Genetics and Genome Sequencing of Childhood-Onset Neuropsychiatric Disorders

Gholson Lyon, M.D. Ph.D.

CSH
STANLEY INSTITUTE FOR COGNITIVE GENOMICS
COLD SPRING HARBOR LABORATORY

UTAH FOUNDATION FOR BIOMEDICAL RESEARCH

@GholsonLyon

The Big Picture

- Over the course of my entire career, I want to help understand the pathophysiology of severe mental disorders, including such things as developmental delay, mental retardation, autism, psychotic disorders (schizophrenia, bipolar, schizoaffective), Tourette Syndrome and obsessive compulsive disorder.

- I expect this to uncover new biology along the way.

The toll with Brain Disorders is tremendous.

Most recent analysis in Europe showed that brain disorders cost almost US $1 trillion year per year, more than cancer, cardiovascular disease and diabetes combined.

These brain disorders include:
Mood disorders
Psychotic Disorders
Addiction
Anxiety
Dementia
Headache
Other- brain tumor, **child/adolescent developmental disorders (autism, ADHD, tics, etc…),** eating disorders, epilepsy, **mental retardation,** multiple sclerosis, neuromuscular disorders, Parkinson's, personality disorders, sleep disorders, somatoform disorder, stroke and traumatic brain injury.

* Cost of disorders of the brain in Europe 2010.
Gustavsson A, et al.
Eur Neuropsychopharmacol. 2011 Oct;21(10):718-779. Epub 2011 Sep 15.

I moved to Utah in July 2009 to find new human genetic syndromes, thus revealing new biology.

- July 2009-December 2009: Attended weekly genetics case conference in which 10-30 genetic cases are presented weekly, led by Dr. Alan Rope and attended by Drs. John Carey and John Opitz.
- There are indeed MANY idiopathic disorders not described in the literature, many of which have neuropsychiatric manifestations. I thought about hundreds of such cases, looking for the ideal first family to sequence.

Discovering a new syndrome and its genetic basis.

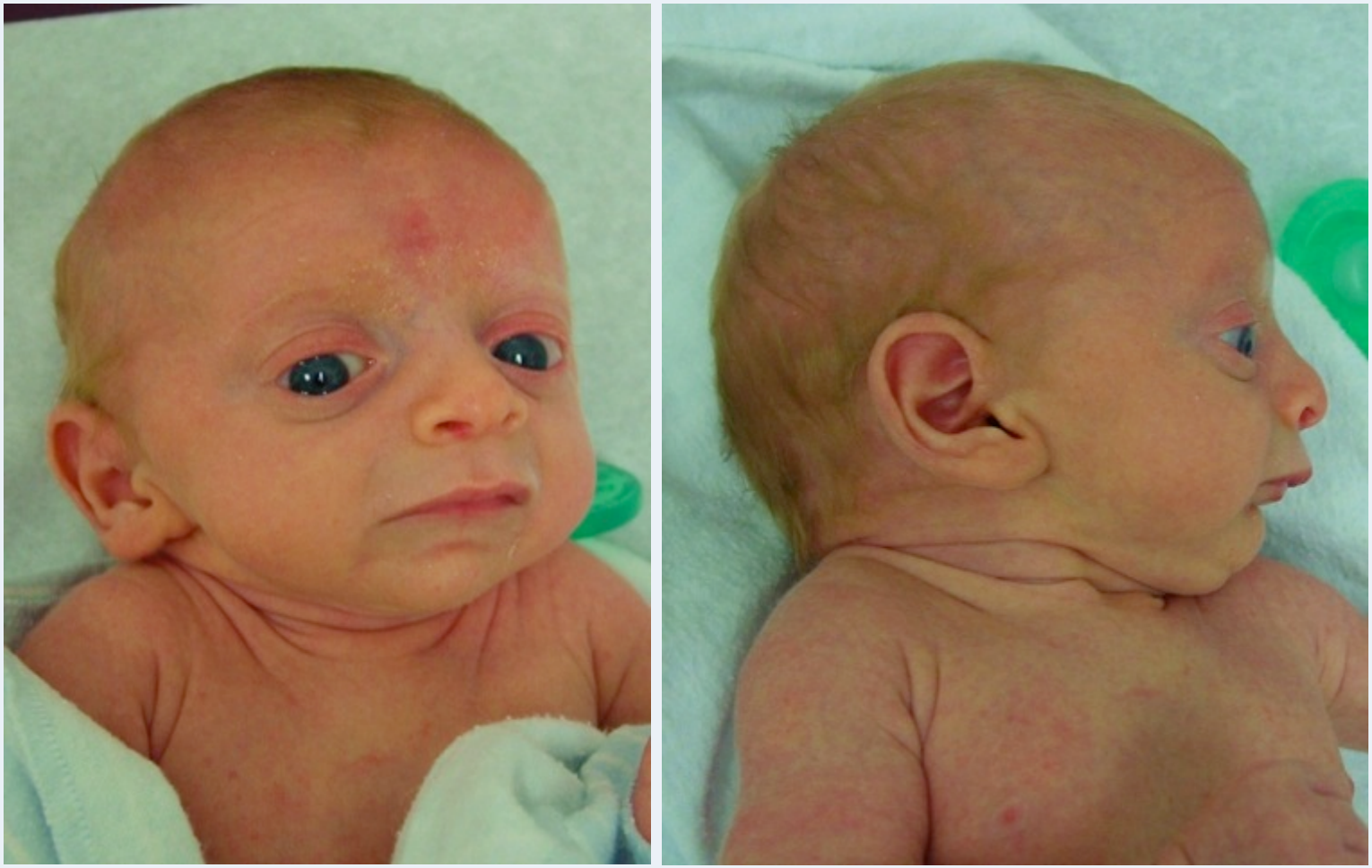
ARTICLE

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,*]

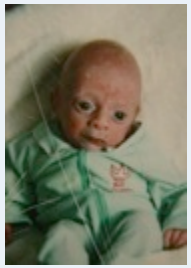
The American Journal of Human Genetics *89*, 1–16, July 15, 2011

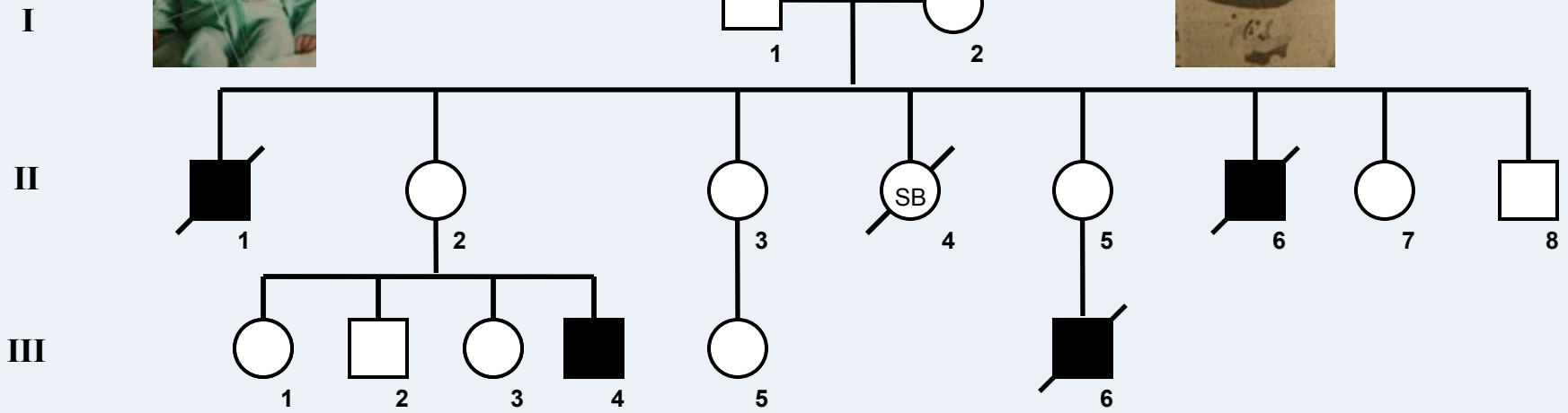
This is the "Proband" photograph presented at Case Conference.

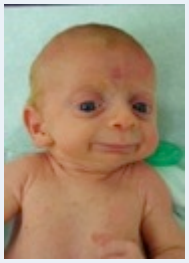


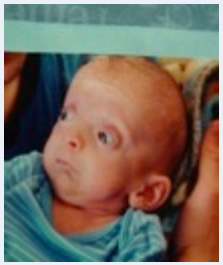
prominence of eyes, down-sloping palpebral fissures, thickened eyelids, large ears, beaking of nose, flared nares, hypoplastic nasal alae, short columella, protruding upper lip, micro-retrognathia

This is the family in Utah in December 2009.









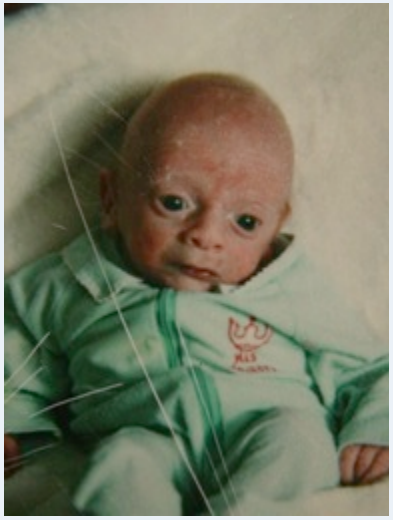
I met the entire family on March 29, 2010

Photo of mother
with son in late 1970's

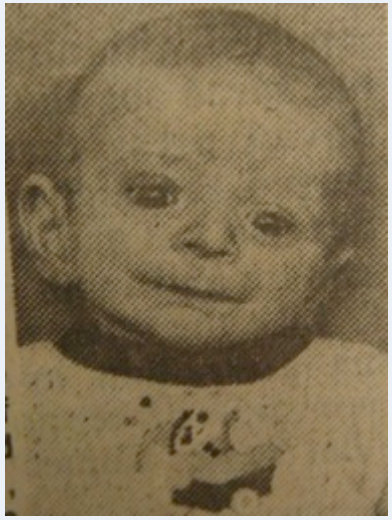
This is the first boy in the late 1970's.

First boy. Called "a little old man" by the family. Died around ~1 year of age, from cardiac arrhythmias.

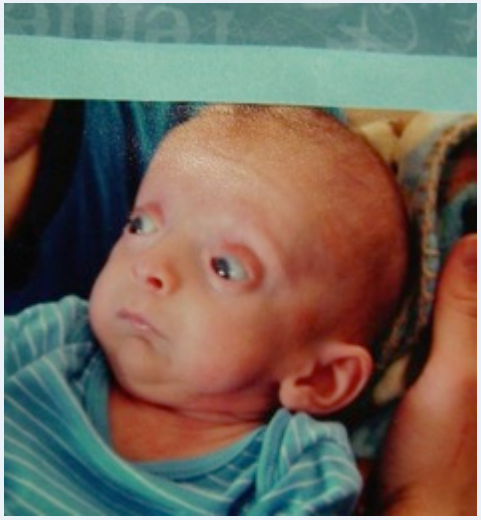
These are the Affected Boys of Family 1 in 2009.



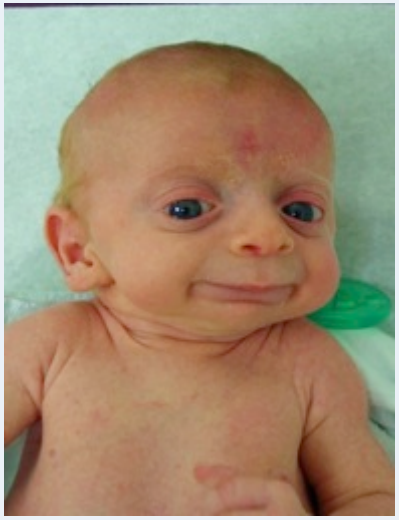
Uncle #1



Uncle #2



cousin



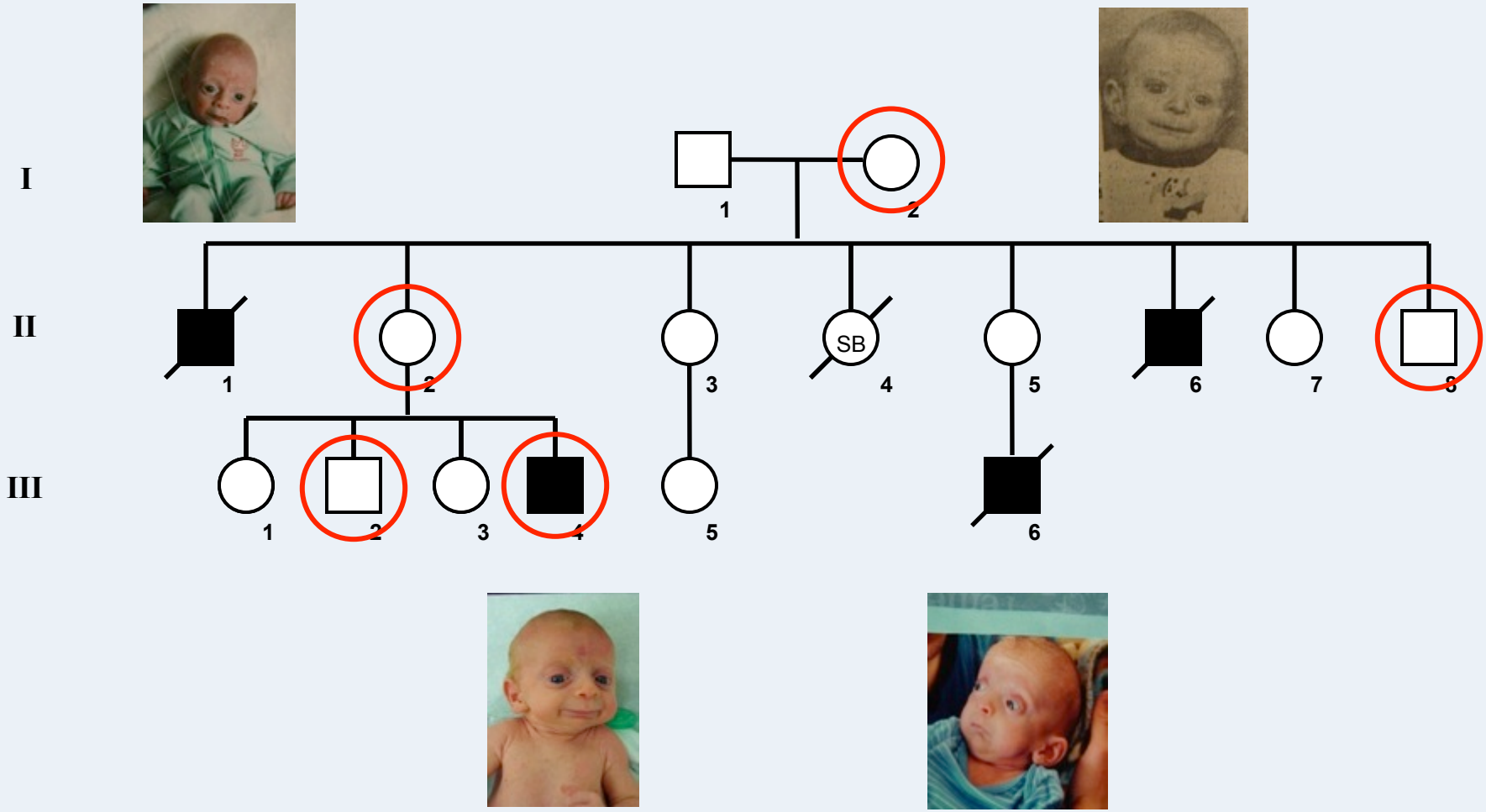
Proband- Sutter

Affected males had the consistent presentation of an aged appearance, a distinct and recognizable combination of craniofacial anomalies, post-natal growth failure, hypotonia, global developmental delays, cryptorchidism, arrhythmia, and eventual death from cardiac failure.

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.	

Experimental Design for Sequencing is Critical.



- **We performed X-chromosome exon capture with Agilent, followed by Next Gen Sequencing with Illumina.**

- **We analyzed the data with ANNOVAR and VAAST (Variant Annotation, Analysis and Search Tool). New computational tools for identifying disease-causing mutations by individual genome sequencing.**

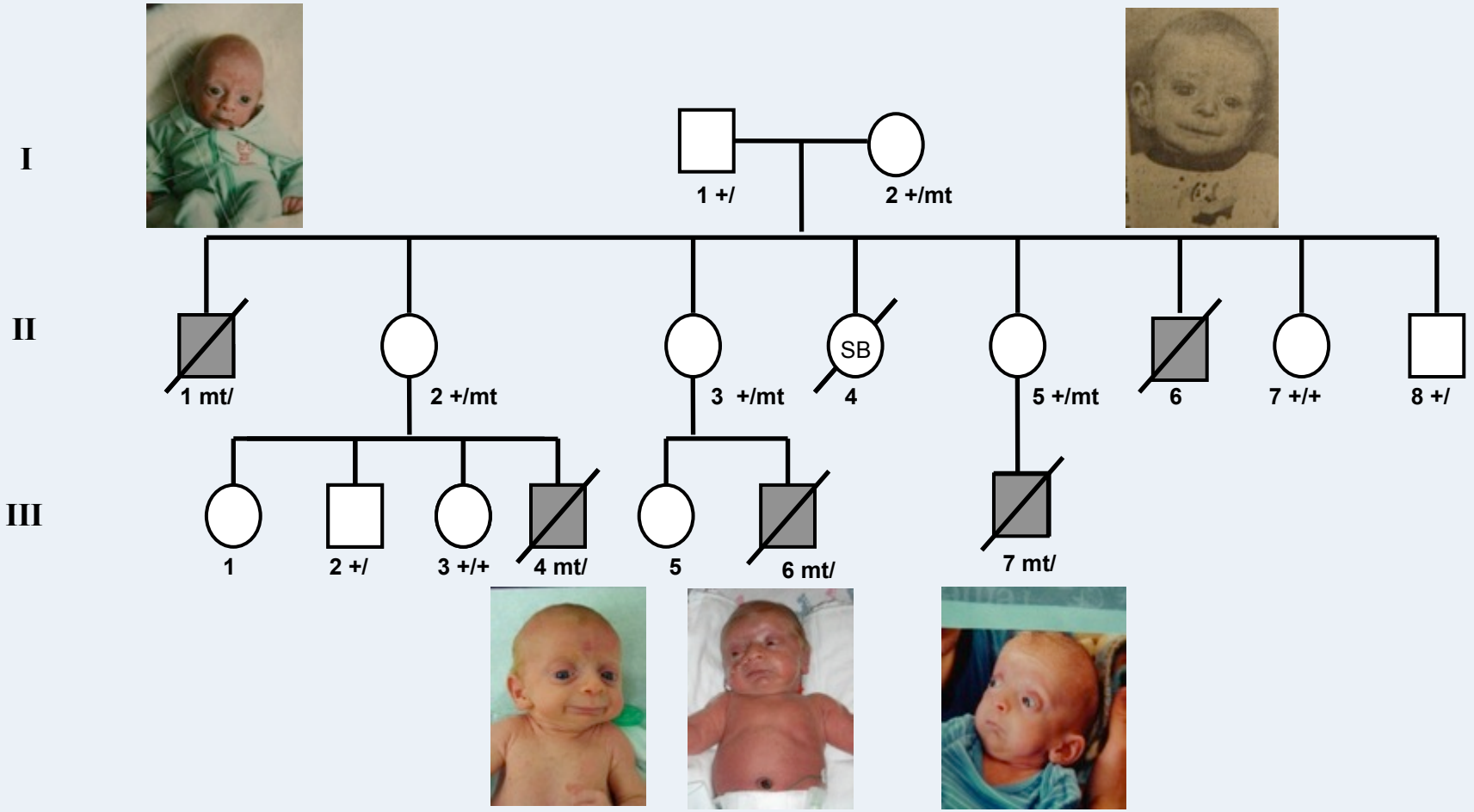
Yandell, M. *et al.* 2011. "A probabilistic disease-gene finder for personal genomes." *Genome Res*. 21 (2011). doi:10.1101/gr.123158.111.

Wang, K., Li, M., and Hakonarson, H. (2010). ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res 38, e164.

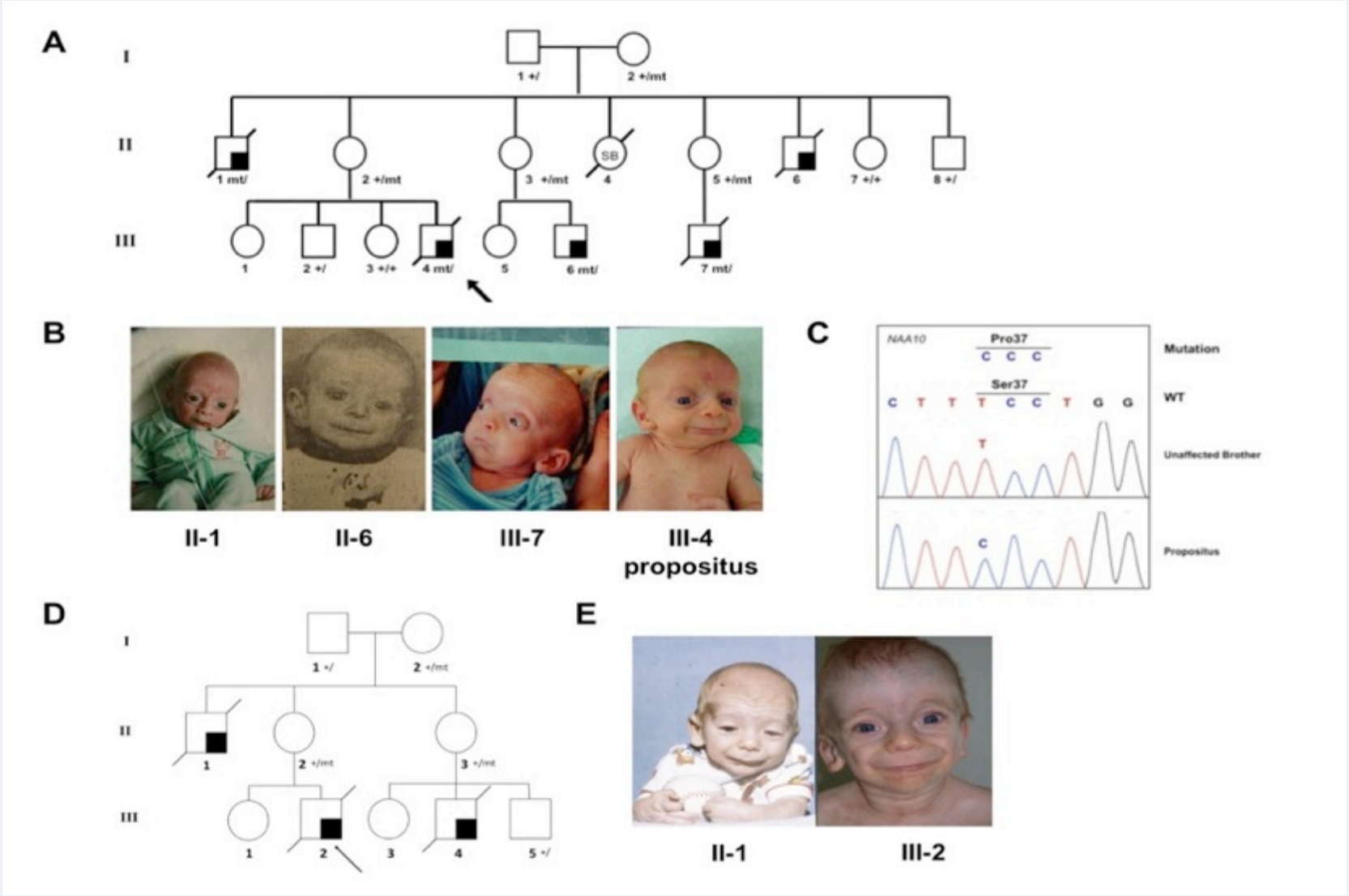
The Exon Capture and Coverage was high depth.

Table 2. Coverage Statistics in Family 1. Based on GNUMAP							
Region	**RefSeq Transcripts**	**Unique Exons**	**Percent Exon Coverage ≥1X**	**Percent Exon Coverage ≥10X**	**Unique Genes**	**Average Base Coverage**	**VAAST Candidate SNVs**
X-chromosome	1,959	7,486	97.8	95.6	913	214.6	1 (*NAA10*)
chrX: 10054434-40666673	262	1,259	98.1	95.9	134	213.5	0
chrX: 138927365-153331900	263	860	97.1	94.9	132	177.1	1 (*NAA10*)
* On chromosome X, there are 8,222 unique RefSeq exons. Of these exons, 736 were excluded from the SureSelect X-Chromosome Capture Kit because they were designated as pseudoautosomal or repetitive sequences (UCSC genome browser).							

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

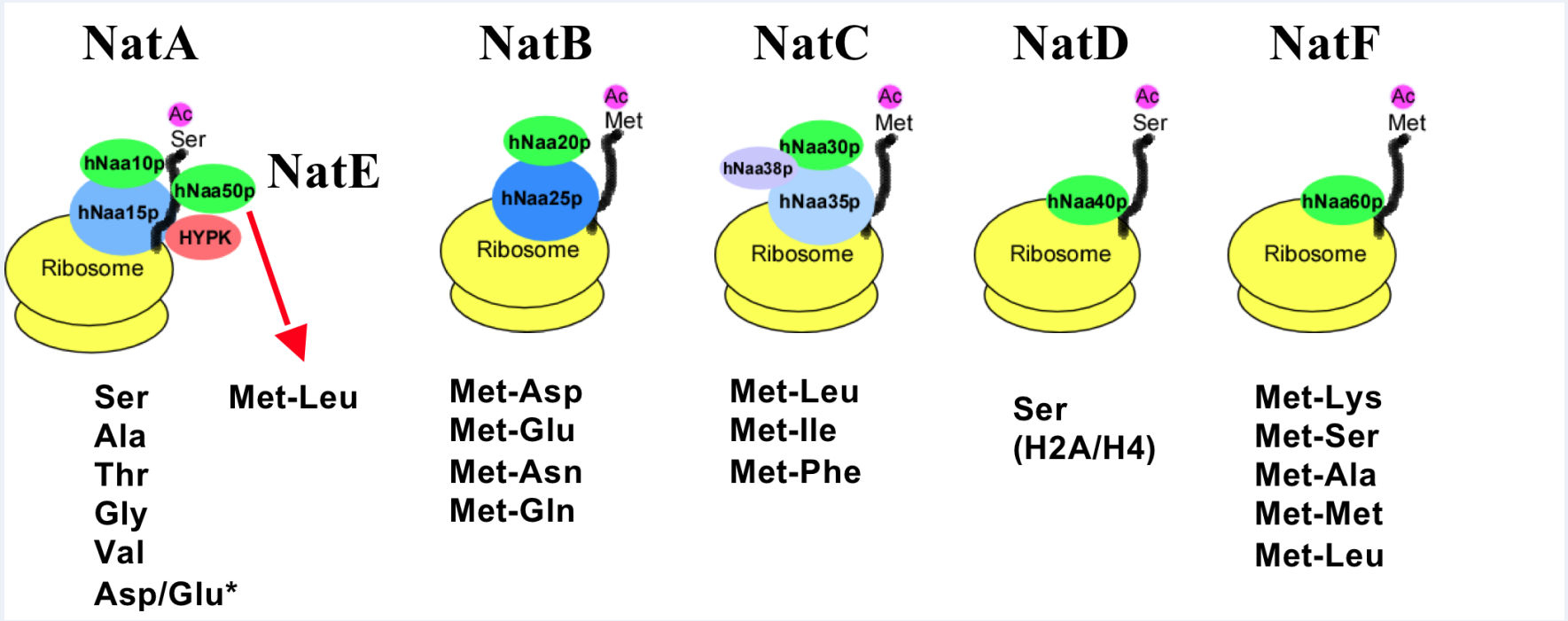


Ancestry Matters! - Ogden Syndrome



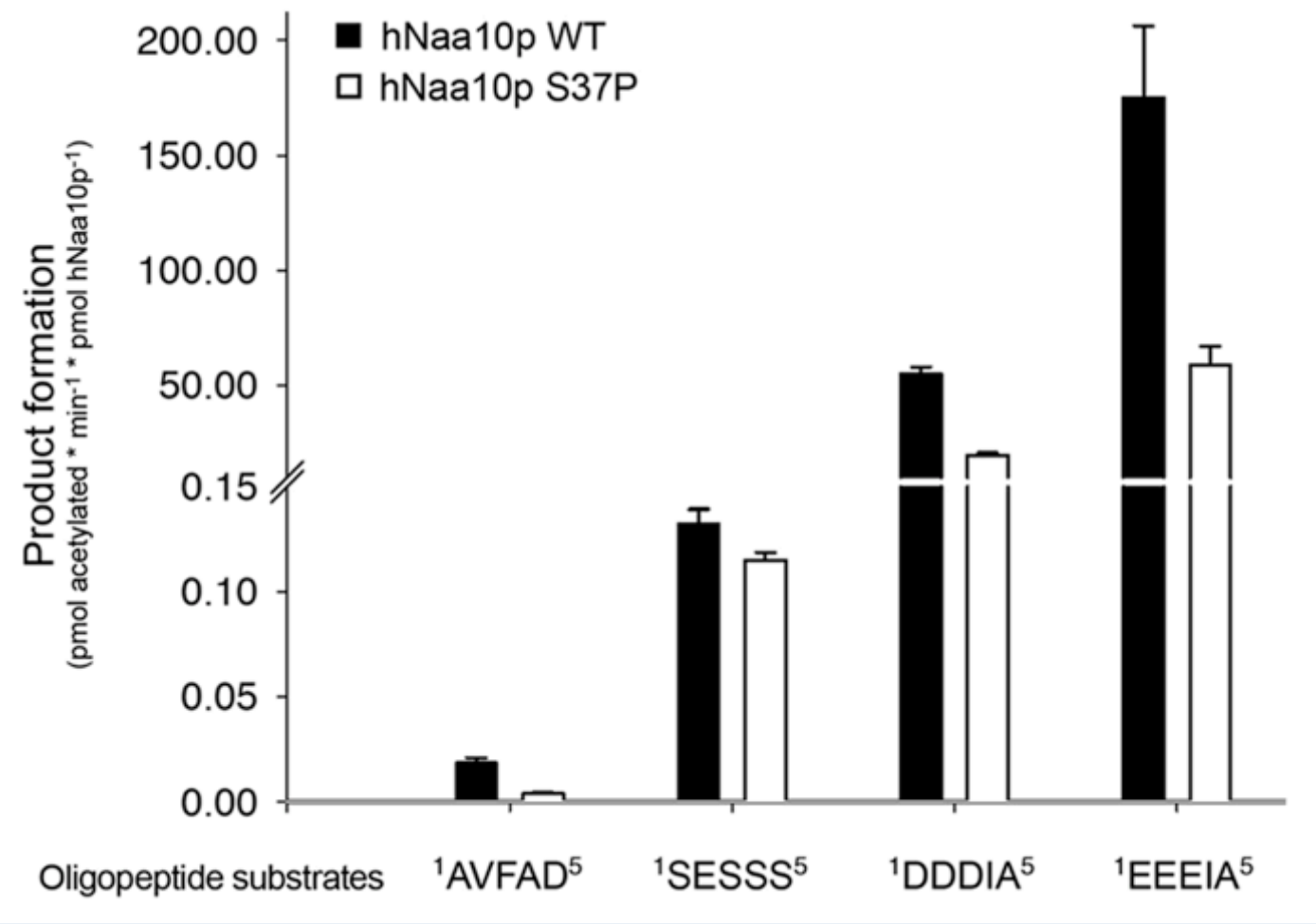
The mutation is **necessary**, but we do not know if it is **sufficient** to cause this phenotype in ANY genetic background. It simply “contributes to” the phenotype.

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

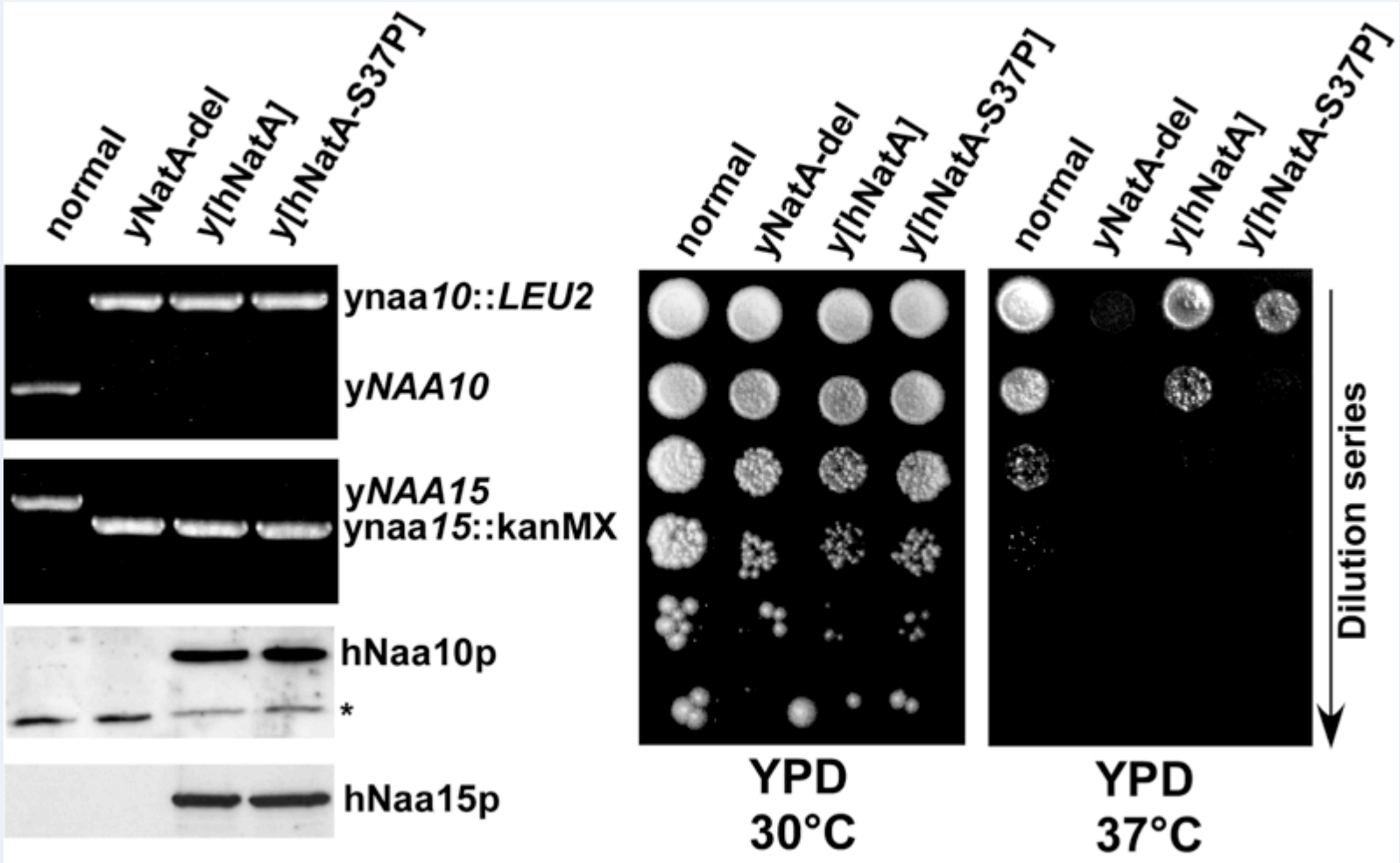


Slide courtesy of Thomas Arnesen

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

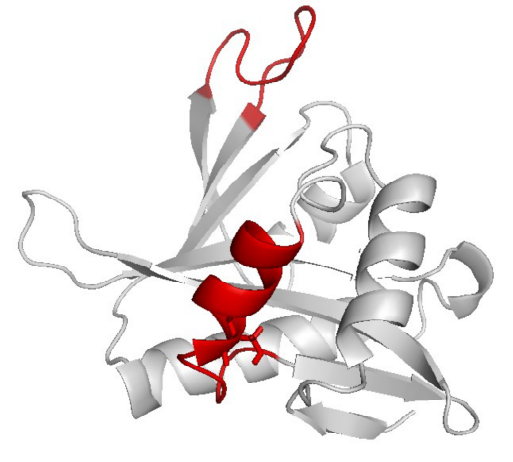


hNaa10p-S37P is functionally impaired *in vivo* using a yeast model.

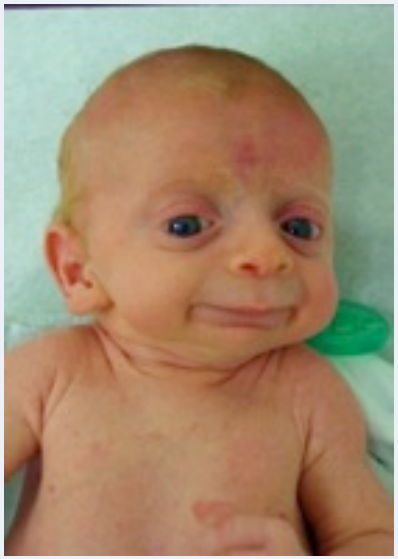


Unpublished data from Thomas Arnesen, do not further distribute.

Big Question:

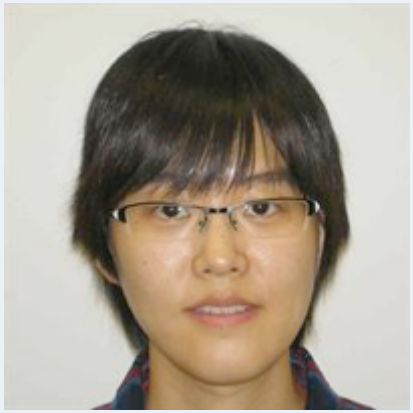


?



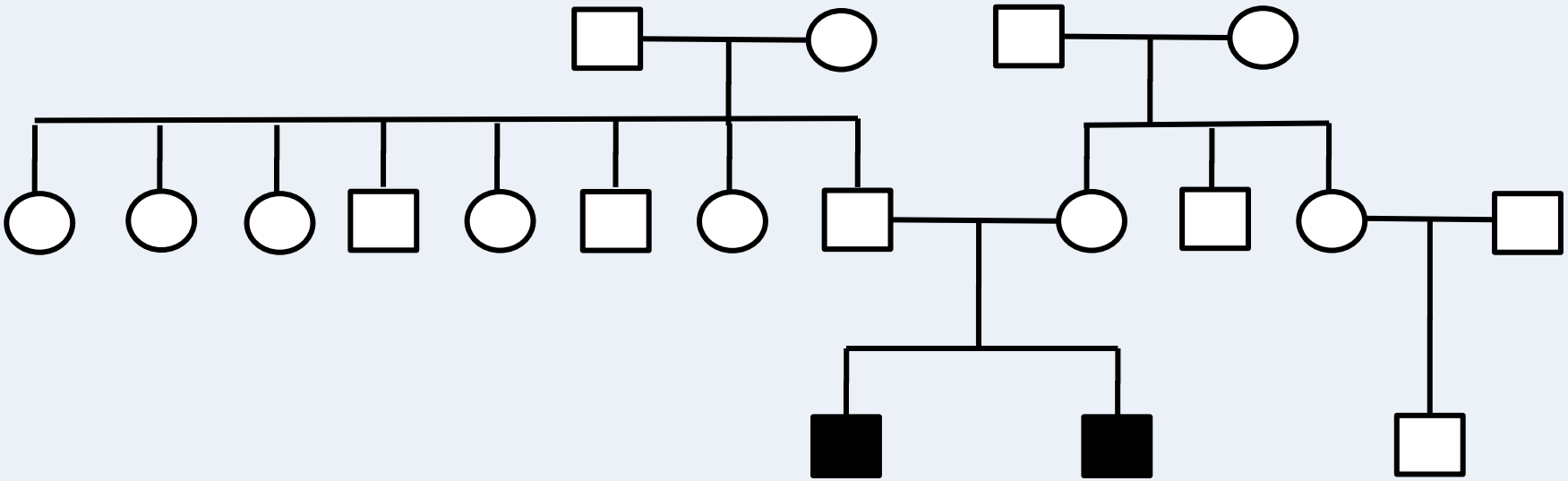
Simulated structure of S37P mutant

Max Doerfel



Yiyang Wu

New Syndrome with Dysmorphology, Mental Retardation, “Autism”, “ADHD”



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

1.5 years old

3.5 years old

7 years old

3 years old

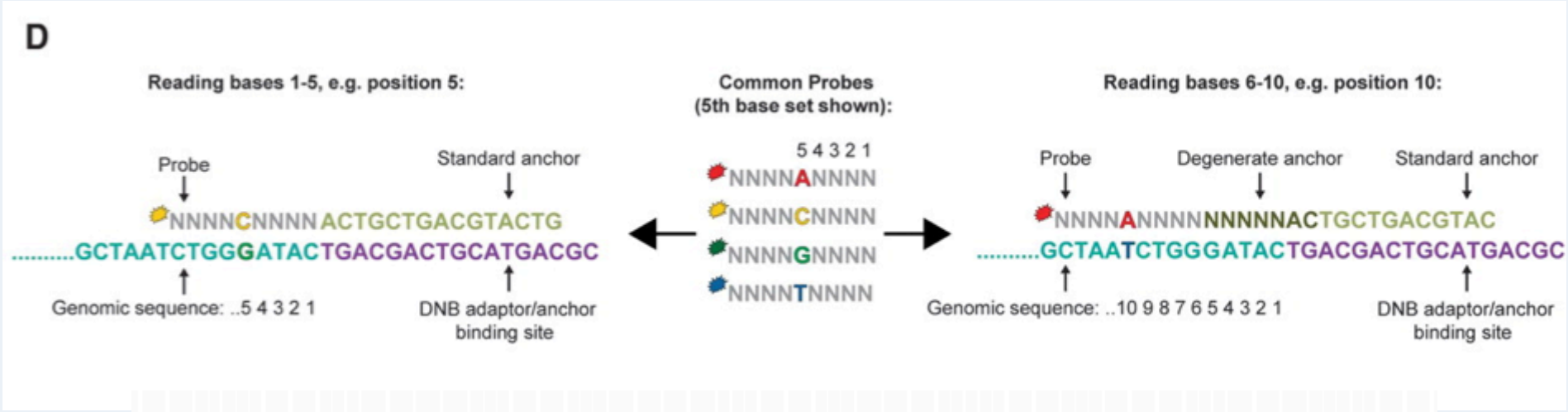
5 years old

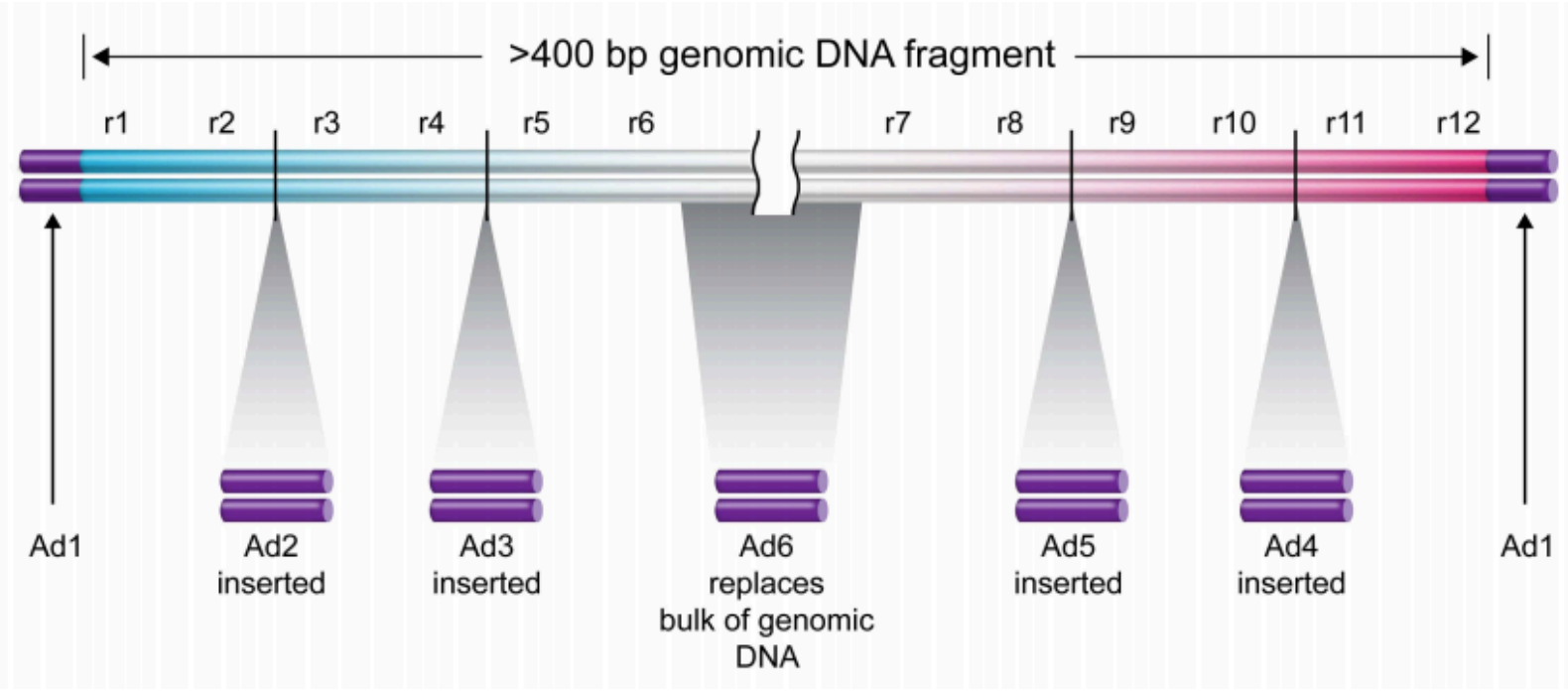
9 years old

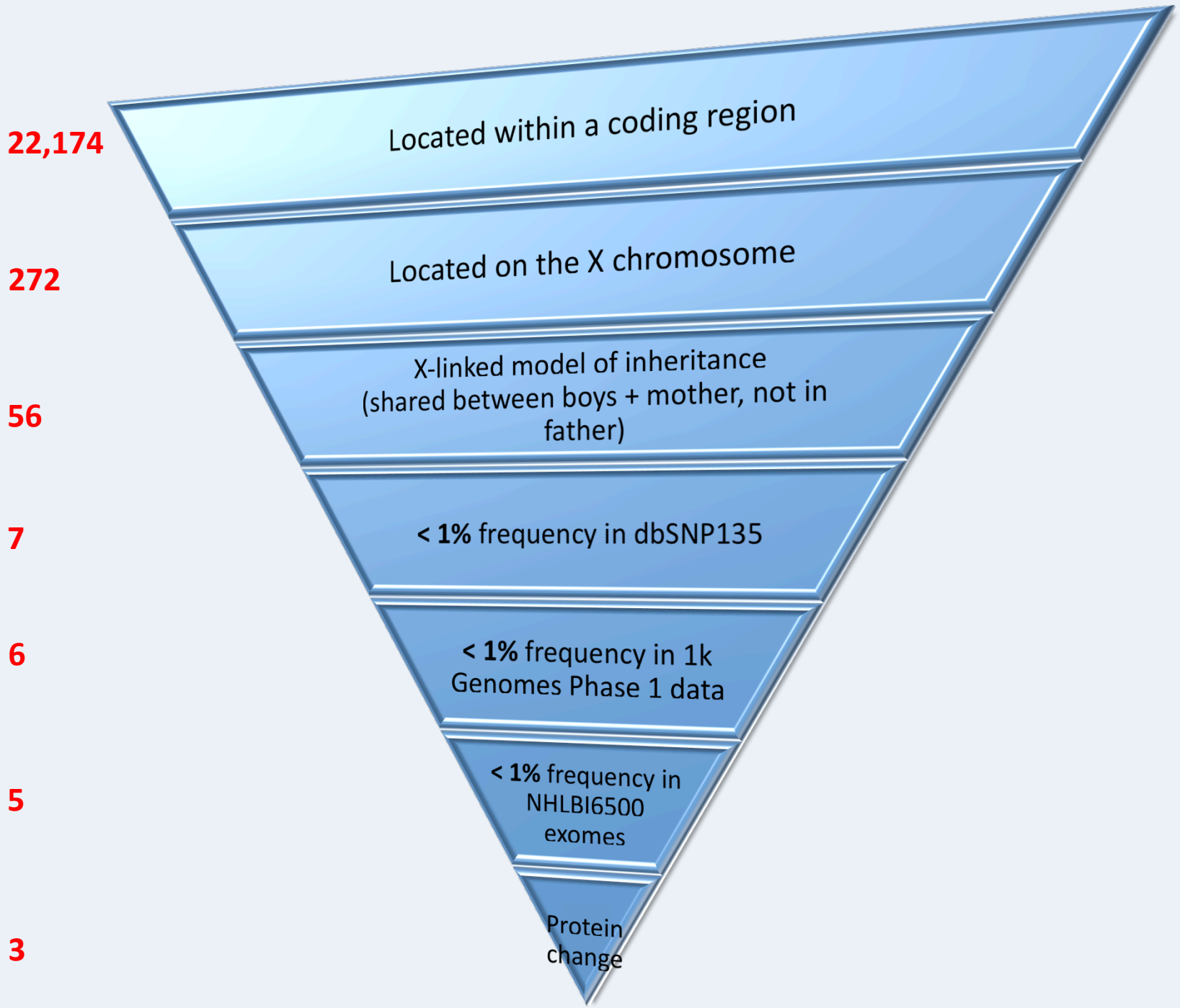
Workup Ongoing for past 10 years

- Numerous genetic tests negative, including negative for Fragile X and many candidate genes.
- No obvious pathogenic CNVs – microarrays normal.
- Sequenced whole genomes of Mother, Father and Two Boys, using Complete Genomics, obtained data in June of this year, i.e. version 2.0 CG pipeline.

Complete Genomics chemistry - combinatorial probe anchor ligation (cPAL)






22,174
Located within a coding region
272
Located on the X chromosome
56
X-linked model of inheritance (shared between boys + mother, not in father)
7
< 1% frequency in dbSNP135
6
< 1% frequency in 1k Genomes Phase 1 data
5
< 1% frequency in NHLBI6500 exomes
3
Protein change

Variant classification

Variant	Reference	Alternate	Classification	Gene 1	Transcript 1	Exon 1	HGVS Coding 1	HGVS Protein 1
X:47307978-SNV	G	T	Nonsyn SNV	ZNF41	NM_007130	5	c.1191C>A	p.Asp397Glu
X:63444792-SNV	C	A	Nonsyn SNV	ASB12	NM_130388	2	c.739G>T	p.Gly247Cys
X:70621541-SNV	T	C	Nonsyn SNV	TAF1	NM_004606	25	c.4010T>C	p.Ile1337Thr

SIFT classification

Chromosome	Position	Reference	Coding?	SIFT Score	Score <= 0.05	Ref/Alt Alleles
X	47307978	G	YES	0.649999976	0	G/T
X	63444792	C	YES	0	1	C/A
X	70621541	T	YES	0.009999999776	1	T/C

VAAST score

RANK	Gene	p-value	p-value-ci	Score	Variants
1	ASB12	1.56E-11	1.55557809307134e-11,0.000290464582480396	38.63056297	chrX:63444792;38.63;C->A;G->C;0,3
2	TAF1	1.56E-11	1.55557809307134e-11,0.000290464582480396	34.51696816	chrX:70621541;34.52;T->C;I->T;0,3
3	ZNF41	1.56E-11	1.55557809307134e-11,0.000290464582480396	32.83011803	chrX:47307978;32.83;G->T;D->E;0,3

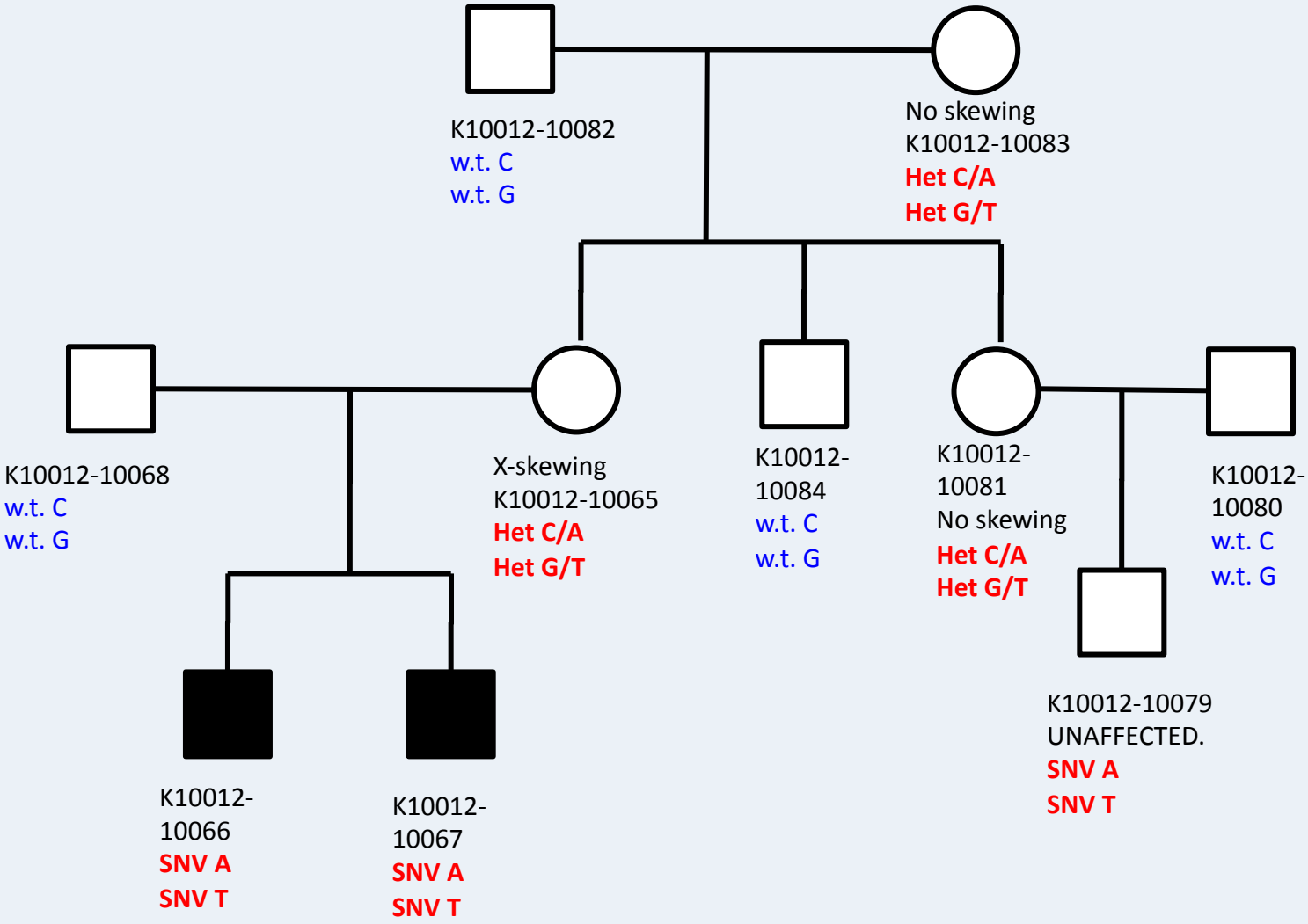
Mutations in the *ZNF41* Gene Are Associated with Cognitive Deficits: Identification of a New Candidate for X-Linked Mental Retardation

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

Sanger validation: ASB12 and ZNF41 mutations



The mutation in ZNF41 may **NOT** be necessary, and it is certainly **NOT** sufficient to cause the phenotype.

So, of course we need baseline whole genome sequencing on everyone to at least understand the DNA genetic background in each pedigree or clan.

Ancestry Matters!

Understand Your Genome Symposium

During this two-day educational event, industry experts will discuss the clinical implementation of whole-genome next-generation sequencing (NGS) technology.

illumina®

Ordering Physician:
Gholson Lyon, MD
Steinmann Institute
10 West Broadway, Suite #820
Salt Lake City, UT 84101

Individual Genome Sequence Results

Clinical Report

www.everygenome.com
CLIA#: 05D1092911

~750 DNA samples from many pedigrees with 455 of these genotyped thus far on Illumina 610K/2.5M arrays and 15 with high-depth exome, and 8 with CG whole genomes.

Table 1. Characteristics of seven new Utah extended pedigrees with preliminary diagnostic information.

Pedigree	# generations	# with DNA	# TS	# CMT	# CVT	# OCD*	# sub OCD**
14349	4	65	13	7	5	29	14
7166	3	27	7	1	0	11	10
13166	3	23	10	2	1	3	6
8115	3	20	9	1	0	9	3
6991	4	15	8	2	0	4	2
8598	3	11	8	0	0	6	0
3695	3	7	3	1	0	4	0
TOTALS		**168**	**58**	**14**	**6**	**66**	**35**

Note. TS=Tourette Syndrome; CMT=Chronic Motor Tics; CVT=Chronic Vocal Tics; OCD= Obsessive Compulsive Disorder; sub OCD=subclinical Obsessive Compulsive Disorder.

*Of the cases with OCD, 39 also have TS or chronic tics, leaving 27 with OCD only.

**Of the cases with sub OCD, 17 also have TS or chronic tics, leaving 18 with sub OCD only.

Some Definitions …

- The words “penetrance” and “expressivity”, defined classically as:
- Penetrance: whether someone has ANY symptoms of a disease, i.e. all or none, 0% or 100%. **Nothing in between.**
- Expressivity: how much disease (or how many symptoms) someone with 100% penetrance has.
- This has led to endless confusion!
- Some just use the word “penetrance” to mean the expressivity of disease, i.e. incomplete penetrance, and maybe we should combine the two terms into ONE word with the full expression from 0-100% of phenotypic spectrum.

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, Foege S413C, 3720 15th Avenue NE, Seattle, WA 98195, USA

Genome-Wide Association Study of Multiplex Schizophrenia Pedigrees

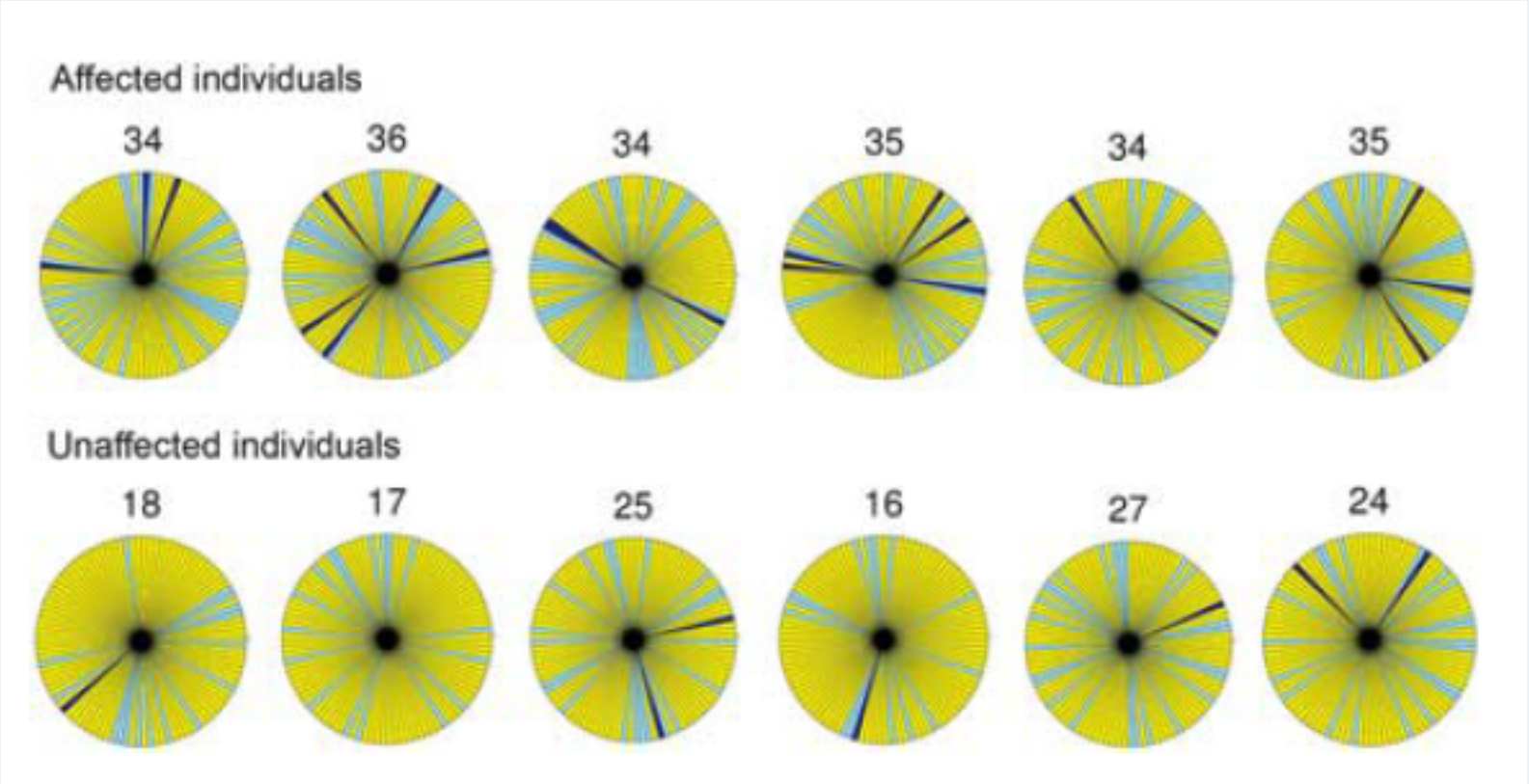
Am J Psychiatry Levinson et al.; AiA:1–11

"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."

Definitions. It is unknown what portion of autism will be oligogenic vs. polygenic

- **Oligogenic** – multiple mutations together contributing to aggregate disease, BUT with only 1 mutation of ~ >10% penetrance (or "effect size) in EACH person.
- **Polygenic** – Dozens to hundreds of mutations in different genes in the SAME person, together contributing to the disease in the SAME person, hence **additive** and/or **epistatic** contribution with ~0.01-1% penetrance for each mutation.

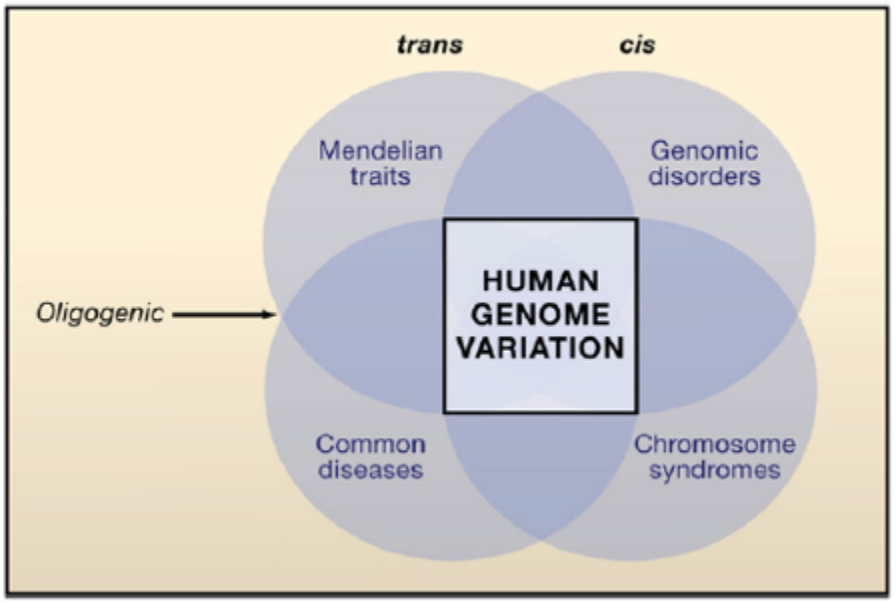
Example of Polygenic Model

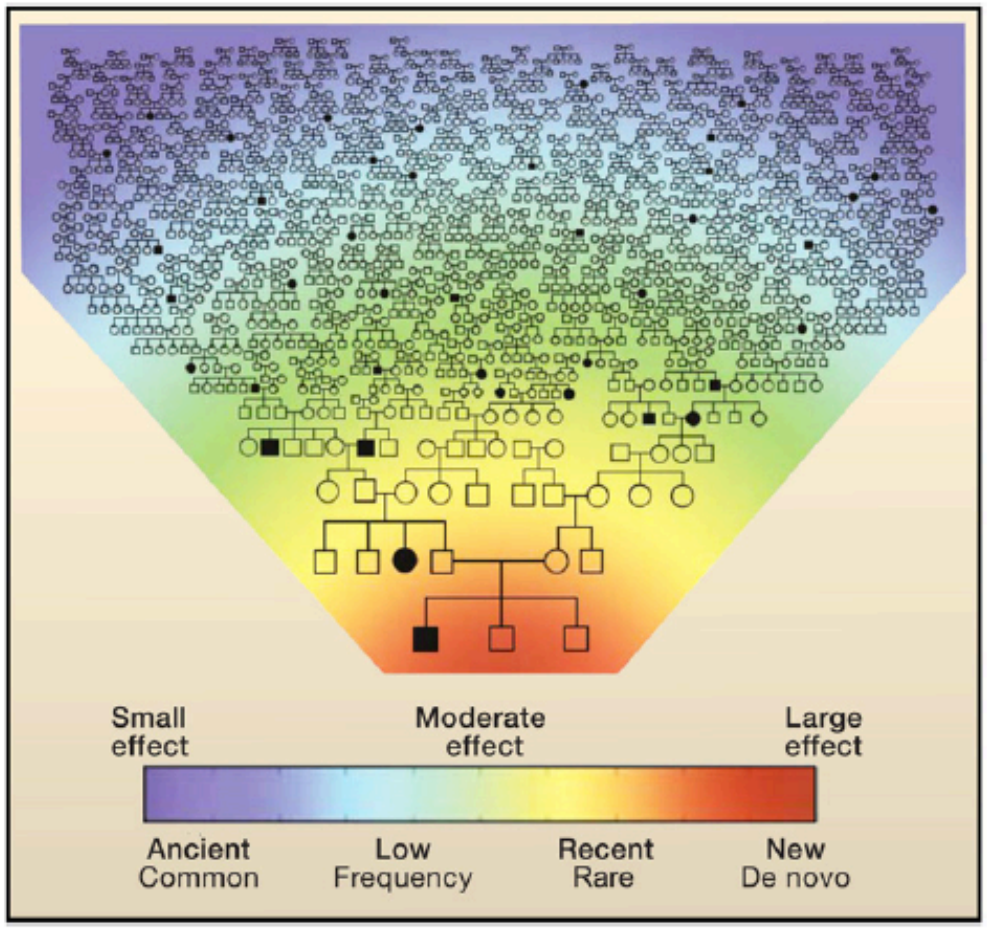


Visscher et al. 2011

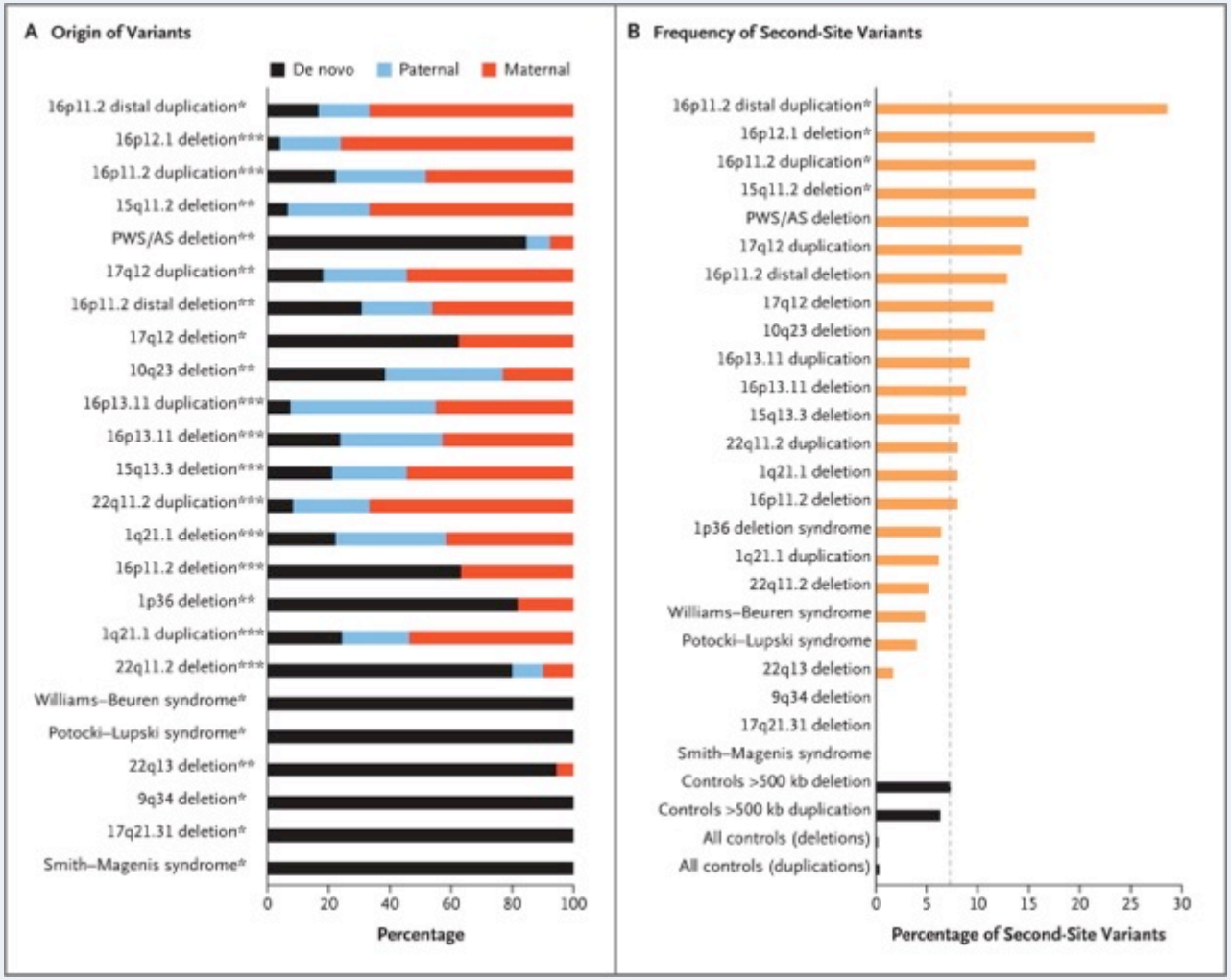
Clan Genomics and the Complex Architecture of Human Disease

James R. Lupski,[1,2,3,*] John W. Belmont,[1,2] Eric Boerwinkle,[4,5] and Richard A. Gibbs[1,5,*]



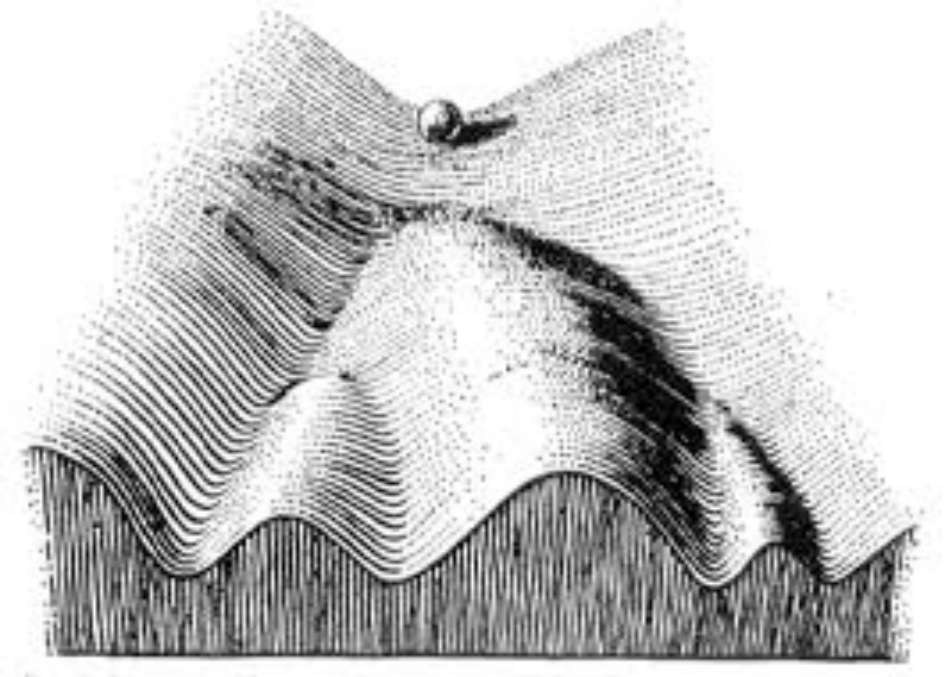


Inheritance Pattern of Copy-Number Variants and Frequency of Second-Site Variants Associated with a Genomic Disorder.



Girirajan S et al. N Engl J Med 2012. DOI: 10.1056/NEJMoa1200395

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.



The canalisation metaphor suggests that phenotypes are very robust to small perturbations, for which development does not exit the canal, and rapidly returns back down, with little effect on the final outcome of development. But perturbations whose magnitude exceeds a certain threshold will break out of the canal, moving the developmental process into uncharted territory. Strong robustness up to a limit, with little robustness beyond, is a pattern that could increase evolvability in a fluctuating environment.

“Biological Indeterminacy”

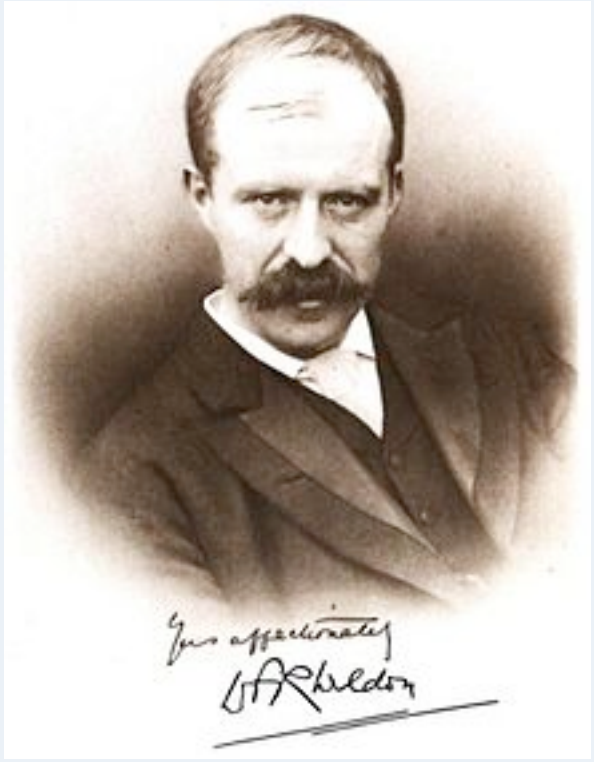
- Bateson became famous as the outspoken Mendelian antagonist of Walter Raphael Weldon, his former teacher, and Karl Pearson who led the biometric school of thinking. This concerned the debate over saltationism versus gradualism (Darwin had been a gradualist, but Bateson was a saltationist). Later, Ronald 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

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

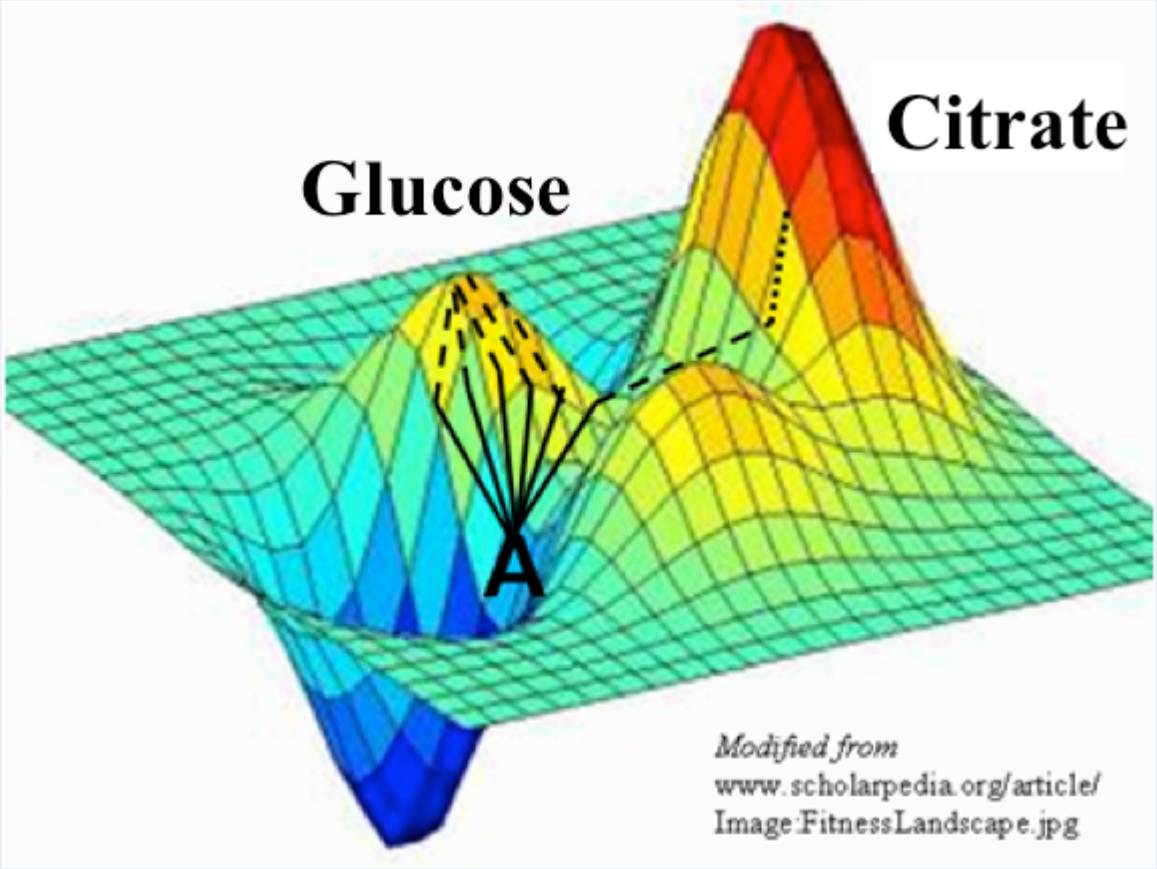


Walter Frank Raphael Weldon

Vs.

William Bateson

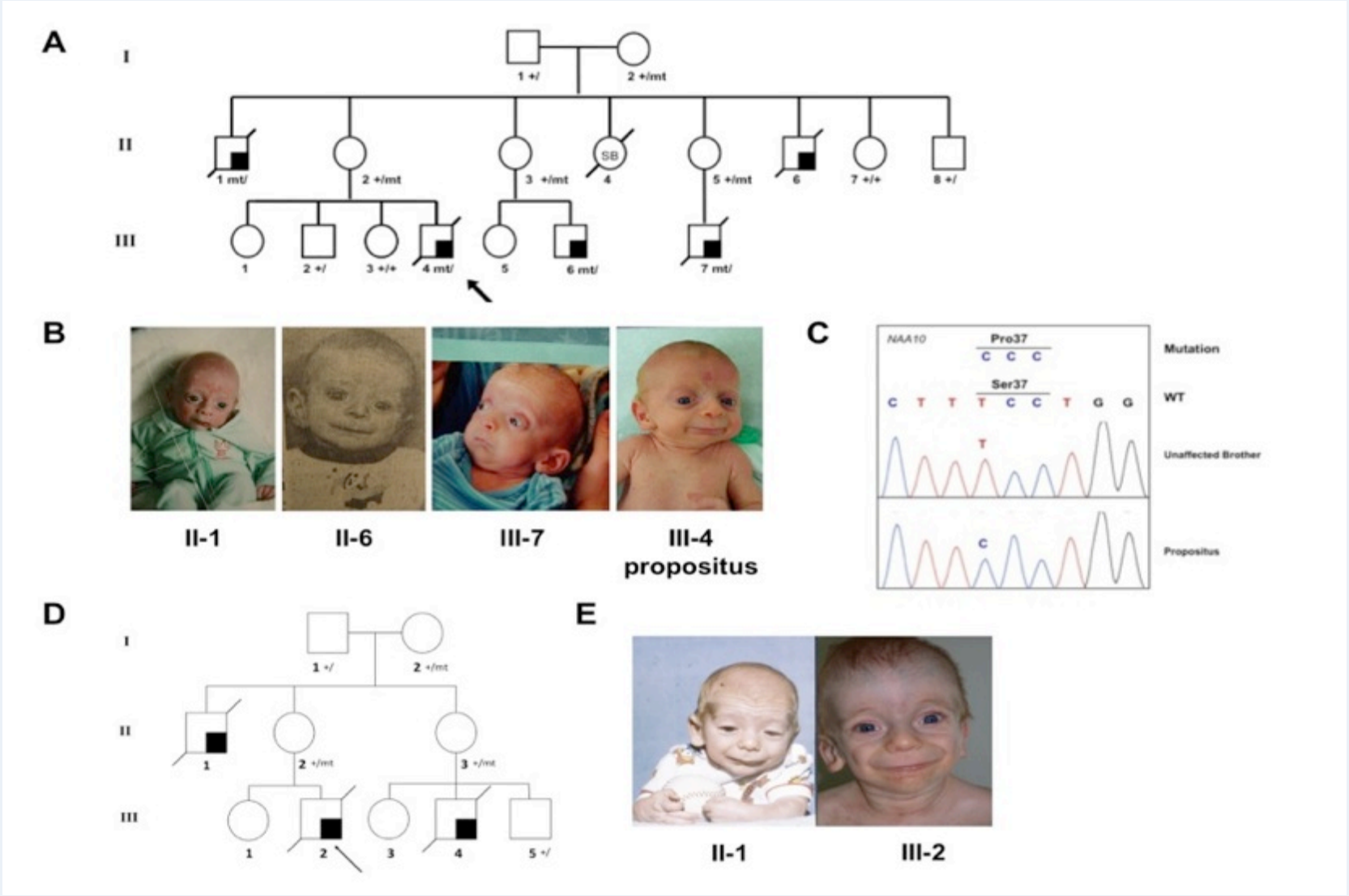
Forthcoming by Greg Radick. Scholarly edition of W. F. R. Weldon's Theory of Inheritance (1904-1905), coedited with Annie Jamieson.



E. coli adapting to low glucose conditions, in the context of media containing citrate. "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

Genomic analysis of a key innovation in an experimental Escherichia coli population.
Blount ZD, Barrick JE, Davidson CJ, Lenski RE.
Nature. 2012 Sep 19. doi: 10.1038/nature11514

Ancestry Matters! - Ogden Syndrome



The mutation is **necessary**, but we do not know if it is **sufficient** to cause this phenotype in ANY genetic background. It simply “contributes to” the phenotype.

Penetrance and Expressivity

- We do not really know the penetrance or expressivity of pretty much ALL mutations in **humans**, as we have not systematically sequenced or karyotyped any genetic alteration in **MILLIONS** of well-phenotyped people.
- Do single mutations drive outcome predominately, or are the results modified substantially by other mutations and/or environment? Is there really such a thing as genetic determinism for **MANY** mutations?

The Biology of MENTAL DEFECT

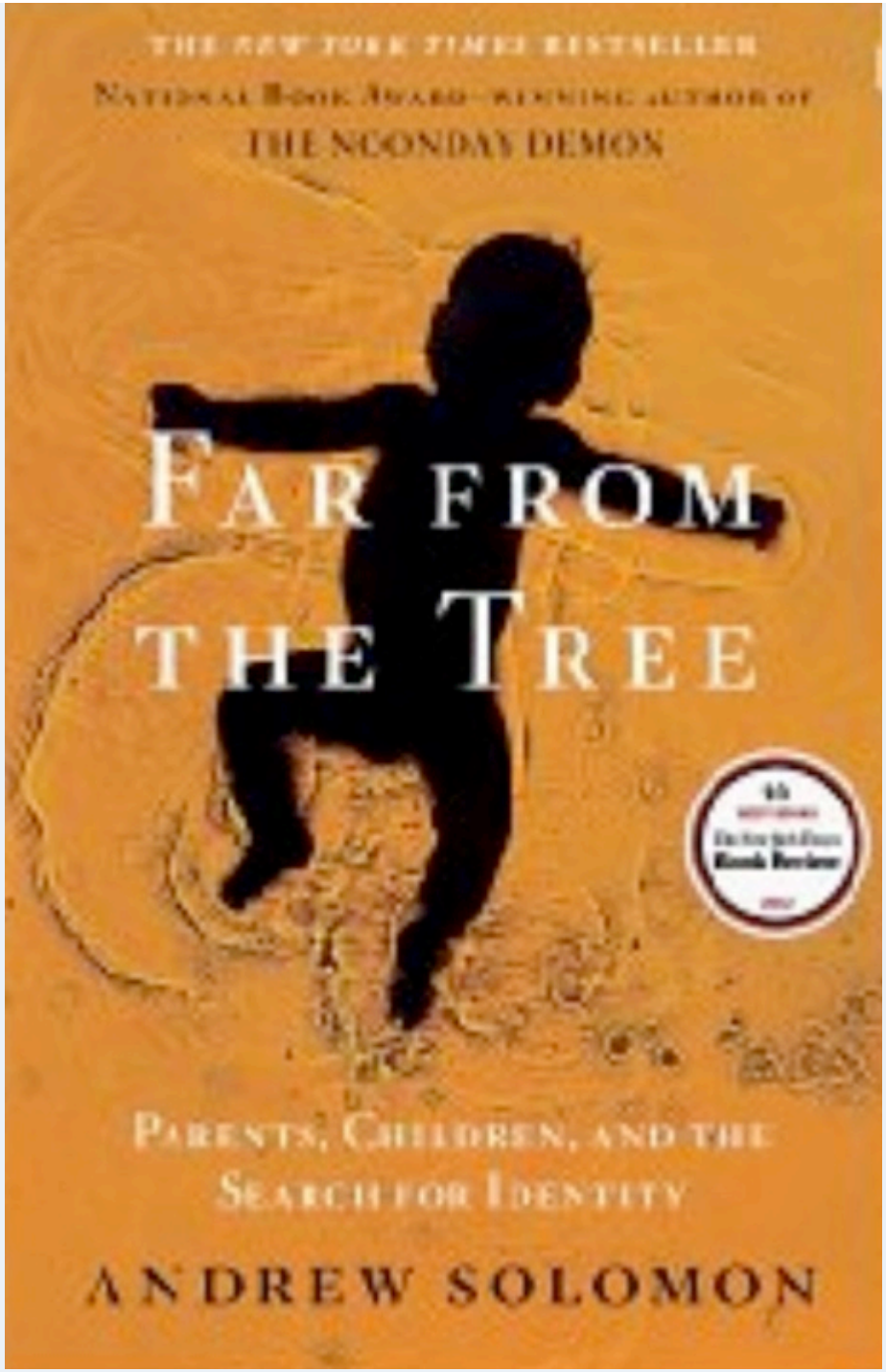
BY

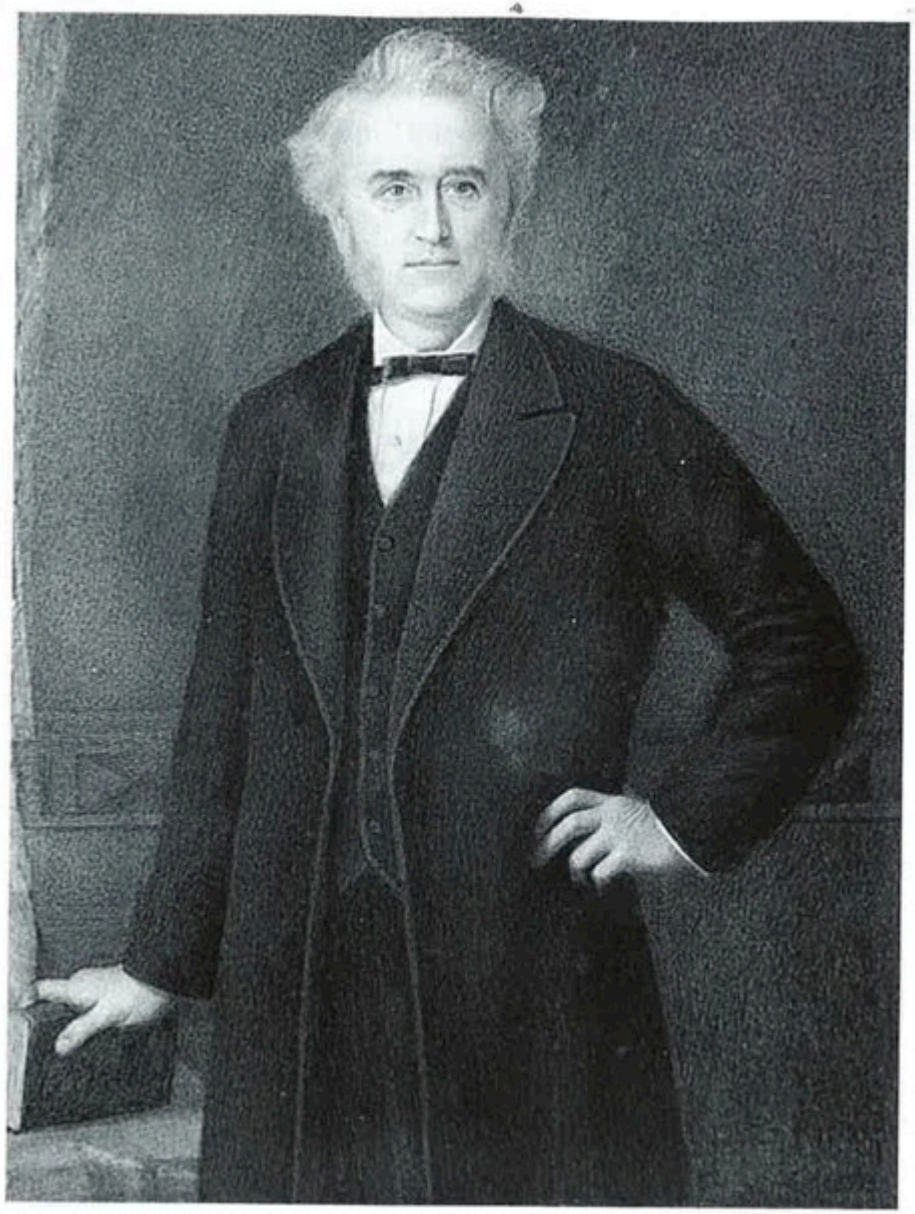
LIONEL S. PENROSE, M.A., M.D.

WITH A PREFACE BY

PROFESSOR J. B. S. HALDANE, F.R.S.

GRUNE & STRATTON
New York
1949





Portrait of Langdon Down, painted by Sydney Hodges in 1883.

OBSERVATIONS ON AN ETHNIC CLASSIFICATION OF IDIOTS *

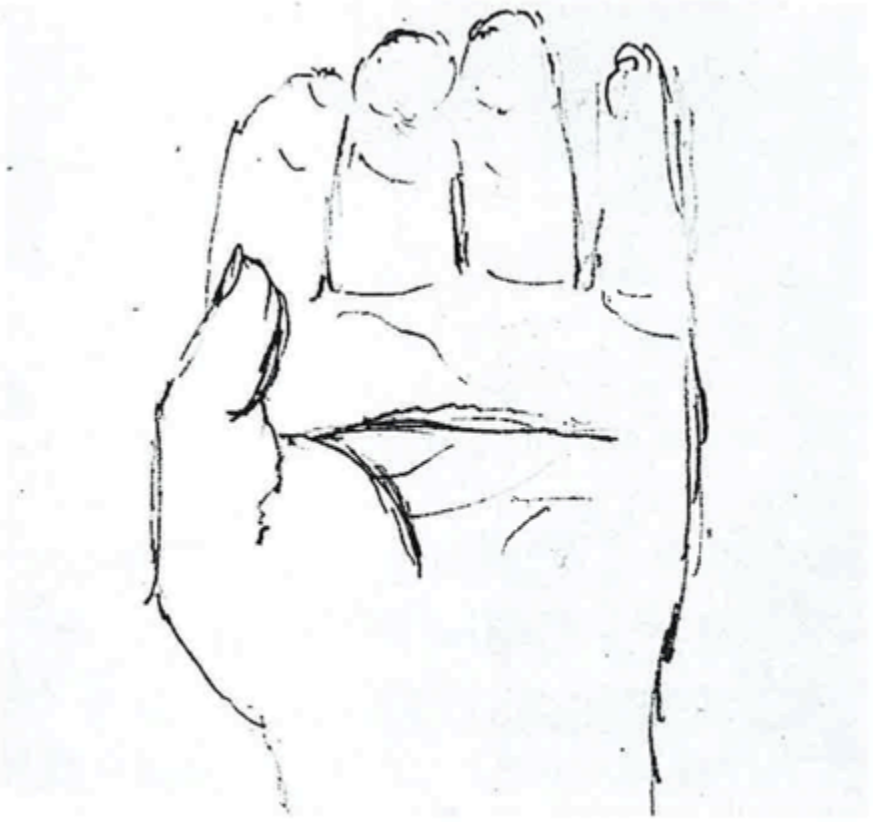
J. LANGDON H. DOWN M.D., *London*

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."

Plate VII—Mongolism in two imbecile brothers aged 10 (Colchester Survey, 1938, Case No. 750) and 5 years, with a normal child aged 2½ years.

As compared with the normal child, the younger mongoloid is seen to have a small head, decreased stature and dysplastic features. The characteristic fold of skin covering the inner canthus of each eye (epicanthic fold) was clearly marked in this case.



Reginald Langdon Down was the first to describe the pattern of creases in the palm in Down's syndrome patients. He drew this sketch in 1908.

Published in “Biology of Mental Defect”, by Lionel Penrose, 1949
And “John Langdon Down: A Caring Pioneer”, by O Conor Ward, 1998.

Mary A, the first Down's syndrome patient admitted to Normansfield, photographed when she was 19 and again when she was 55. She lived to the age of 58.

Florence T, a Down's syndrome patient at Normansfield. Photographed in 1886 when she was seven and again in 1899 aged 20.

Published in “John Langdon Down: A Caring Pioneer”, by O Conor Ward, 1998.

Langdon Down began to take clinical photographs in 1862. His first photograph of an Earlswood resident with Down's syndrome was this unnamed girl in the 1865 series. She was probably the first ever Down's syndrome patient to be photographed.

Published in "John Langdon Down: A Caring Pioneer", by O Conor Ward, 1998.

Four Down's syndrome patients. Part of the Earlswood series, photographed in 1865.

Dr Reginald Langdon Down with his daughters Stella and Elspie. Stella married Russell Brain and became Lady Brain. Elspie was an artist. The only son was John, who had Down's syndrome.

Dr Percival Langdon Down with his wife and children. His son Norman, was to be the last Langdon Down superintendent of Normansfield, ending a family connection that had lasted for 102 years. The elder daughter, Molly, was also a doctor and worked in Normansfield.

Langdon Down's personal patients with his syndrome[2]

Name	Age Admitted	Date Admitted	Outcome	Comment
Mary A	19	12.5.68	Died 1907, age 58	Cardiac failure, Alzheimer's
Cecelia GA	10	7.6.68	Died 31.1.70, age 12	Fatal scarlet fever
Herbert H	8	15.7.68	Discharged 10.10.68	Improved
Edward GP	11	1.5.69	Died 1908, age 50	
Laura M	7	5.4.69	Died 5.4.77, age 15	Tuberculosis: Query
Walter AP	4	4.11.75	Discharged 27.1.77	Masturbation cured
Margaret DE	11	14.4.74	Died 15.5.74, age 11	Fatal scarlet fever
Norah MT	12	23.4.74	Died 26.6.74, age 12	Acute Bronchitis
James DKW	5	10.1.77	Died 30.12.77, age 12	Bronchitis and Pneumonia
Norman MB	10	14.2.77	Died 12.1.12, age 45	Alzheimer's?
Thomas N	6	13.11.77	Died 1896, age 25	Cardiac failure
Margaret AW	4	11.3.80	Died 1885, age 9	Sudden death on holiday
George HW	6	27.3.80	Died 27.11.80, age 7	Laryngo bronchitis, croup
Cathy MS	9	28.3.82	Died 20.8.82, age 9	Bronchitis and pneumonia
Lucy EN	11	22.8.82	Died 3.11.85, age 14	Broncho-pneumonia, cardiac failure
Ada FH	15	2.12.82	Alive 1895	
Elizabeth G	5	27.10.83	Discharged 16.2.87	Improved
Florence ET	7	8.3.86	Alive 1895	
David AH	6	5.4.72	Died 1915. age 49	Late onset of blindness and deafness
Constance AW	13	31.7.86	Discharged 12.5.88	Improved
Ann MR	17	18.11.86	Discharged 26.5.91	Improved
John GT	15	6.7.74	Died 4.6.18, age 59	Alzheimer's?

Down Syndrome

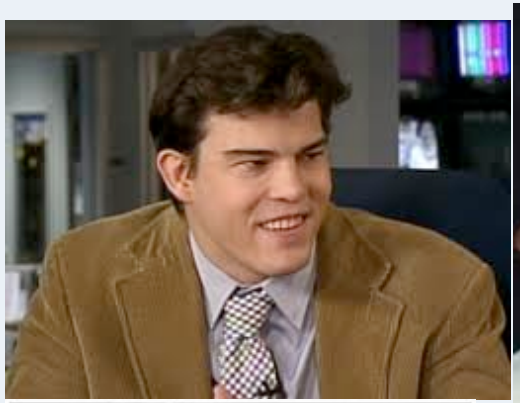
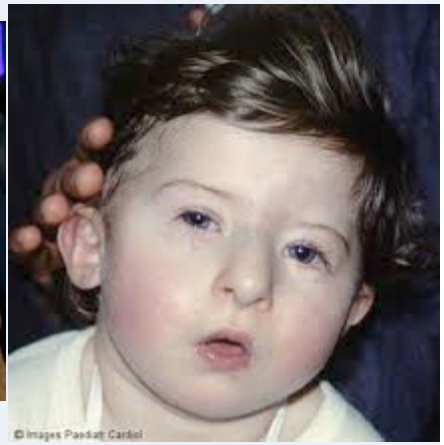
ARTICLE

An Excess of Deleterious Variants in VEGF-A Pathway Genes in Down-Syndrome-Associated Atrioventricular Septal Defects

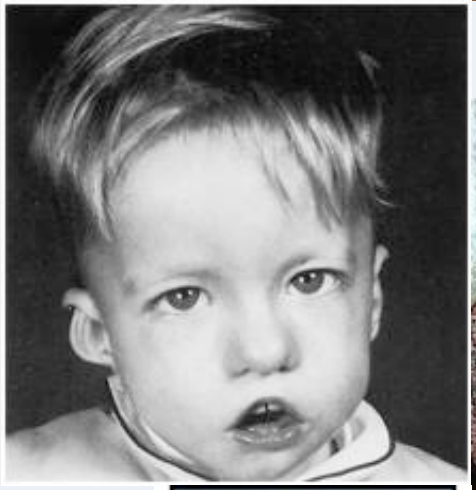
Christine Ackerman,[1] Adam E. Locke,[2,8] Eleanor Feingold,[3] Benjamin Reshey,[1] Karina Espana,[1] Janita Thusberg,[4] Sean Mooney,[4] Lora J.H. Bean,[2] Kenneth J. Dooley,[5] Clifford L. Cua,[6] Roger H. Reeves,[7] Stephanie L. Sherman,[2] and Cheryl L. Maslen[1,*]

About half of people with trisomy 21 have a congenital heart defect (CHD), whereas the remainder have a structurally normal heart, demonstrating that trisomy 21 is a significant risk factor but is not causal for abnormal heart development. Atrioventricular septal defects (AVSD) are the most commonly occurring heart defects in Down syndrome (DS), and ~65% of all AVSD is associated with DS. We used a candidate-gene approach among individuals with DS and complete AVSD (cases = 141) and DS with no CHD (controls = 141) to determine whether rare genetic variants in genes involved in atrioventricular valvuloseptal morphogenesis contribute to AVSD in this sensitized population. We found a significant excess ($p < 0.0001$) of variants predicted to be deleterious in cases compared to controls. At the most stringent level of filtering, we found potentially damaging variants in nearly 20% of cases but fewer than 3% of controls. The variants with the highest probability of being damaging in cases only were found in six genes: *COL6A1*, *COL6A2*, *CRELD1*, *FBLN2*, *FRZB*, and *GATA5*. Several of the case-specific variants were recurrent in unrelated individuals, occurring in 10% of cases studied. No variants with an equal probability of being damaging were found in controls, demonstrating a highly specific association with AVSD. Of note, all of these genes are in the VEGF-A pathway, even though the candidate genes analyzed in this study represented numerous biochemical and developmental pathways, suggesting that rare variants in the VEGF-A pathway might contribute to the genetic underpinnings of AVSD in humans.

Velocardiofacial (22q11.2) Syndrome

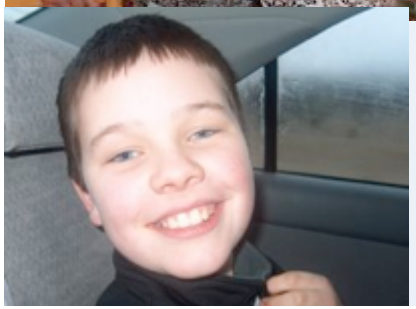





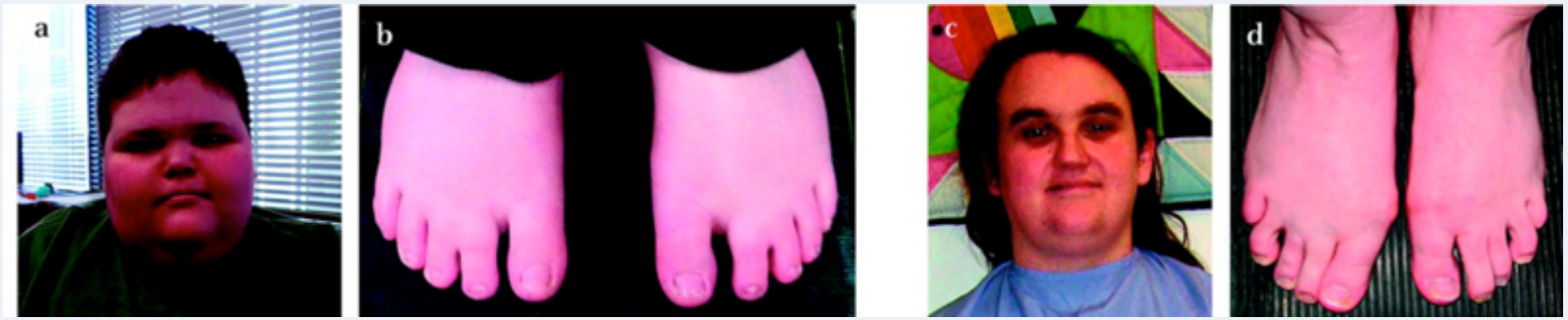








16p11.2 deletion



Clinical photographs. (a and b) Proband 2 (de novo deletion 16p11.2). Note long narrow palpebral fissures, short delicate nose, short neck and brachydactyly with 2–3 cutaneous toe syndactyly. (c and d) Mother of proband 3 (both with deletions). Note her large ears, smooth philtrum and short fifth toes.

Fernandez B A et al. J Med Genet 2010;47:195-203

JMG

16p11.2 duplication

Clinical photographs. (e) Proband 5 who has a maternally inherited duplication. (f) Proband 5 (note smooth philtrum) and her healthy duplication positive sister. (g) Duplication positive mother of proband 5, who also has a smooth philtrum. (h) Proband 6 (inherited duplication and oliogohydramnios sequence). Note her frontal bossing, receding hairline, hypoplastic supraorbital ridges and smooth philtrum. (i) Proband 6's right hand showing fifth finger clinodactyly.

Fernandez B A et al. J Med Genet 2010;47:195-203

16p11.2 deletion, not in mother or father, only in child.

5 years old, but developmental age of 2 year old.
Speaks a few words, almost unintelligible.
Very hyperactive.
Can be withdrawn and has at times been diagnosed with “autism”.

Current Diagnoses under Evaluation (DSM IV-TR)

AXIS I	299.00	Autism Disorder
	314.01	Attention-Deficit-Hyperactivity Disorder, Combined Type
AXIS II	V71.09	No Diagnosis
AXIS III	16p11.2	Microdeletion
AXIS IV		Psychosocial Stressors: Moderate(Adaptive/Behavioral and Educational/Learning Problems)
AXIS V		Current GAF: 60

Assessment Procedures:

Wechsler Preschool and Primary Scale of Intelligence (WPPSI)
Wide Range Achievement Test 4rd Edition (WRAT-4)
Test of Memory and Learning 2 (TOMAL, 2)
Beery VMI 6th Edition (Beery-Buktenica Developmental Test of Visual-Motor Integration, 6th Edition; Visual Perception, 6th Edition; Motor Coordination, 6th Ed)
Wide Range Assessment of Visual Motor Abilities (WRAVMA)
Conners' Comprehensive Behavior Rating Scales (CBRS) (Parent Report)
The Social Responsiveness Scale
Autism Diagnostic Interview Revised (ADI-R)
Mental Status Examination
Steinmann Neuropsychiatric Developmental Questionnaire
CNS Vital Signs Neuropsychological Screening
Clinical Interview with Patient
Clinical Interview with Parent
Clinical Observations
Review of Medical, Psychiatric, and Scholastic Records

Clinical Management and Genetics

Genet Med. 2011 Sep;13(9):770-6.

Chromosomal microarray testing influences medical management.

Coulter ME, Miller DT, Harris DJ, Hawley P, Picker J, Roberts AE, Sobeih MM, Irons M.

PURPOSE:

Chromosomal microarray (CMA) testing provides the highest diagnostic yield for clinical testing of patients with developmental delay (DD), intellectual disability (ID), multiple congenital anomalies (MCA), and autism spectrum disorders (ASD). Despite improved diagnostic yield and studies to support cost-effectiveness, concerns regarding the cost and reimbursement for CMA have been raised because it is perceived that CMA results do not influence medical management.

METHODS:

We conducted a retrospective chart review of CMA testing performed during a 12-month period on patients with DD/ID, ASD, and congenital anomalies to determine the proportion of cases where abnormal CMA results impacted recommendations for clinical action.

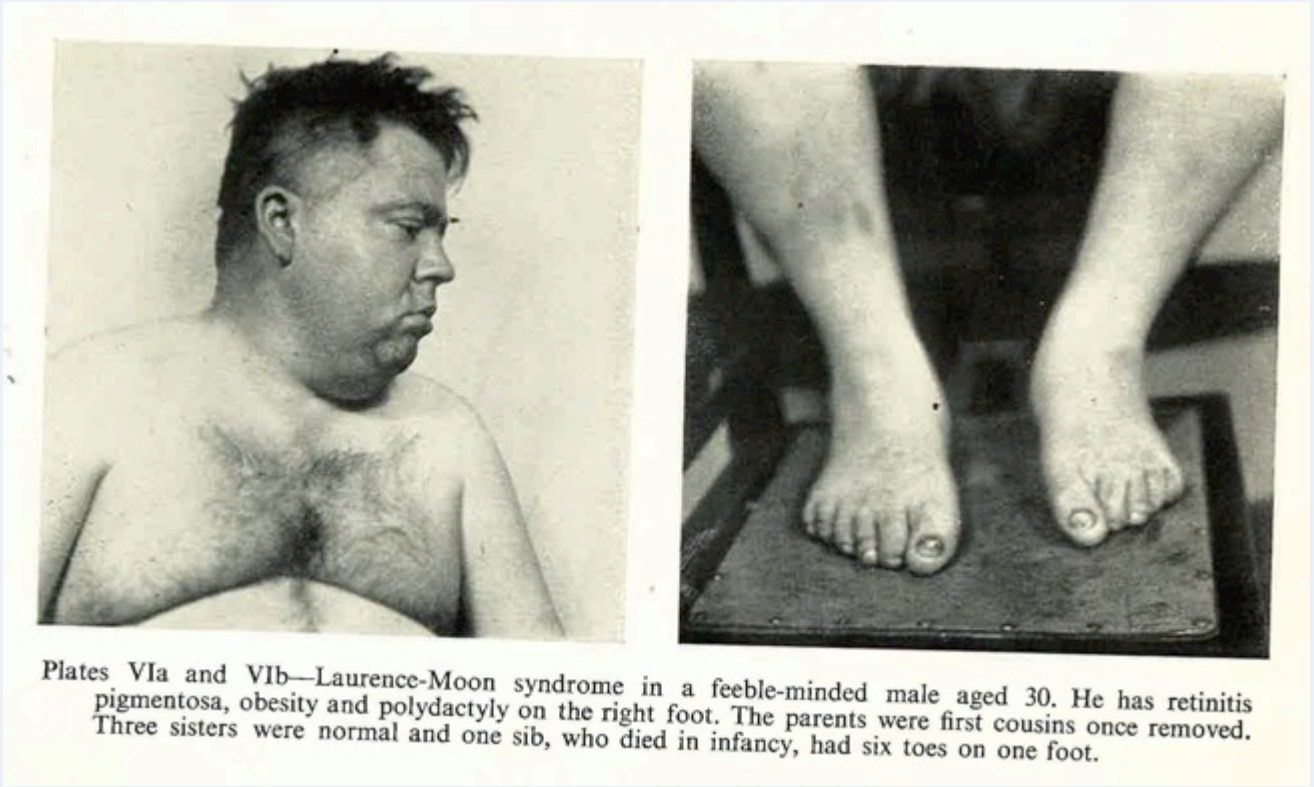
RESULTS:

Among 1792 patients, 13.1% had clinically relevant results, either abnormal (n = 131; 7.3%) or variants of possible significance (VPS; n = 104; 5.8%). Abnormal variants generated a higher rate of recommendation for clinical action (54%) compared with VPS (34%; Fisher exact test, P = 0.01). CMA results influenced medical care by precipitating medical referrals, diagnostic imaging, or specific laboratory testing.

CONCLUSIONS:

For all test indications, CMA results influenced medical management in a majority of patients with abnormal variants and a substantial proportion of those with VPS. These results support the use of CMA as a clinical diagnostic test that influences medical management for this patient population.

Laurence-Moon Syndrome, now known as Bardet-Biedl Syndrome



Plates VIa and VIb—Laurence-Moon syndrome in a feeble-minded male aged 30. He has retinitis pigmentosa, obesity and polydactyly on the right foot. The parents were first cousins once removed. Three sisters were normal and one sib, who died in infancy, had six toes on one foot.

Langdon Down's patient Elizabeth C. She has the short stature, severe obesity and characteristic facial appearance of Prader–Willi syndrome.

Published in "John Langdon Down: A Caring Pioneer", by O Conor Ward, 1998.

James Henry Pullen, the idiot savant *who designed the prize winning exhibit for the Paris exhibition in 1867, dressed in the admiral's uniform which he accepted in return for not pursuing his plan to marry. He also designed a realistic model of the* Great Eastern, *a famous transatlantic vessel built by Brunel.*

Master Craftsman
Most famously designed The Great Eastern, a 10 foot long model ship with incredible detail.

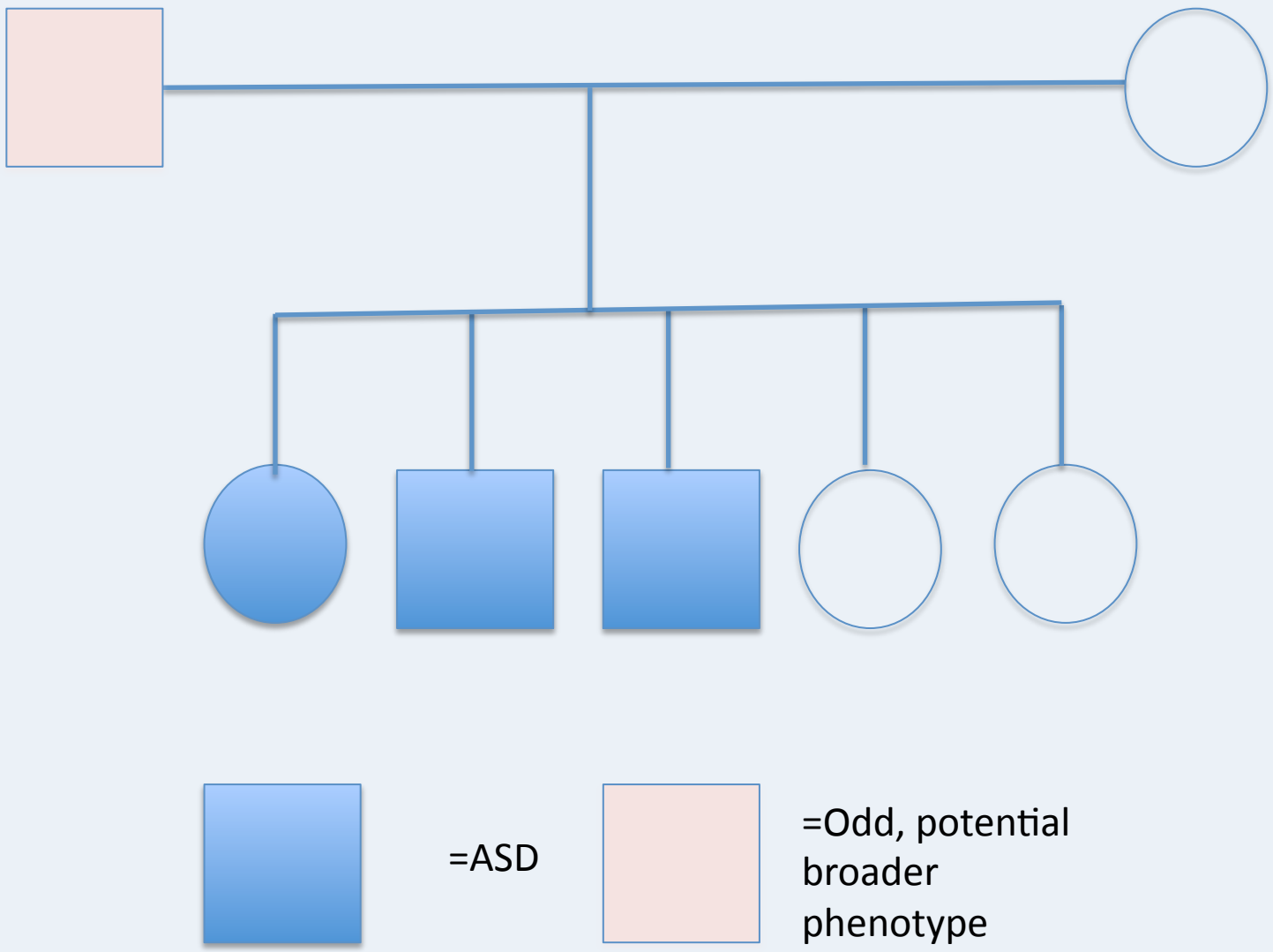
Deaf and nearly mute – Nonverbal,
Obsessed with one topic of building things.
Thought to be mentally retarded.
Usually quiet and reserved, but sometimes was intolerant of advice, suspicious of strangers, and ill-tempered and violent.

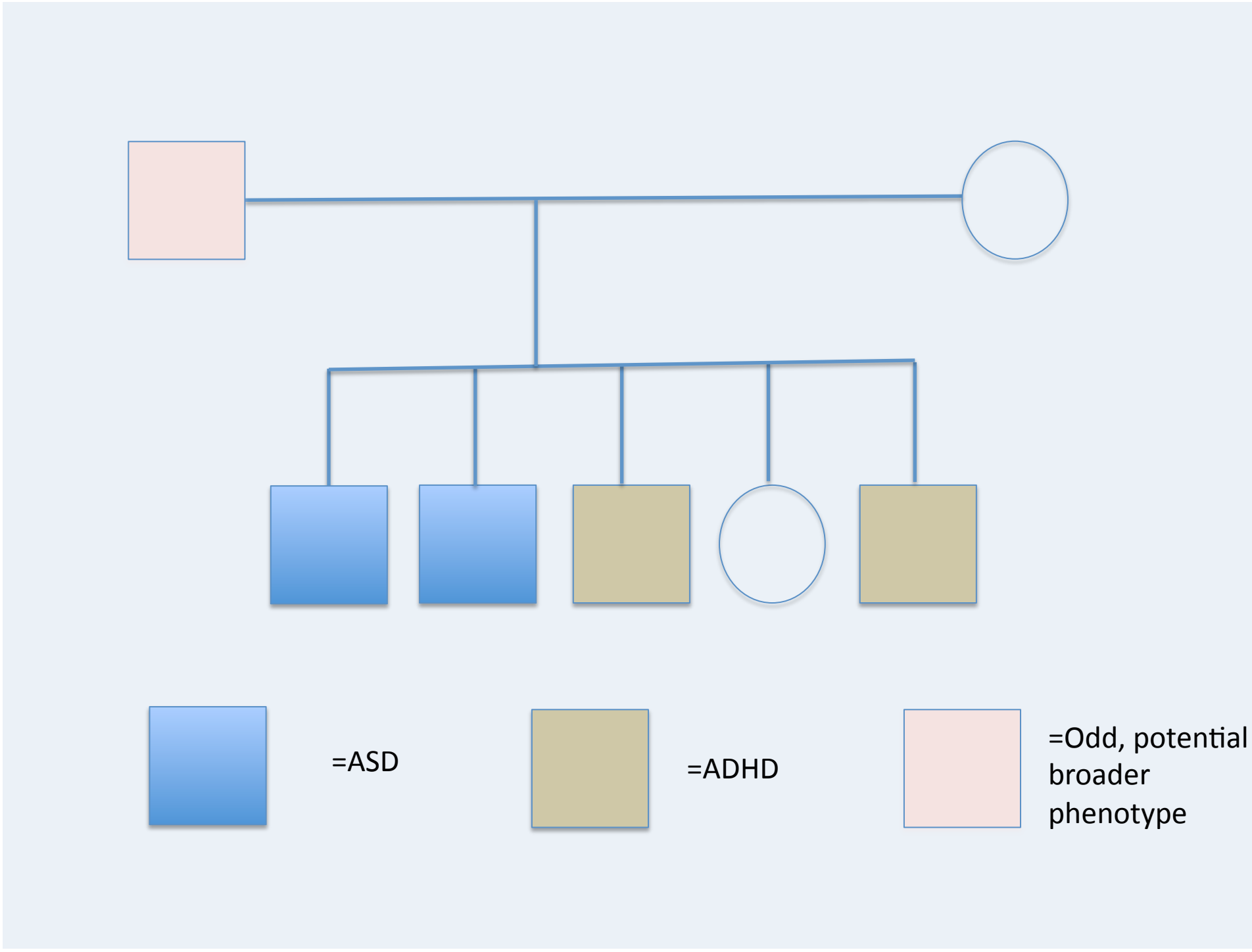
"The clinical and pathological evidence of a pervasive developmental disorder points to a retrospective diagnosis of autism."

Ir J Psych Med 2005; 22(4): 151-155

Sibling Defense Theory

- Defense or modifier Genes? – mutations that somehow protect against or modify the effects of a primary mutation.
- Or, can female gender also somehow be protective with certain mutations?
- Henry Pullen was one of 13 children, but only 3 lived to adult life. His brother, William, was also institutionalized and had exceptional artistic skills. Their parents were first cousins.


=ASD
=Odd, potential broader phenotype


=ASD
=ADHD
=Odd, potential broader phenotype

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

Genome Medicine

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]

For now, more effort should be placed on the following:

- Implementing Standards for a “clinical-grade” exome, and promoting the “networking of science” model.
- Focusing on rare, highly penetrant mutations running in families, with cascade carrier testing of even more relatives as needed.
- The genomic background is much more constant in families.
- The environmental background is sometimes more constant in families.
- This allows one to figure out penetrance of rare variants in these families, along with other issues, such as somatic mosaicism.

Acknowledgments

Alan Rope
John C. Carey
Chad D. Huff
W. Evan Johnson
Lynn B. Jorde
Barry Moore
Jeffrey J Swensen
Jinchuan Xing
Mark Yandell

Golden Helix
Gabe Rudy

Sage Bionetworks
Stephen Friend
Lara Mangravite

Reid Robison
Edwin Nyambi

Kai Wang

Zhi Wei
Lifeng Tian
Hakon Hakonarson

our study families

Thomas Arnesen
Rune Evjenth
Johan R. Lillehaug

Jason O'Rawe
Michael Schatz
Giuseppe Narzisi

Tao Jiang
Guangqing Sun
Jun Wang