G->T
C
I
II
III
IV
V
VI



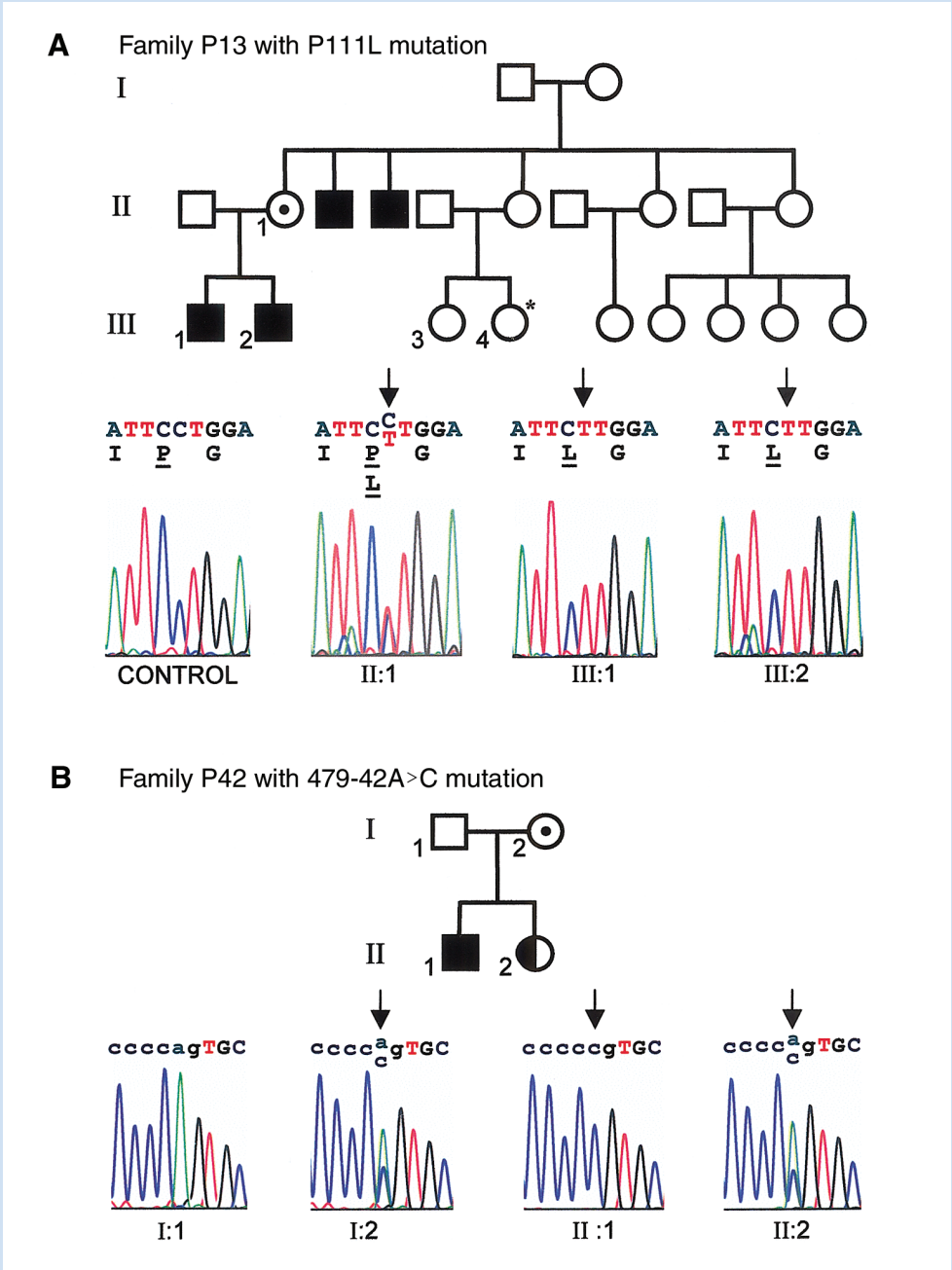
X-linked

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



Non-coding

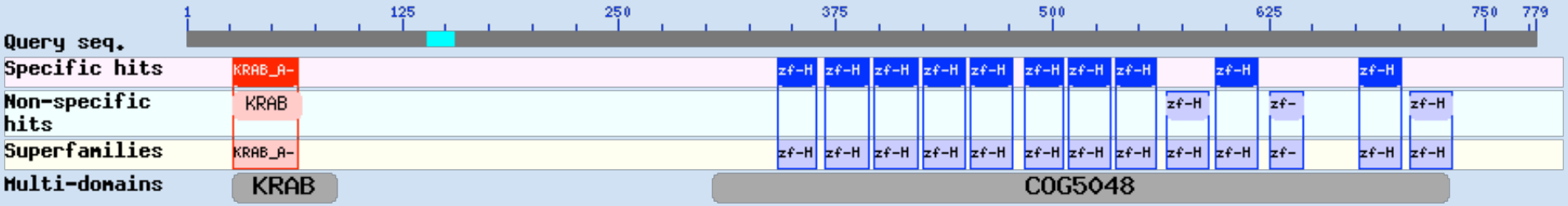
Gene	Locus	Exon	Protein
UTR3 AR	X:66945414	-------	-------
FAM155B (*dist=271971*)	X:68453113	-------	-------
MIR221 (*dist=35606*)	X:45569979	-------	-------
DMD-AS2 *intronic*	X:31284835	-------	-------
MID1 (*dist=30252*)	X:10383096	-------	-------



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.

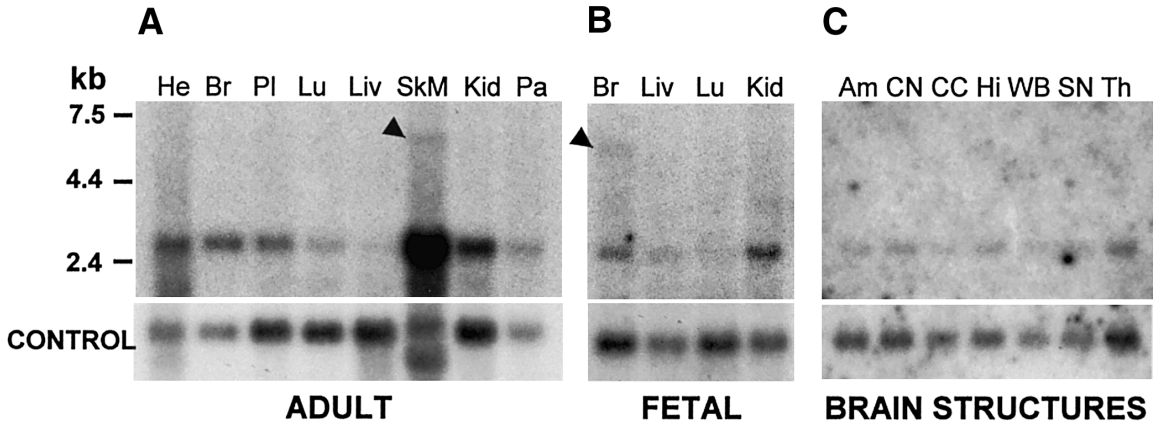


Figure 6 Northern blot hybridization of *ZNF41*, by use of a probe corresponding to nucleotides 621–1099 of *ZNF41* transcript variant 1. *A*, Adult tissues (left to right): heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas. *B*, Fetal tissues (left to right): brain, lung, liver, 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

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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

Lyon and Wang *Genome Medicine* 2012, 4:58
http://genomemedicine.com/content/4/7/58

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]

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

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The End

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

Genome Medicine

RESEARCH Open Access

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

Jason O'Rawe[1,2], Tao Jiang[3], Guangqing Sun[3], Yiyang Wu[1,2], Wei Wang[4], Jingchu Hu[3], Paul Bodily[5], Lifeng Tian[6], Hakon Hakonarson[6], W Evan Johnson[7], Zhi Wei[4], Kai Wang[8,9*] and Gholson J Lyon[1,2,9*]

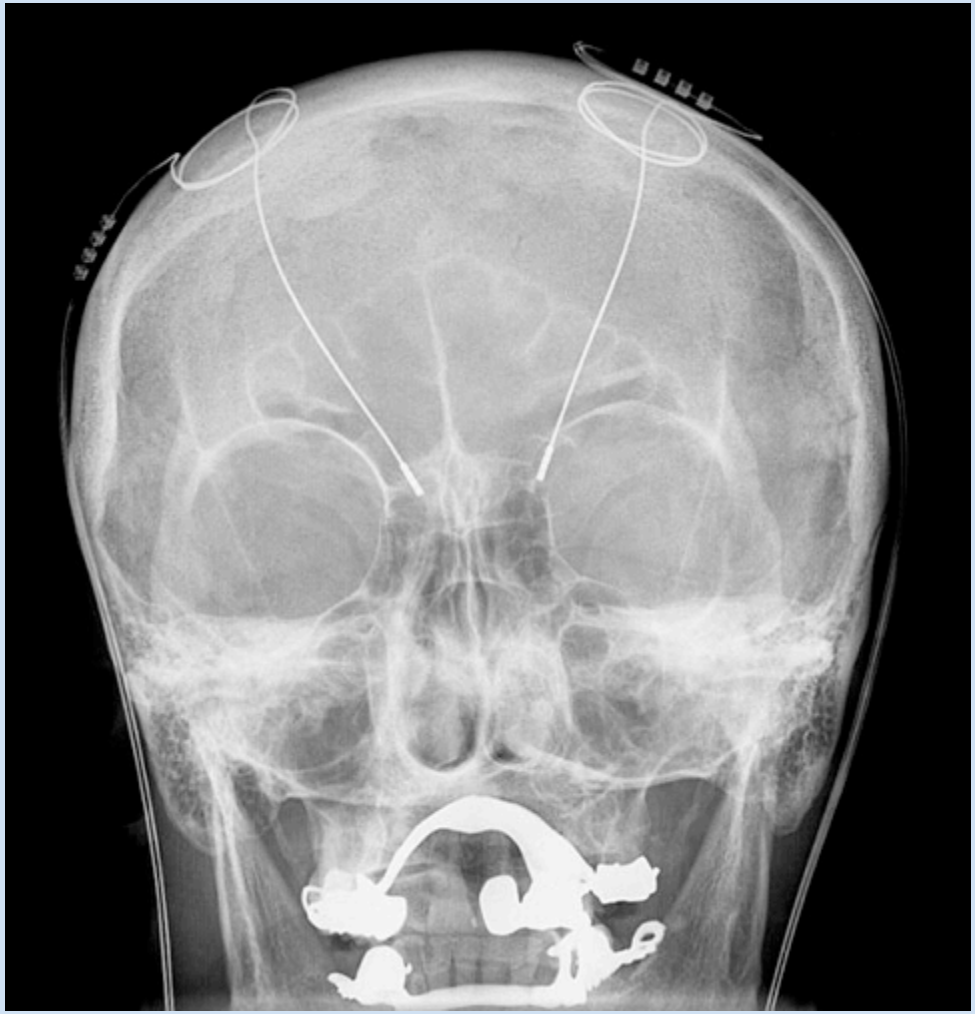
Major Conclusion: Clinical Validity?

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.

Biological Indeterminacy. Greenspan RJ. Sci Eng Ethics. 2012 Jul 3

- 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."