The Implementation of Clinical Genomics: Ethical, Societal and Regulatory Considerations

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UTAH FOUNDATION FOR BIOMEDICAL RESEARCH

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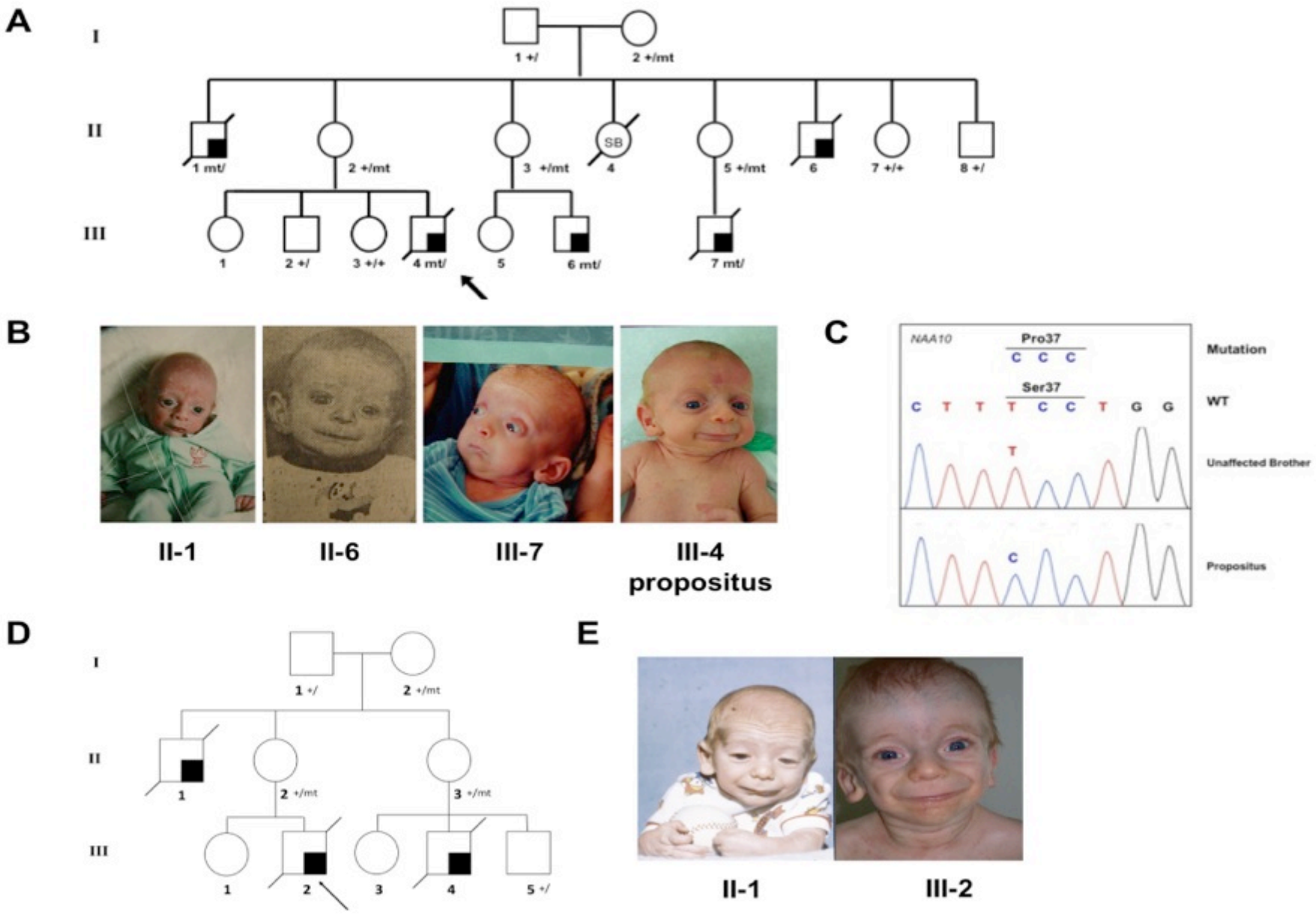
Conflicts of Interest

- I do not accept salary from anyone other than my current employer, CSHL.
- Any revenue that I earn from providing medical care is donated to UFBR for genetics research.
- I worked on the Clarity Challenge as an unpaid medical consultant to: Omicia Unlocking Individualized Medicine

- Tweeting about my talk is ok, but please do not take photographs of the various people pictured in this presentation. They have given me permission to show their photos, but I do not want to widely distribute these photos.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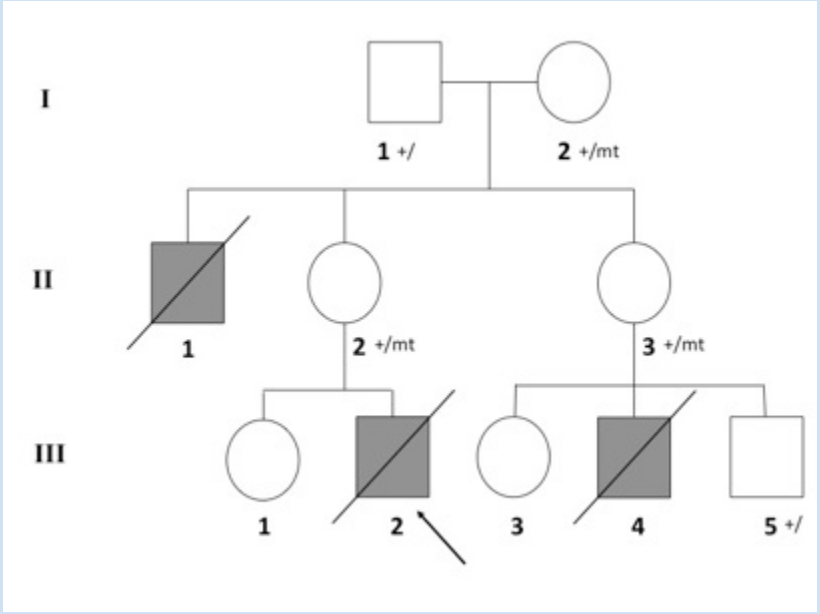
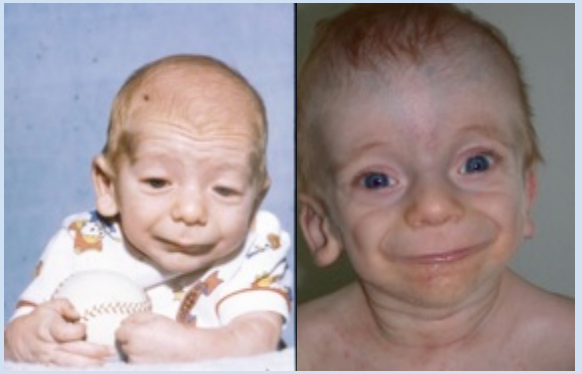
Proving Causality

- Need to find the EXACT same mutation in another unrelated family, i.e. in a different genetic background and environment.

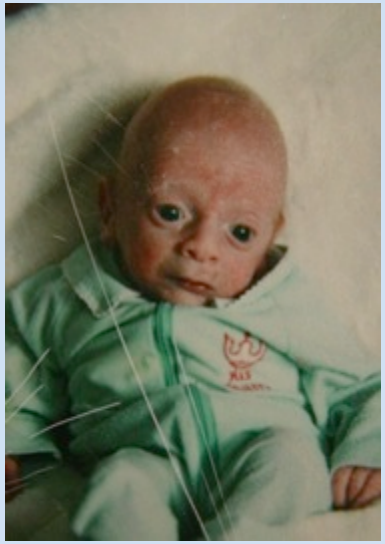
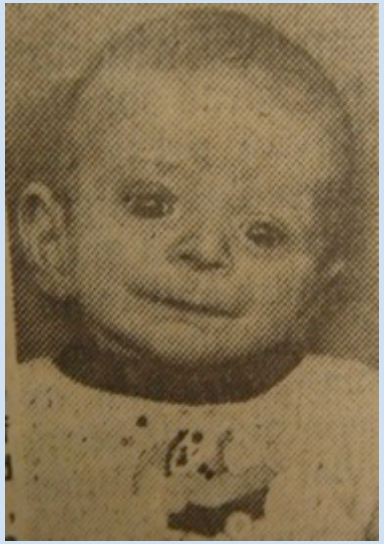
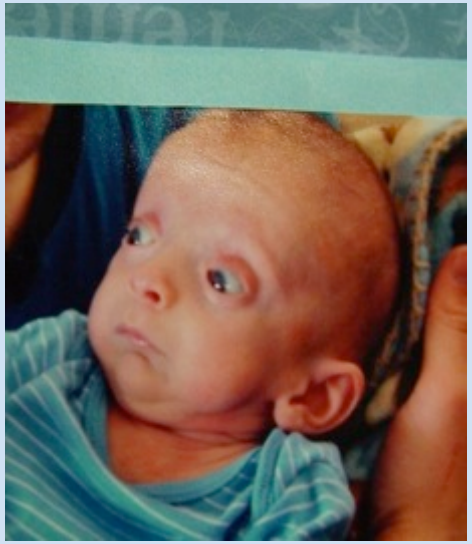
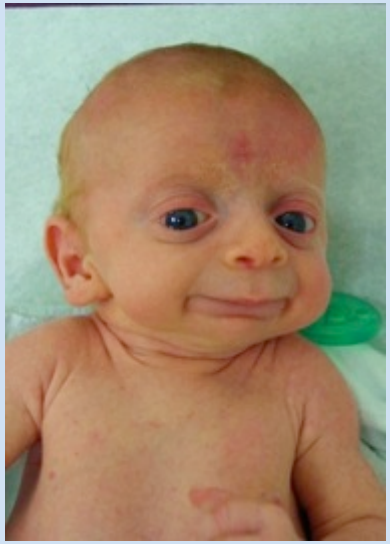
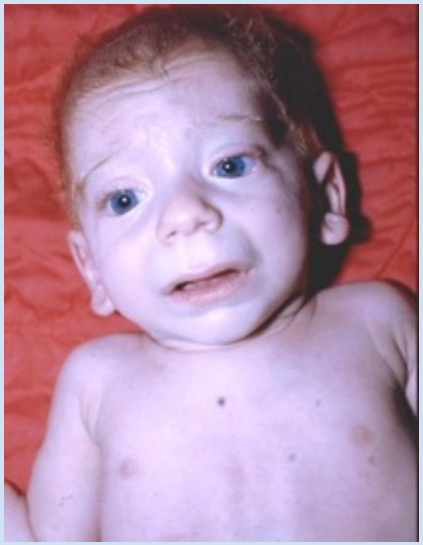
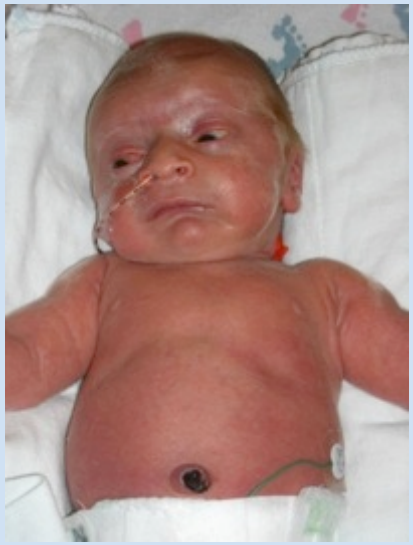
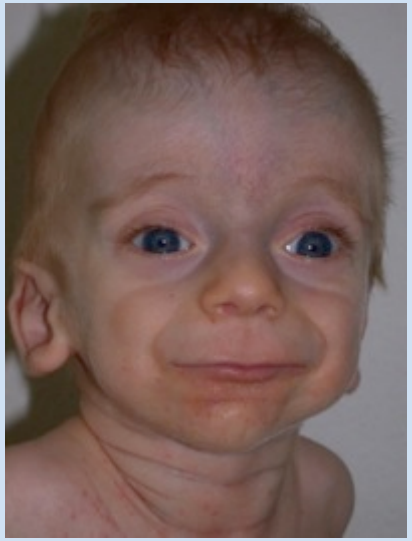
- How did we find the second family?

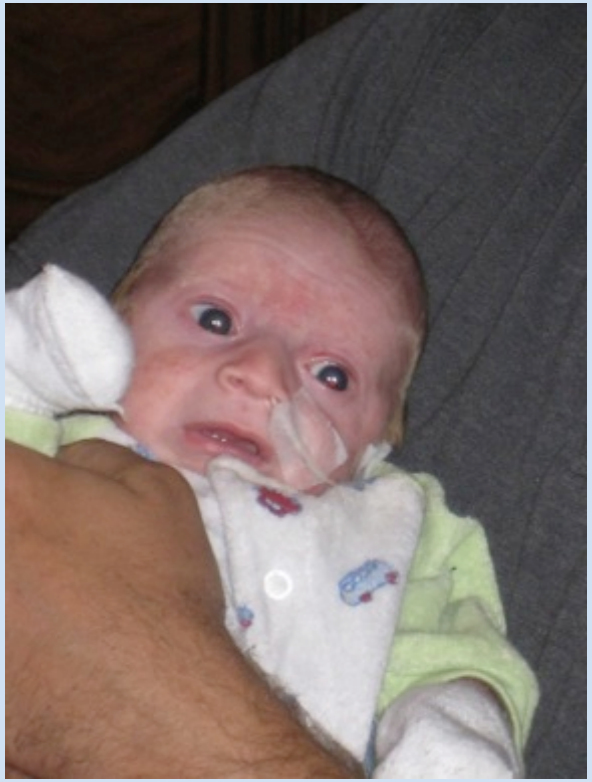




II-1 III-2

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



Many barriers in the way of CLIA-certified genetic testing for anyone to use.

Mother four months pregnant Nov 2010

Baby born March 2011.

Affected with Disease.

He died June 2011, same week as publication of our paper in AJHG.

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**.

In Choosing a Sperm Donor, a Roll of the Genetic Dice
Sarah Phipps for The New York Times

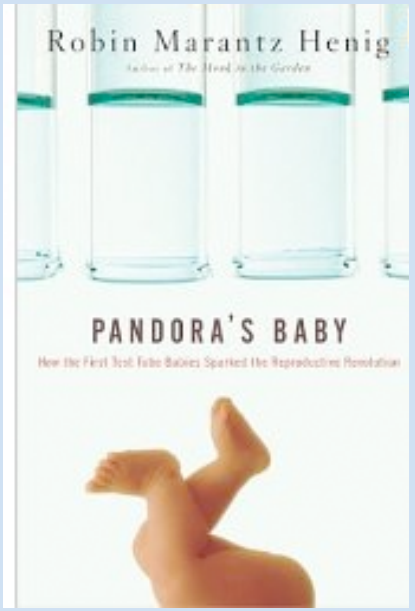
Jaxon Kretchmar, 2, who was conceived with donated sperm, has cystic fibrosis.

Whole Genome Sequencing in centralized facilities is a “Disruptive Technology”

- This is only true with economy of scale, done well and with CLIA certification.
- It can and should replace single gene diagnostic tests for patients presenting with severe genetic illnesses, which can themselves each cost $3000.
- Sometimes, physicians spend $30,000-100,000 on diagnostic odysseys with numerous single gene tests.

Pandora's Baby

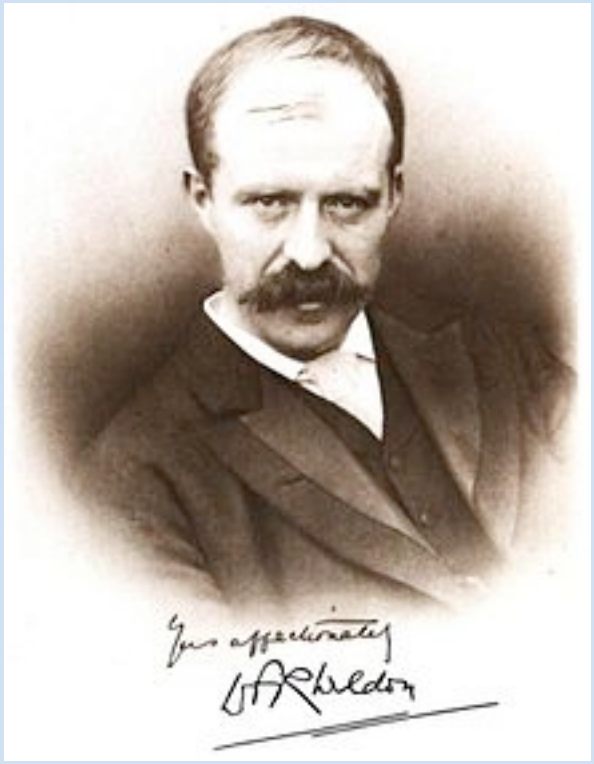
- "It seemed to boil down to a struggle between two competing impulses: the creative drive to understand nature versus the conservative drive to impose limits and maintain the status quo."



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

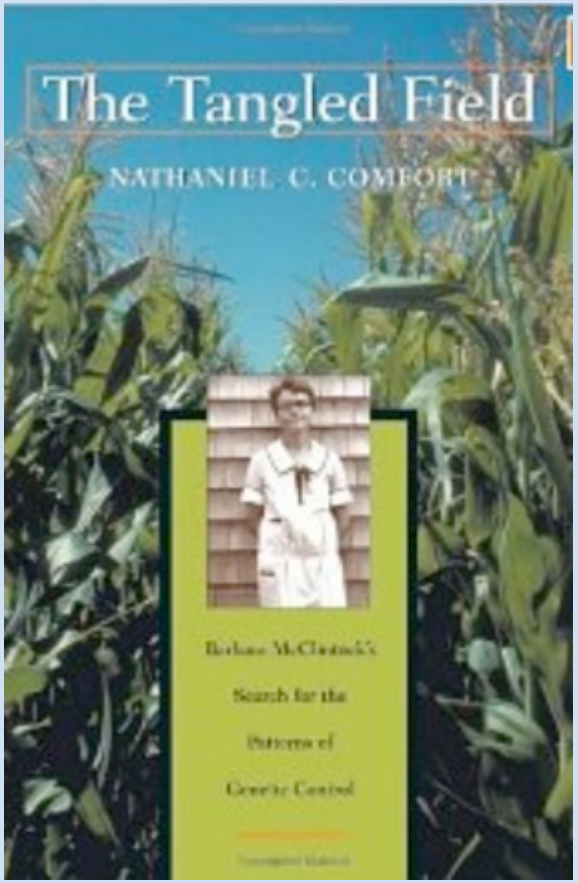
Vs.

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.

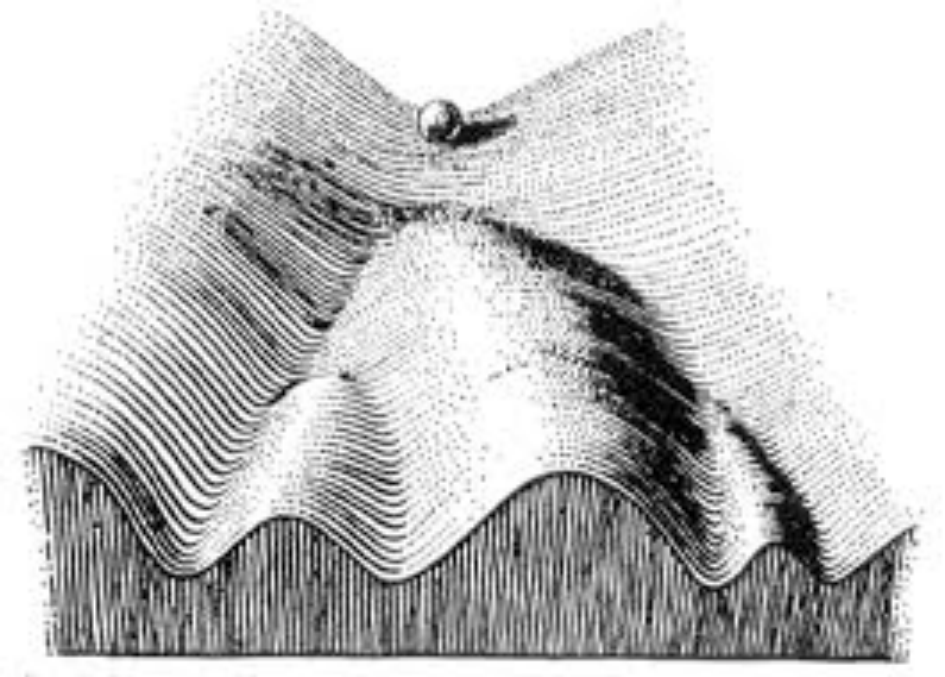
Weldon, W. F. R. 1902. Mendel's laws of alternative inheritance in peas. *Biometrika*, 1:228-254.


The Tangled Field
NATHANIEL C. COMFORT

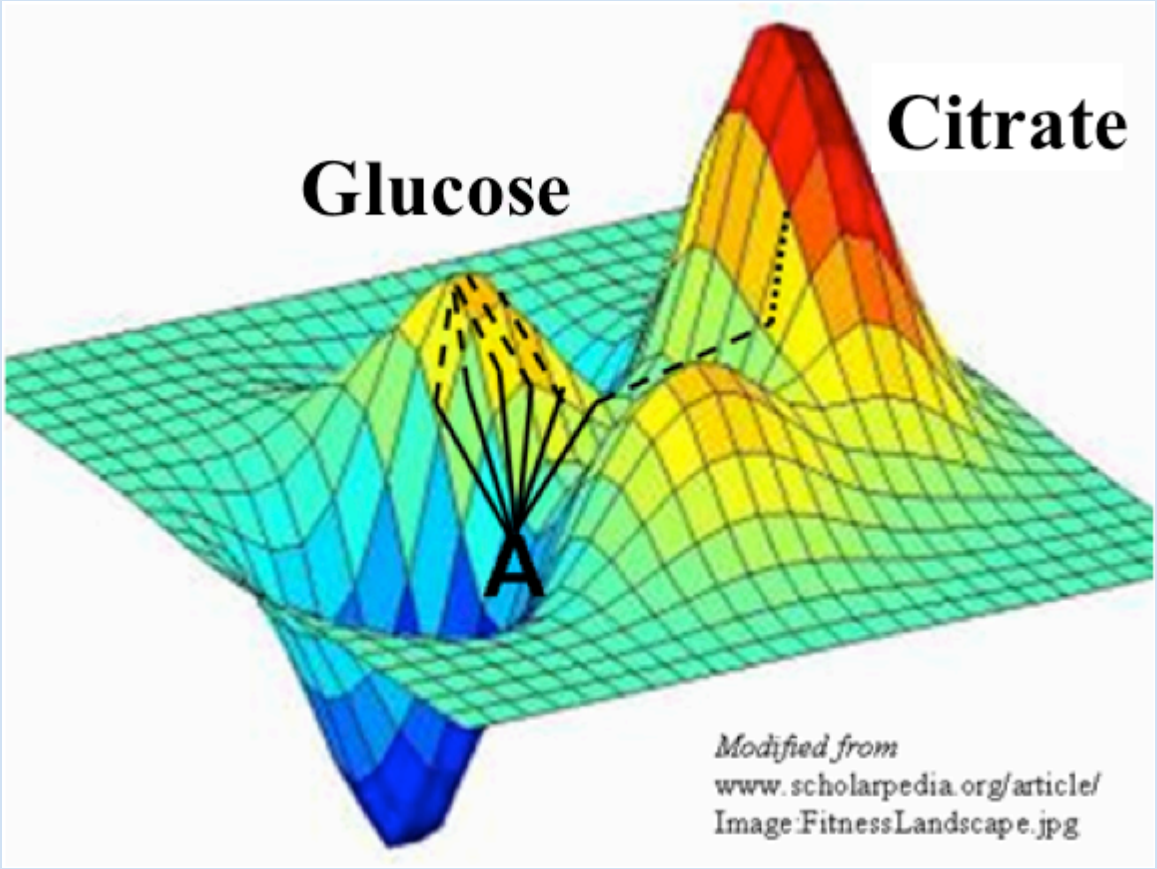
Barbara McClintock was also the first scientist to correctly speculate on the basic concept of epigenetics—and she did so before the molecular structure of DNA was even discovered. Mainly, she recognized that genes can be expressed and silenced during mitosis in genetically identical cells. Thus, in these few sentences, McClintock summarized epigenetic regulation by way of chromatin remodeling, a concept not formally described until more than 40 years later.

Barbara McClintock and the Discovery of Jumping Genes (Transposons)
Citation: Pray, L. & Zhaurova, K. (2008) Barbara McClintock and the discovery of jumping genes (transposons). Nature Education 1(1)

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.



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

Penetrance Issues

- We do not really know the penetrance 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?

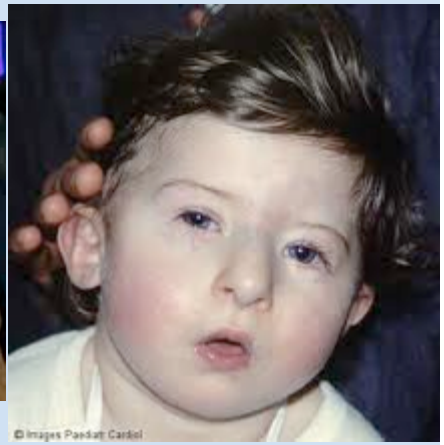
Down Syndrome

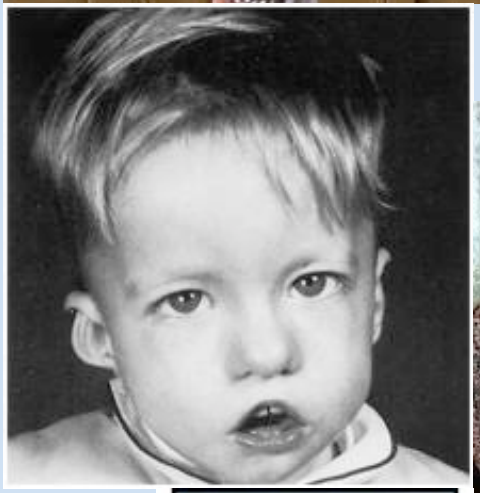
Down Syndrome

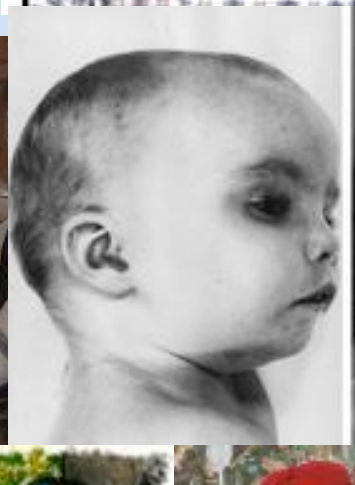
Christopher Joseph "Chris" Burke (born August 26, 1965) is an American actor and folk singer, who lives with Down syndrome, who has become best known for his character Charles "Corky" Thacher on the television series Life Goes On.

And there are people with Mosaic Down Syndrome, who are much less affected.

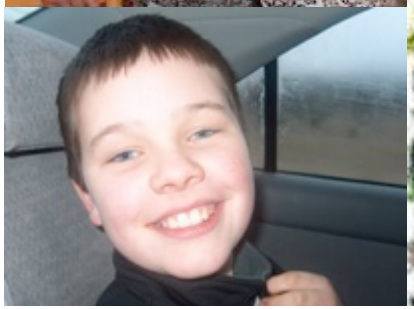
Velocardiofacial (22q11.2) Syndrome

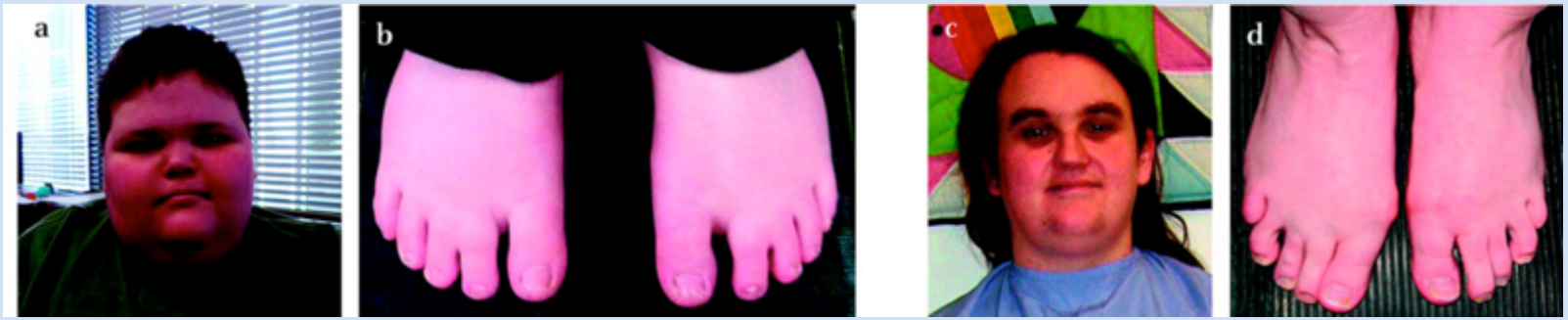









16p11.2 deletion



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

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

JMG

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”.

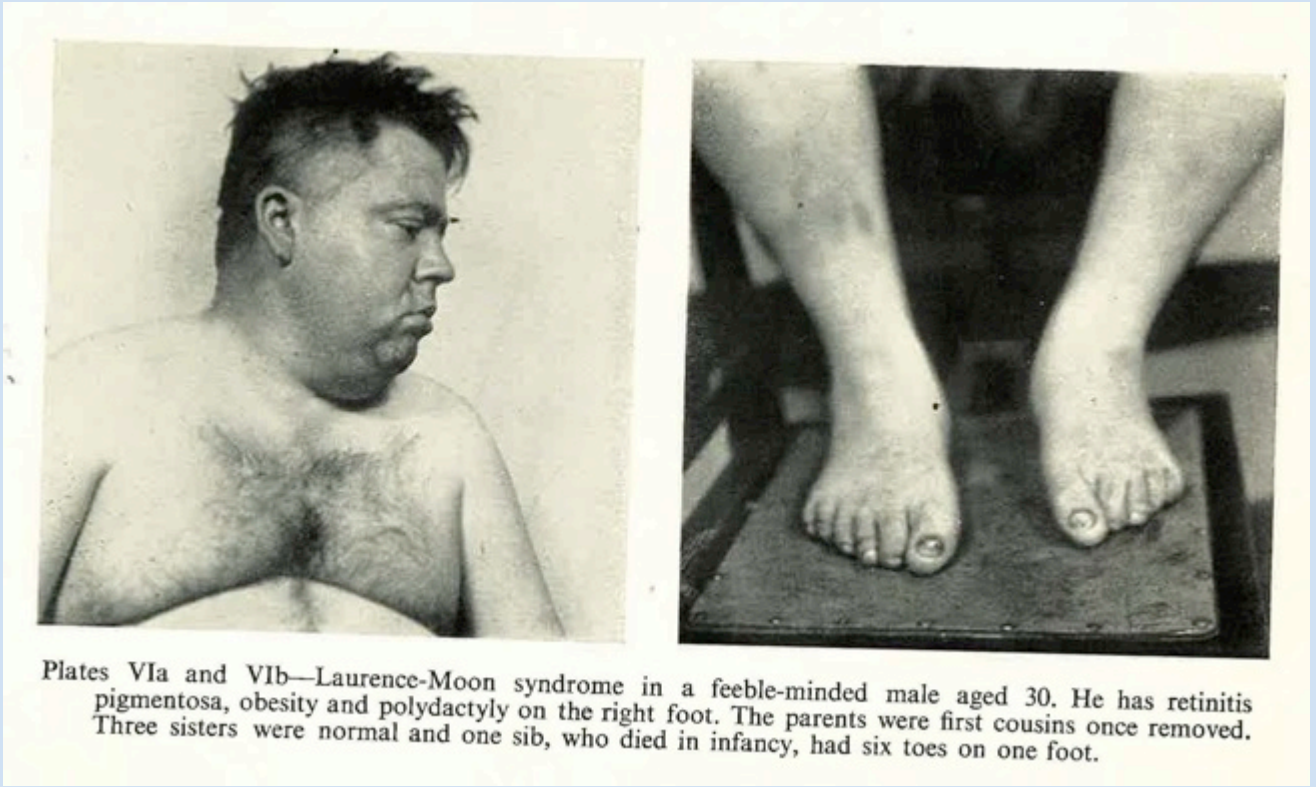
Current Diagnoses under Evaluation (DSM IV-TR)

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

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.

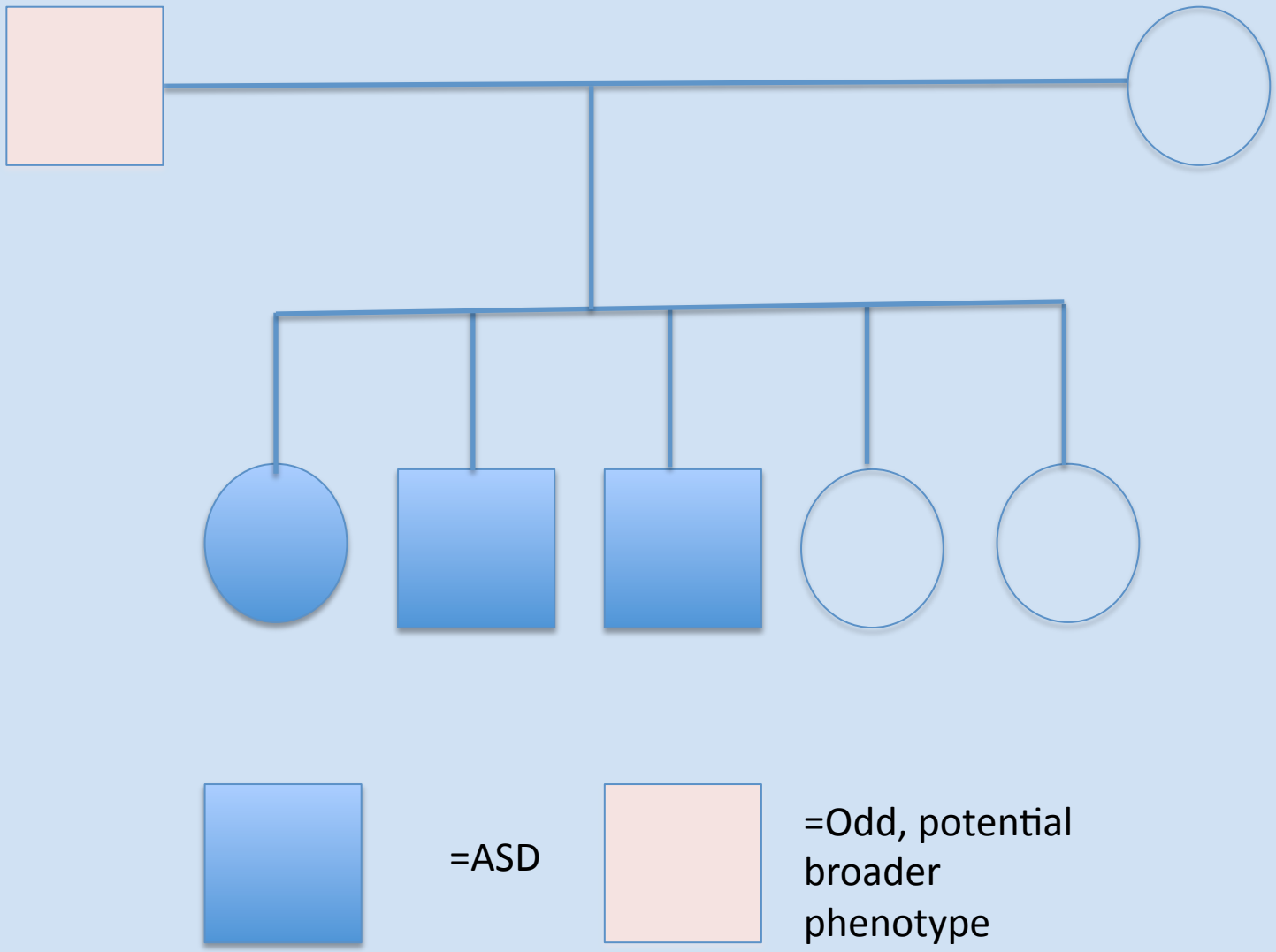
Deaf and nearly mute – Nonverbal,
Obsessed with one topic of building things.
Thought to be mentally retarded.
Usually quiet and reserved, but sometimes was intolerant of advice, suspicious of strangers, and ill-tempered and violent.

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

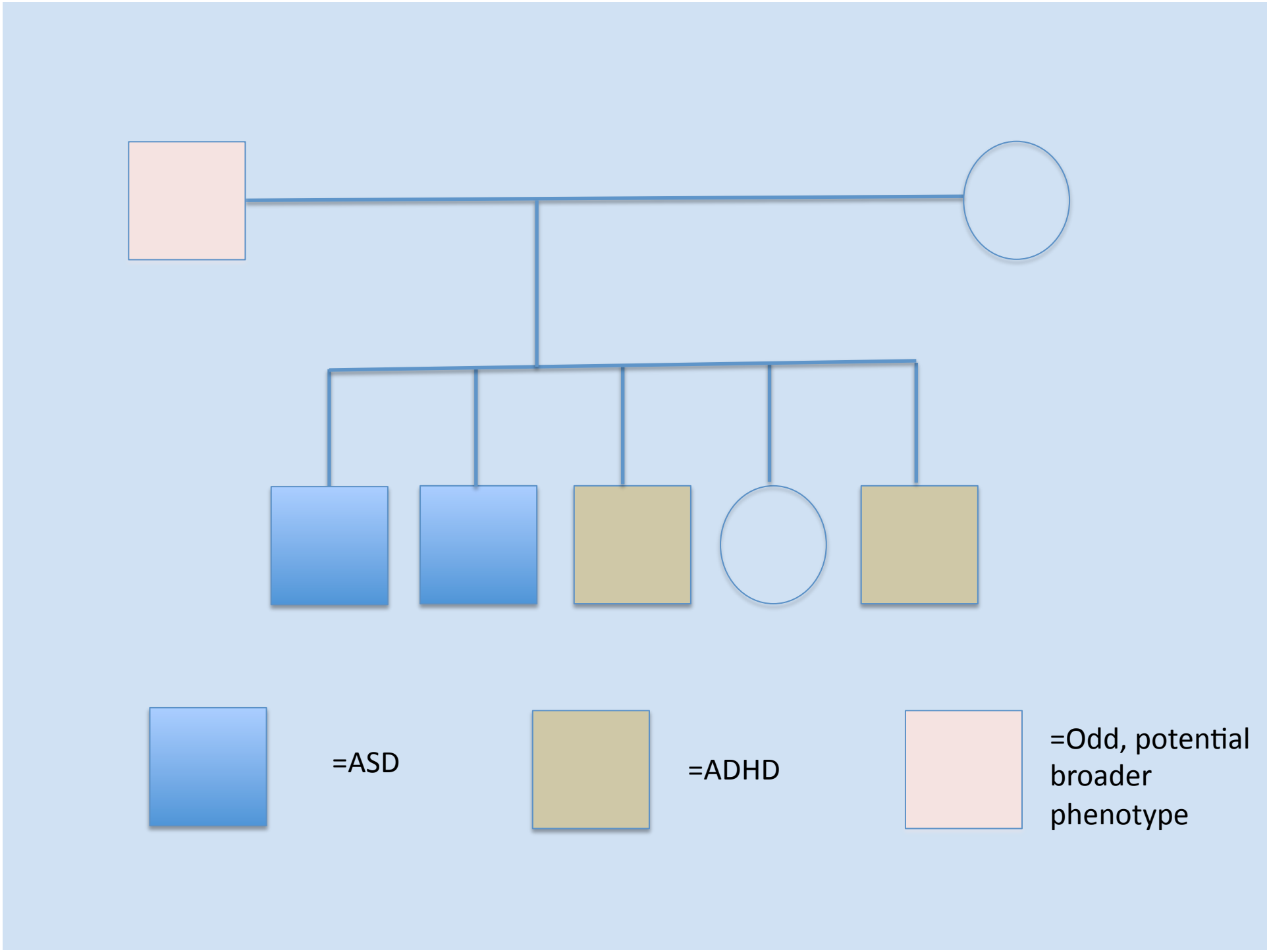
Ir J Psych Med 2005; 22(4): 151-155

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.

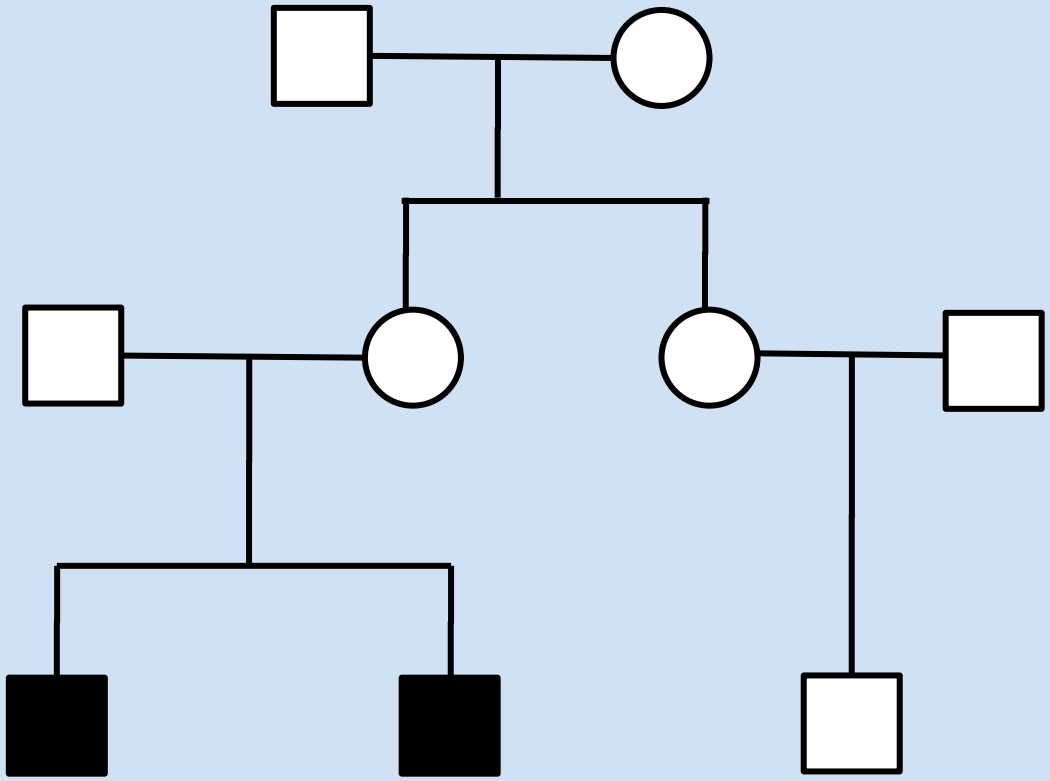

=ASD
=Odd, potential broader phenotype

*Private Photographs – Do not further distribute.


=ASD
=ADHD
=Odd, potential broader phenotype

*Private Photographs – Do not further distribute.

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

1.5 years old

3.5 years old

Dysmorphic
Mental Retardation
“autism”
“ADHD”
Hearing difficulties

3 years old

5 years old

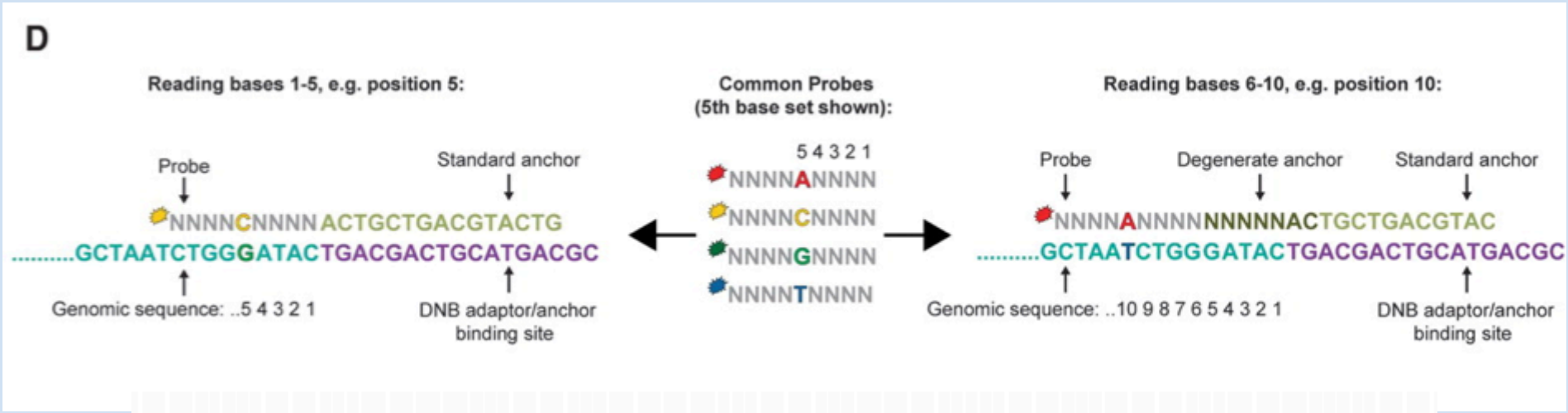
Workup Ongoing for past 10 years

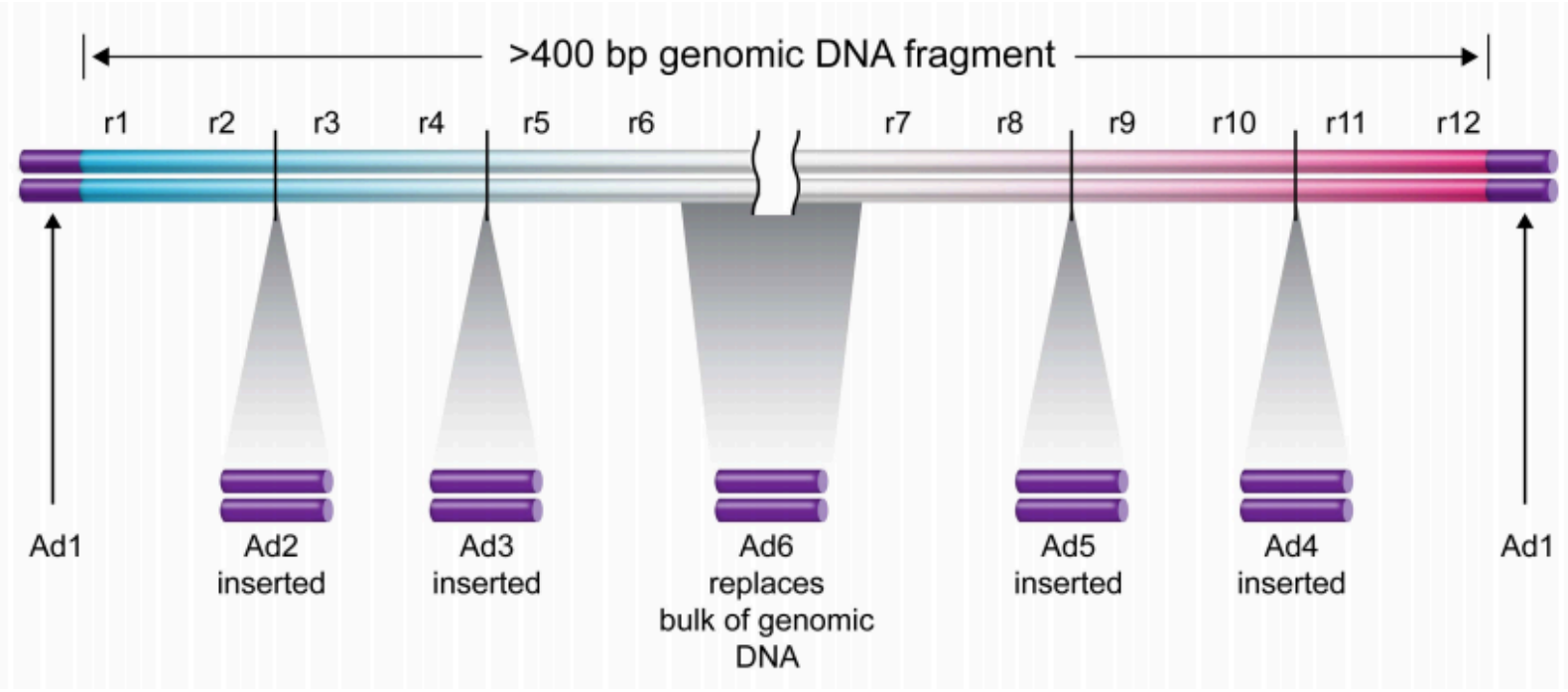
- Numerous genetic tests negative, including negative for Fragile X and MANY candidate genes.
- Found one missense mutation in a known mental retardation gene, but the mutation is a very conservative nonsynonymous Asp to Glu. Is it relevant or not? What about the whole rest of the genome?

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

- Sequenced “whole” genomes to obtain high-quality exomes and noncoding and other non-exonic regions.

Complete Genomics chemistry - combinatorial probe anchor ligation (cPAL)





Accuracy of Complete Genomics Whole Human Genome Sequencing Data

Analysis Pipeline v2.0

	FALSE POSITIVES	EST FPs	FALSE NEGATIVES	TOTAL DISCORDANCES	CONCORDANCE
Discordant SNVs per called MB	1.56 x 10-6	4,450	1.67 x 10-6	3.23 x 10-6	99.9997% of bases

Table 2. *Concordance of Technical Replicates.*

COMPLETE GENOMICS CALL	OTHER PLATFORM	PLATFORM-SPECIFIC SNVs	VALIDATION RATE	EST FPs	FPR
Het or Hom SNV	No SNV Reported	99K	17/18 = 94.4%	5,577	0.16%
No-call or Hom-Ref	SNV Reported	345K	2/15 = 13.3%	299,115	8.2%

Table 3. *False Positive Rate.*

Analyzing Variants with Omicia Opal System

Overview
Proband: **Son 1**
Affected Sibling: Son2
Unaffected Mother: Mother
Unaffected Father: Father
Report Filter: AnySupportingEvidence

Note Hide

Variant Class	Gene	Position dbSNP	Change	Proband Zygosity	Sibling Zygosity	Father Zygosity	Mother Zygosity	Effect	Global MAF	Omicia Score	Polyphen	Mutation-Taster	SIFT PhyloP	Evidence
Unknown Significance B	ABCG8	chr2 44071743 rs4148211	c.161A>G p.Tyr54Cys	het	het	het	het	non-synon	A:56% G:44%	0.755	benign	benign	0.05 3.02	HGMD
Unknown Significance B	ABCG8	chr2 44104925 rs6544718	c.1895T>C p.Val632Ala	het	het	het	hom	non-synon	T:11% C:89%	0.085	benign	benign	1 0.02	HGMD
Unknown Significance B	NPAS2	chr2 101591304 rs2305160	c.1180A>G p.Thr394Ala	het	het	het	het	non-synon	A:23% G:77%	0.082	benign	benign	0.46 -0.55	HGMD
Unknown Significance B	NPAS2	chr2 101594191 rs11541353	c.1412C>T p.Ser471Leu	het	het	het	-	non-synon	C:91% T:9%	0.506	benign	benign	0.33 2.2	HGMD
Known Pathogenic	CX3CR1	chr3 39307162 rs3732378	c.839C>T p.Thr280Met	het	-	het	-	non-synon	G:90% A:10%	0.32	damaging	benign	0.04 0.31	OMIM HGMD
Known Pathogenic	CX3CR1	chr3 39307256 rs3732379	c.745G>A p.Val249Ile	het	het	het	het	non-synon	C:83% T:17%	0.087	benign	benign	0.85 -0.33	OMIM HGMD
Unknown Significance B	PTPN13	chr4 87690998 rs2230600	c.4581A>G p.Ile1527Met	het	-	het	-	non-synon	A:82% G:18%	0.305	-	benign	0.22 0.1	HGMD

Analyzed Data with Many Methods

- 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

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]

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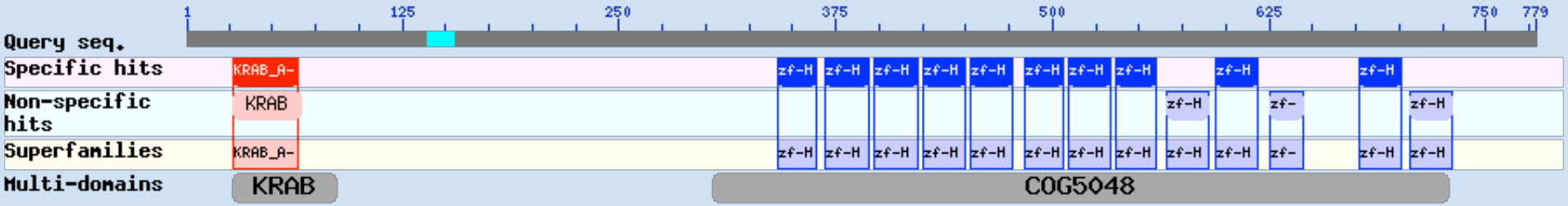
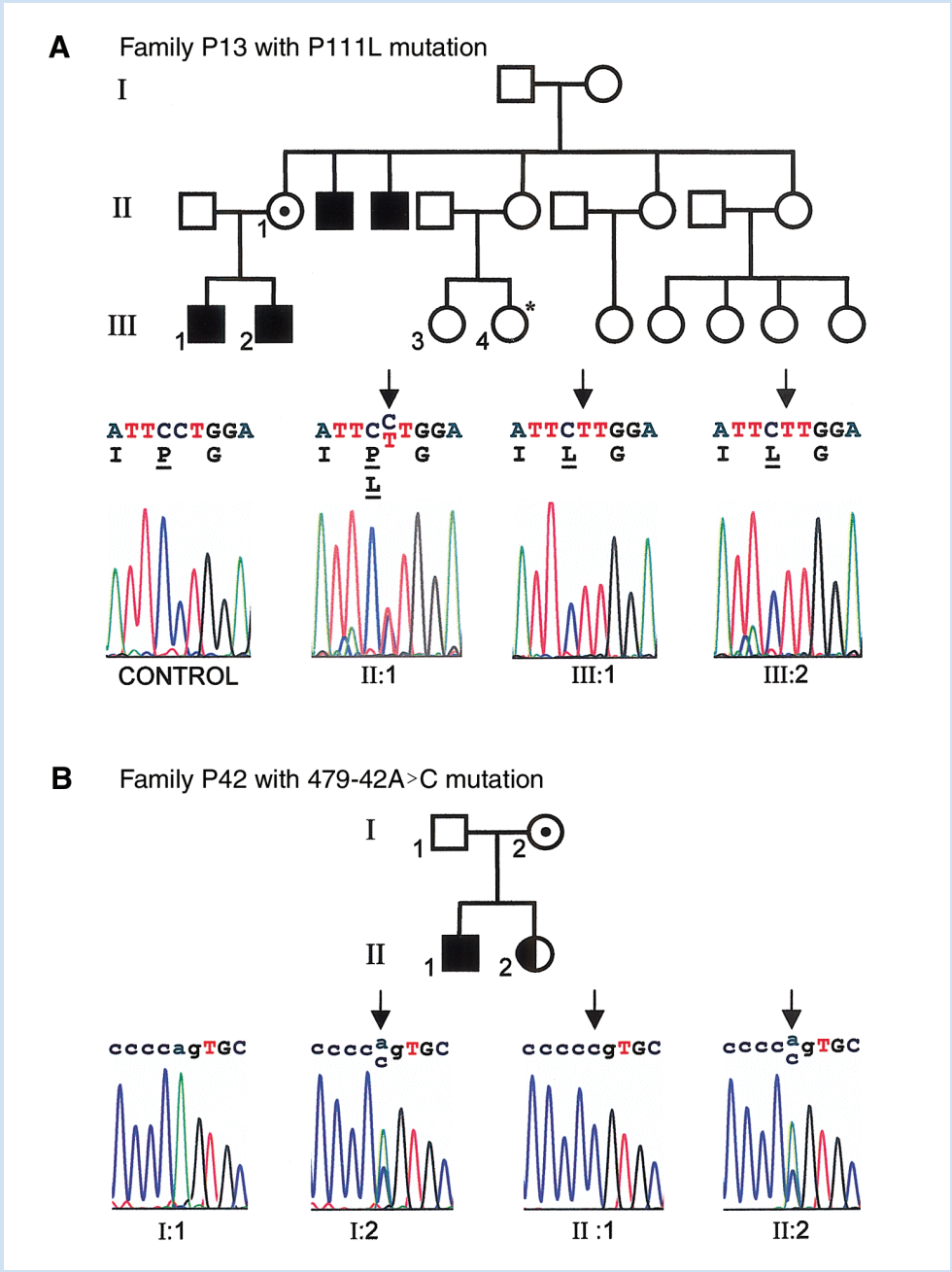
Am. J. Hum. Genet. 73:1341–1354, 2003



- 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.


A
Family P13 with P111L mutation
I
II
III
1
2
3
4
*
ATTCCTGGA
I P G
ATTCCTTGGA
I P L G
ATTCTTGGA
I L G
ATTCTTGGA
I L G
CONTROL
II:1
III:1
III:2
B
Family P42 with 479-42A>C mutation
I
II
1
2
1
2
ccccagTGC
ccccagTGC
ccccgTGC
ccccagTGC
I:1
I:2
II :1
II:2

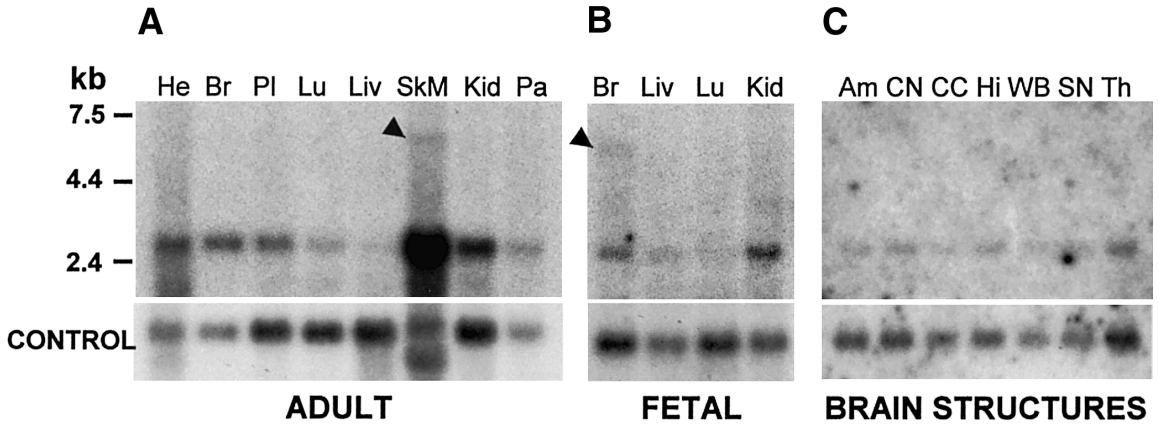


Figure 6 Northern blot hybridization of *ZNF41*, by use of a probe corresponding to nucleotides 621–1099 of *ZNF41* transcript variant 1. *A*, Adult tissues (left to right): heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas. *B*, Fetal tissues (left to right): brain, lung, liver,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

- But how I am going to find a second family with this same mutation and same 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]

Major Barriers to the implementation of Genomic Medicine in the clinic:

1) Lack of public education – consumer not sure it matters.
2) Lack of physician knowledge about genetics.
3) Apathy on the part of populace, as they have been lulled into being apathetic and not empowered about their own health.
4) Refusal of insurance companies to pay for “not useful” genetic testing.
5) Focus in our society on Treatment, NOT on early diagnosis and prevention.

Emphasis Should be on Diagnosis and Prevention, NOT just on Treatment

- 15 year old girl with Type I diabetes, hospitalized dozens of times with diabetic ketoacidosis. Millions spent to save life repeatedly, but very little on therapy or education – WHY?
- 14 year old boy hospitalized >10 times with pancreatitis over > ten years. Finally, someone gets genetics consult. Patient has cystic fibrosis, undiagnosed till then. Benefits from pancreatic enzyme supplementation, plus therapy and education. WHY so LONG to diagnose?

I would suggest that researchers working on DNA samples from living humans perform CLIA-certified sequencing UP FRONT, either with exomes or whole genomes, so that we can network the genomes and exomes & return results to consumers, “patients”, research participants and families.

Clinical grade exome and whole genome DNA testing should mean:

1) Blood or saliva collected with rigorous, automated sample tracking.

2) DNA isolated in a CLIA-certified facility.

3) Sequencing performed in a CLIA-certified facility.

4) Analysis performed with a CLIA-certified bioinformatics pipeline.

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

- Rare, highly penetrant mutations running in families, with cascade carrier testing.
- The genomic background is much more constant in families.
- The environmental background is sometimes more constant in families.
- This allows one to know much more about issues with penetrance of rare variants in these families.

An alternate universe

- Genomes sequenced by companies and academics with the minimal standards in place (i.e. CLIA in America).
- All data, including variant lists, added to “the cloud” that consumers can access.
- Consumers can go back and repeatedly look at their own genome.
- Consumers own and manage these data, and they can pay anyone they like to help them interpret the data with them.
- These are CONSUMERS, not patients, and we need to **move away from paternalistic medicine**.
- I am concerned that regulation requiring delivery of genetic data by “physicians” will choke off and kill the genomic revolution and individualized medicine.

One Solution

- I pray and hope that we will move toward collating and distributing mutations in an international human variation database, allowing for calculation of penetrance and extensive burden testing.
- CLIA-certified (clinical grade) sequencing up front allows return of all data, including rare, highly penetrant mutations, to families, facilitating carrier screening and counseling.
- Require return of genomic data to participants, allowing the participants to distribute and "crowd-source" their own data.
- Government should divert funds toward a 10 to 100 fold increase for genetic counselors, so that we can have compassionate engagement with families.

Ancestry.com *meets* 23andMe *meets* PatientsLikeMe *meets* WGS?

Clinical Validity with Worldwide Human Genetic Variation "database"?

PatientsLikeMe

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our study families

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Michael Schatz
Giuseppe Narzisi

Tao Jiang
Jun Wang

- END OF THE TALK
- EXTRA SLIDES NEXT

"The fundamental mistake which vitiates all work based upon Mendel's method is the neglect of ancestry, and the attempt to regard the whole effect upon offspring, produced by a particular parent, as due to the existence in the parent of particular structural characters; while the contradictory results obtained by those who have observed the offspring of parents apparently identical in certain characters show clearly enough that not only the parents themselves, but their race, that is their ancestry, must be taken into account before the result of pairing them can be predicted."

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

“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