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

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Conflicts of Interest

- I do not receive salary compensation from any for-profit companies.
- I do some consulting work for free, i.e. I do not accept $$$ as 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.

Stan Nelson, Clinical Applications

- Exome seq – competing w/ current paradigms
- Clinical exome seq – 4-12 week turnaround time, UCLA has a Genomics Data Board
- Claims 50% diagnosis rate. But finding many de novo mutations, with N=1 & many novel gene discoveries.
- TCF4 – Pitt Hopkins Syndrome
- Duchenne Muscular Dystrophy – developmental delay but also diagnostic delay.
- Walker Warburg Syndrome
- Possible Juvenile ALS case
- Technology issues, with coverage and mis-alignment stuff.

How does a technology spread and get implemented clinically?

- WGS with Sanger sequencing **~2001** – Venter and Collins/Lander teams

- Exon capture and sequencing with short-read sequencing techology **~2007.**

Hodges E, Xuan Z, Balija V, Kramer M, Molla MN, Smith SW, Middle CM, Rodesch MJ, Albert TJ, Hannon GJ, McCombie WR. Genome-wide in situ exon capture for selective resequencing. *Nature genetics* 2007, 39:1522-1527.

Albert TJ, Molla MN, Muzny DM, Nazareth L, Wheeler D, Song X, Richmond TA, Middle CM, Rodesch MJ, Packard CJ, Weinstock GM, Gibbs RA. Direct selection of human genomic loci by microarray hybridization. *Nature methods* 2007, 4:903-905.

Evolution and Application of Exome and "Whole Genome" Sequencing

2008

Ng PC, Levy S, Huang J, Stockwell TB, Walenz BP, Li K, Axelrod N, Busam DA, Strausberg RL, Venter JC: Genetic variation in an individual human exome. *PLoS genetics* 2008, 4:e1000160.

2009

Ng SB, Turner EH, Robertson PD, Flygare SD, Bigham AW, Lee C, Shaffer T, Wong M, Bhattacharjee A, Eichler EE, et al: Targeted capture and massively parallel sequencing of 12 human exomes. *Nature* 2009, 461:272-276.

Choi M, Scholl UI, Ji W, Liu T, Tikhonova IR, Zumbo P, Nayir A, Bakkaloglu A, Ozen S, Sanjad S, et al: Genetic diagnosis by whole exome capture and massively parallel DNA sequencing. *Proc Natl Acad Sci U S A* 2009, 106:19096-19101.

Evolution and Application of Exome and "Whole Genome" Sequencing, cont…

2010

Ng SB, Buckingham KJ, Lee C, Bigham AW, Tabor HK, Dent KM, Huff CD, Shannon PT, Jabs EW, Nickerson DA, et al: Exome sequencing identifies the cause of a mendelian disorder. *Nat Genet* 2010, 42:30-35.

Ng SB, Bigham AW, Buckingham KJ, Hannibal MC, McMillin MJ, Gildersleeve HI, Beck AE, Tabor HK, Cooper GM, Mefford HC, et al: Exome sequencing identifies MLL2 mutations as a cause of Kabuki syndrome. *Nature genetics* 2010, 42:790-793.

Roach JC, Glusman G, Smit AF, Huff CD, Hubley R, Shannon PT, Rowen L, Pant KP, Goodman N, Bamshad M, et al: Analysis of genetic inheritance in a family quartet by whole-genome sequencing. *Science* 2010, 328:636-639.

By July 2012 – Explosion of Research

- As of July 2012, 540 articles in Pubmed with word “exome” and “human” in them.
- And 358 article with “whole human genome sequencing & disease” in them.
- Hundreds of new mutations in known disease genes, and dozens of new disease genes discovered.

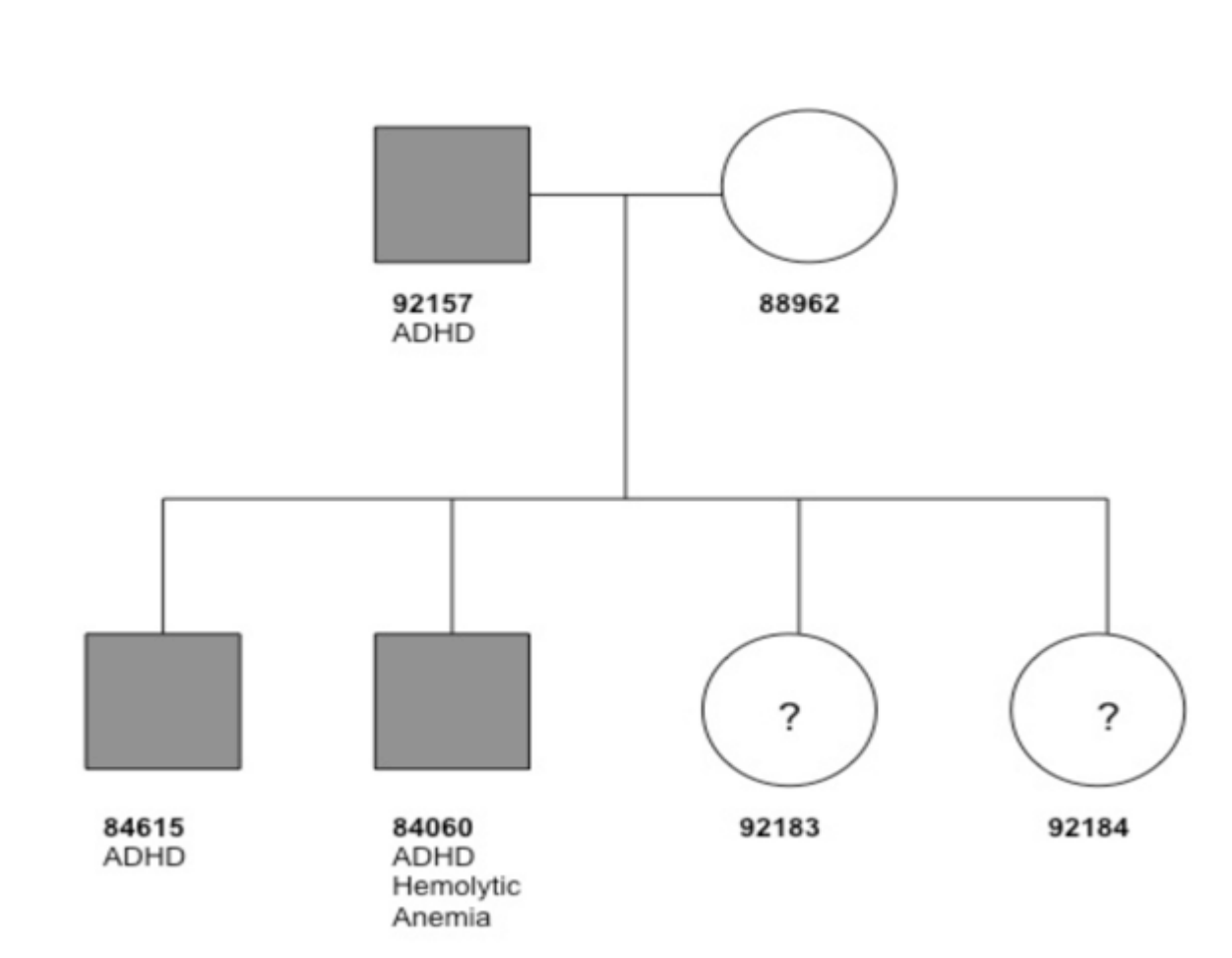
Story # 1

Exome Sequencing and Unrelated Findings in the Context of Complex Disease Research: Ethical and Clinical Implications

Gholson J. Lyon, Tao Jiang, Richard Van Wijk, Wei Wang, Paul Mark Bodily, Jinchuan Xing, Lifeng Tian, Reid J. Robison, Mark Clement, Lin Yang, Peng Zhang, Ying Liu, Barry Moore, Joseph T. Glessner, Josephine Elia, Fred Reimherr, Wouter W. van Solinge, Mark Yandell, Hakon Hakonarson, Jun Wang, William Evan Johnson, Zhi Wei, and Kai Wang

Discov Med. 2011 Jul;12(62):41-55.

Exome sequencing of one pedigree in a research setting.



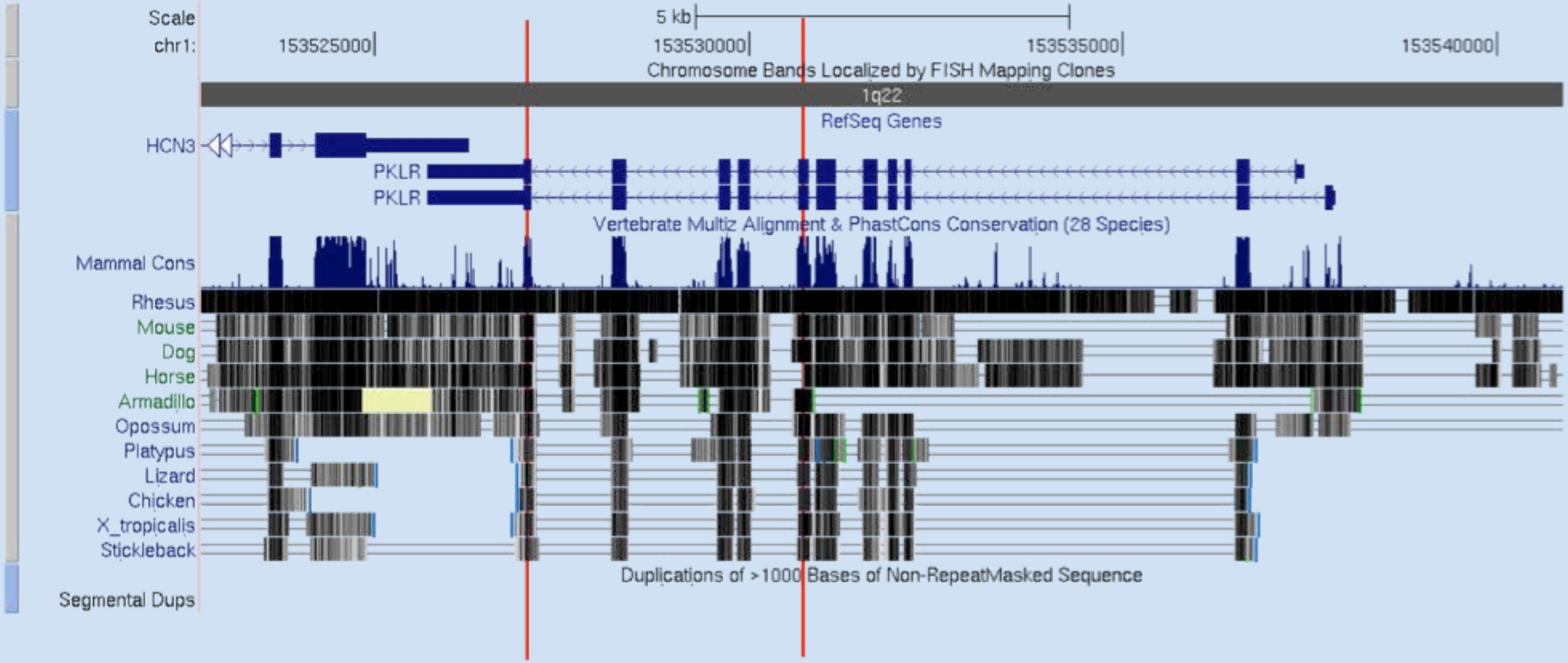
Exome Sequencing performed early 2010

While analyzing the exome data, research participant (age ~24) informs me that he recently had his spleen removed!

He has idiopathic hemolytic anemia, since childhood.

Although I am not his physician, I still feel an ethical and moral obligation to try to figure out what is going on.

Compound Heterozygote in *PKLR*, with each mutation inherited from one parent.



Some Additional Data to support the causation of these variants for idiopathic hemolytic anemia.

Table 2: Biochemical assays of enzyme activities in the patient affected with idiopathic hemolytic anemia confirmed *PKLR* deficiency. PK, pyruvate kinase; HK, hexokinase; G6PD, glucose-6-phosphate dehydrogenase.

	Patient 84060	Control	Reference values
PK (U/gHb)	3.3 L	8.6	6.1 – 12.3
HK (U/gHb)	3.2 H	1.1	0.8 – 1.5
G6PD (U/gHb)	15.8 H	9.2	6.4 – 10.5

Table 3: Bioinformatics prediction on the functional impact of two PKLR mutations. A mutation is regarded as deleterious if the SIFT<0.05, or PolyPhen>0.85, or PhyloP>0.95, or MutationTaster/LRT prediction as "D" (deleterious).

Mutation	SIFT	PolyPhen 2	PhyloP	LRT	MutationTaster
R569Q	0.03	0.84	0.97	D	D
G341A	0	0.889	1	D	D

Structural Modeling is also consistent with deleterious effects of these mutations.

Secondary Variants in Individuals Undergoing Exome Sequencing: Screening of 572 Individuals Identifies High-Penetrance Mutations in Cancer-Susceptibility Genes

Jennifer J. Johnston,[1,7] Wendy S. Rubinstein,[1,2,3,7,8] Flavia M. Facio,[1] David Ng,[1] Larry N. Singh,[1] Jamie K. Teer,[1,4] James C. Mullikin,[1,4,5,6] and Leslie G. Biesecker[1,4,*]

The American Journal of Human Genetics *91*, 97–108, July 13, 2012

Table 3. Participant Information for Pathogenic Variants Identified in *BRCA1* and *BRCA2*

Gene	Mutations and RefSeq Accession Numbers	Sex	Age	Ethnicity	Mutation Results Prior to Study	BRCAPRO Score	Met NCCN Guidelines	Met USPSTF Referral Criteria (Women Only)
BRCA1	c.547+2T>A[a] (NM_007294.3)	female	48 years	northern European	yes	N/A	yes	yes
BRCA1	c.68_69del (p.Glu23Valfs*17) (NM_007294.3)	male	61 years	Ashkenazi Jewish	no	0.3%	no	N/A
BRCA2	c.5482_5486 del (p.Lys1828Valfs*4) (NM_000059.3)	female	56 years	Japanese	no	0.0%	no	no
BRCA2	c.5946del (p.Ser1982Argfs*22) (NM_000059.3)	male	57 years	Ashkenazi Jewish	no	0.9%	yes	N/A
BRCA2	c.5946del (p.Ser1982Argfs*22) (NM_000059.3)	male	60 years	Ashkenazi Jewish	no	42.3%	yes	N/A
BRCA2	c.5946del (p.Ser1982Argfs*22) (NM_000059.3)	male	55 years	Ashkenazi Jewish	yes	N/A	yes	N/A
BRCA2	c.8297del (p.Thr2766Asnfs*11) (NM_000059.3)	male	59 years	Irish	no	0.6%	no	N/A

The following abbreviations are used: NCCN, National Comprehensive Cancer Network; and USPSTF, U.S. Preventive Services Task Force.
[a]The predicted protein alteration is not provided for this splice-site mutation.

I suggest we get rid of this term “incidental finding”, and replace it with “secondary”, “unrelated”, or “unanticipated” findings

- Why does it matter?
- Your child has a “terminal deletion” ???
- The “incidentalome”– just putting “-ome” on the end does not make this interesting or useful!
- There is NOTHING “Incidental” about Unrelated, Unanticipated or Secondary Findings.
- Sequencing a bunch of exomes and finding random rare variants MIGHT be “incidental”, but actually proving that these variants CAUSE the disease is NOT simple or “incidental” or “accidental” or “coincidental”.
- I would suggest calling these “unrelated, unanticipated or secondary findings”, rather than “incidental**”.**
- Continuing to call these “incidental findings” trivializes the amount of work that ought to go into proving causality, plus it confuses the public at large. Lyon, *Personalized Medicine*, 2012.

Yet, it is July 2012 and my research participant still has not come back to give blood for CLIA-certified results. Why?

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 "learned" to be apathetic and to not be 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?

One Conclusion that I started to come to from this story alone:

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 return results to consumers, “patients”, research participants and families.

Story #2

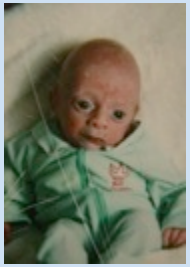
ARTICLE

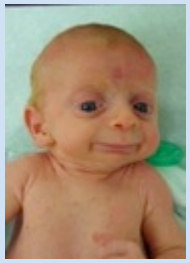
Using VAAST to Identify an X-Linked Disorder Resulting in Lethality in Male Infants Due to N-Terminal Acetyltransferase Deficiency

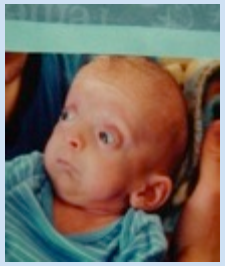
Alan F. Rope,[1] Kai Wang,[2,19] Rune Evjenth,[3] Jinchuan Xing,[4] Jennifer J. Johnston,[5] Jeffrey J. Swensen,[6,7] W. Evan Johnson,[8] Barry Moore,[4] Chad D. Huff,[4] Lynne M. Bird,[9] John C. Carey,[1] John M. Opitz,[1,4,6,10,11] Cathy A. Stevens,[12] Tao Jiang,[13,14] Christa Schank,[8] Heidi Deborah 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,*]

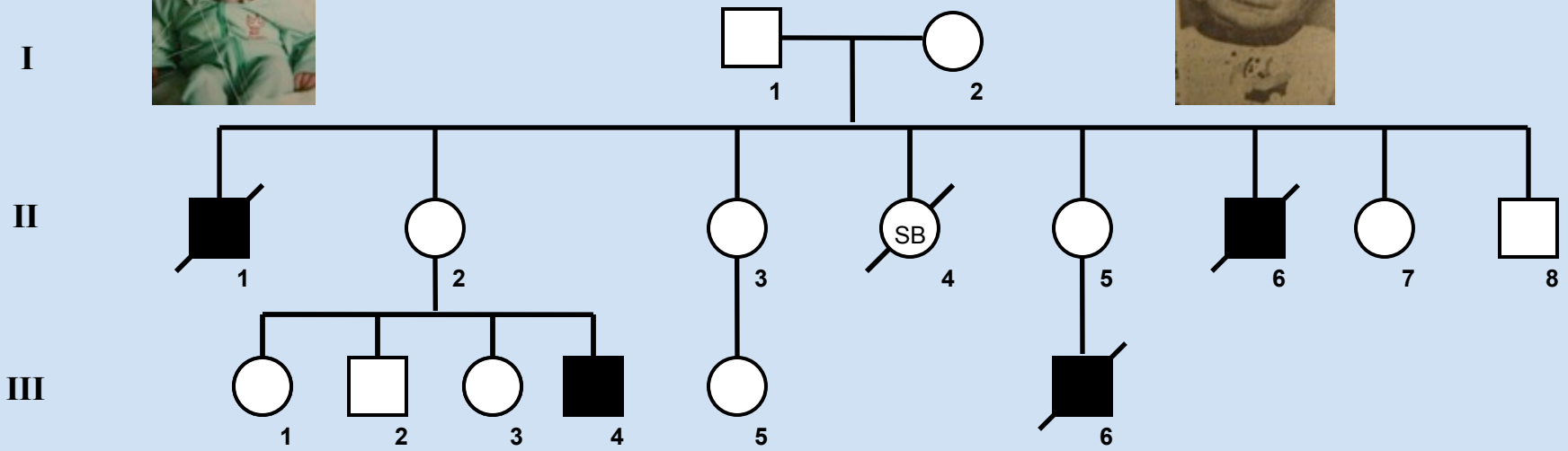
The American Journal of Human Genetics *89*, 1–16, July 15, 2011

Family Pedigree

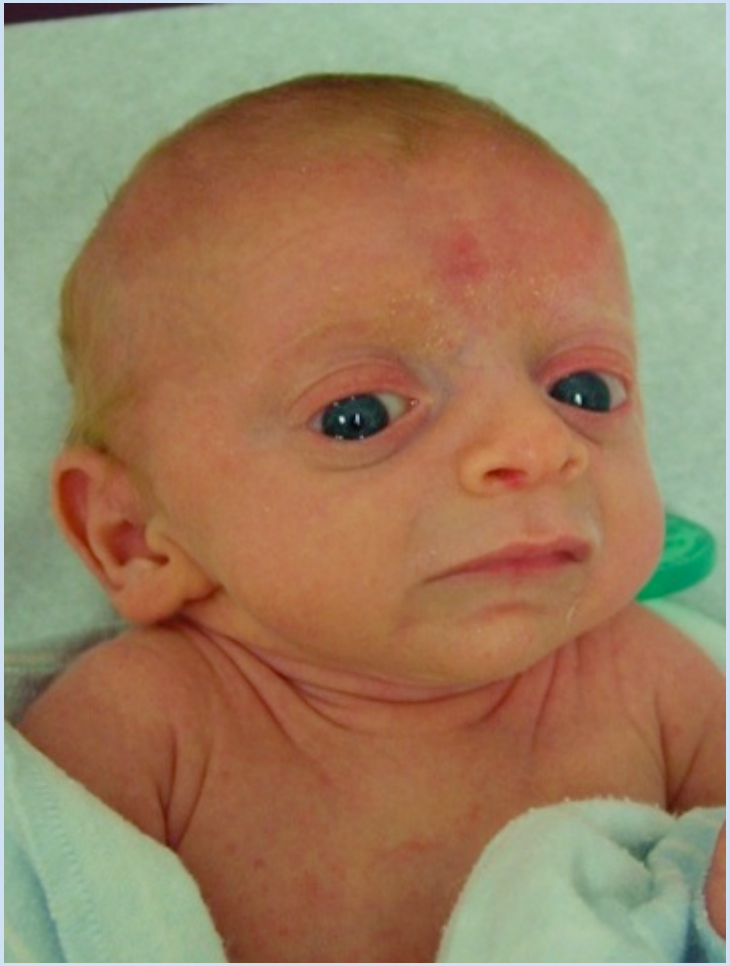


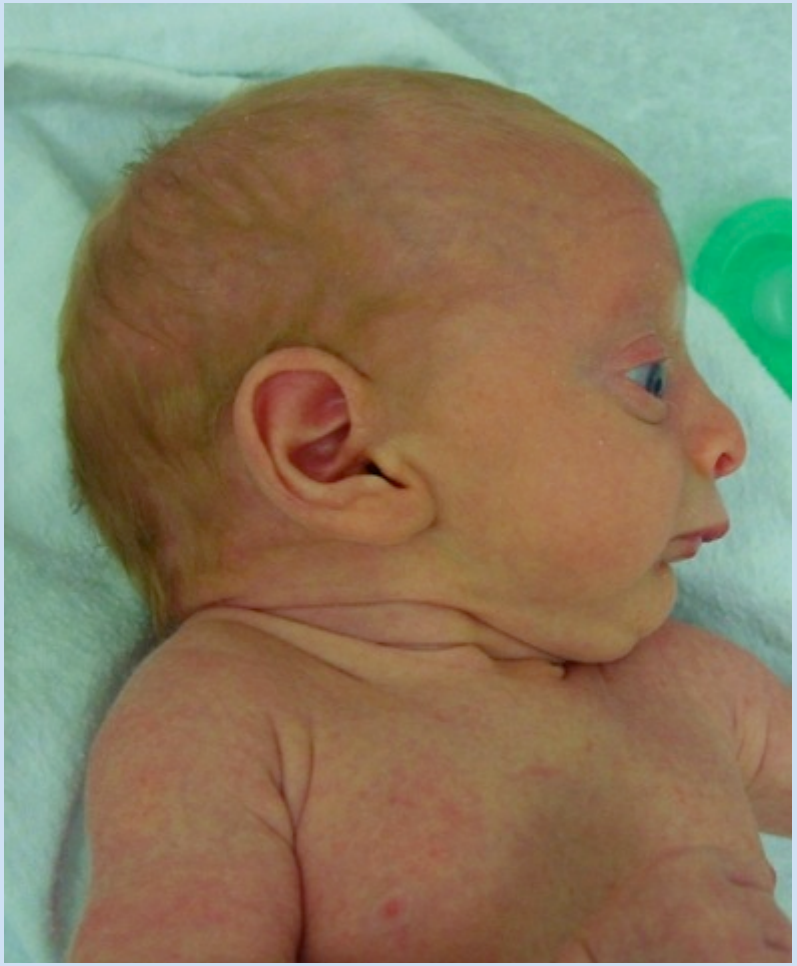






Using Next Gen Seq to figure out genetic basis of a New Disease





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

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.

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

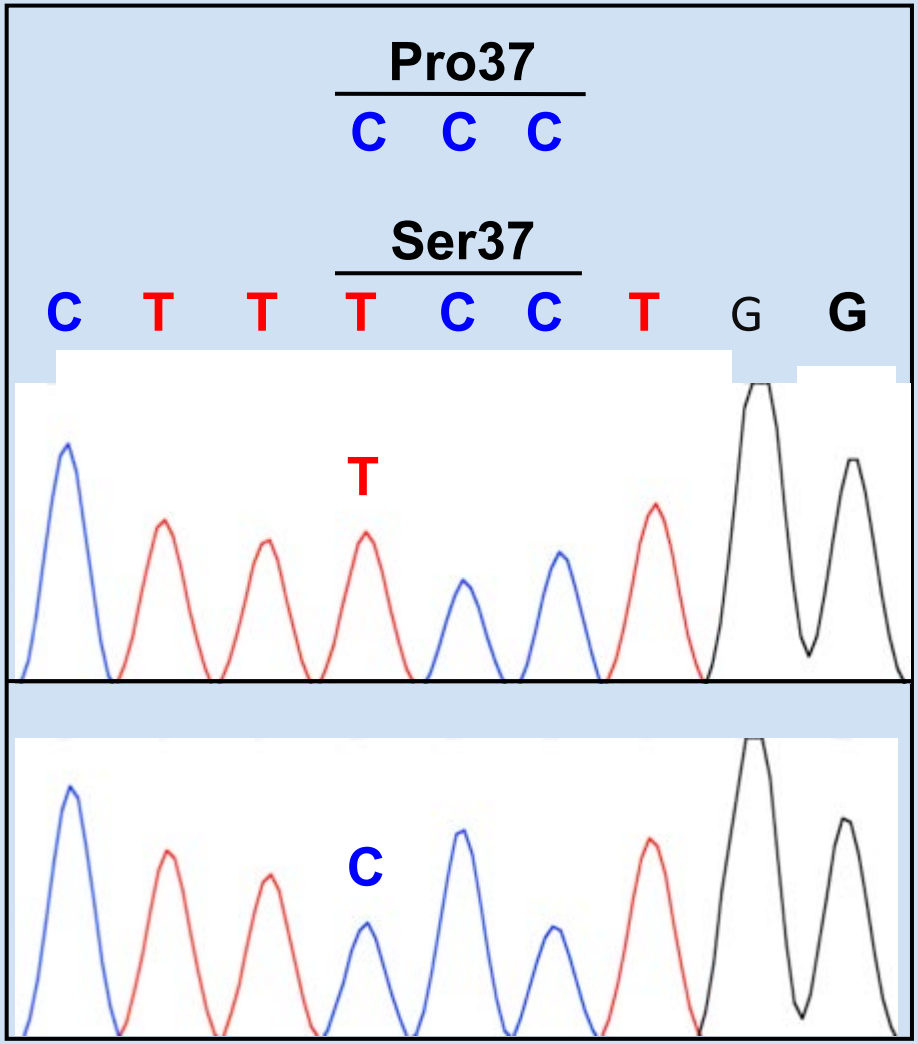
- **Analysis with ANNOVAR and VAAST (Variant Annotation, Analysis and Search Tool).**

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

Rope, A.F., Wang, K., Evjenth, R., Xing, J., Johnston, J.J., Swensen, J.J., Johnson, W.E., Moore, B., Huff, C.D., Bird, L.M., et al. (2011). Using VAAST to Identify an X-Linked Disorder Resulting in Lethality in Male Infants Due to N-Terminal Acetyltransferase Deficiency. American Journal of Human Genetics.

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

This is the causative mutation. There are ~6 billion nucleotide in a diploid human genome, but this one REALLY matters!



Mutation

WT

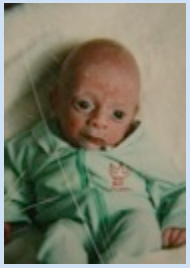
Unaffected Brother

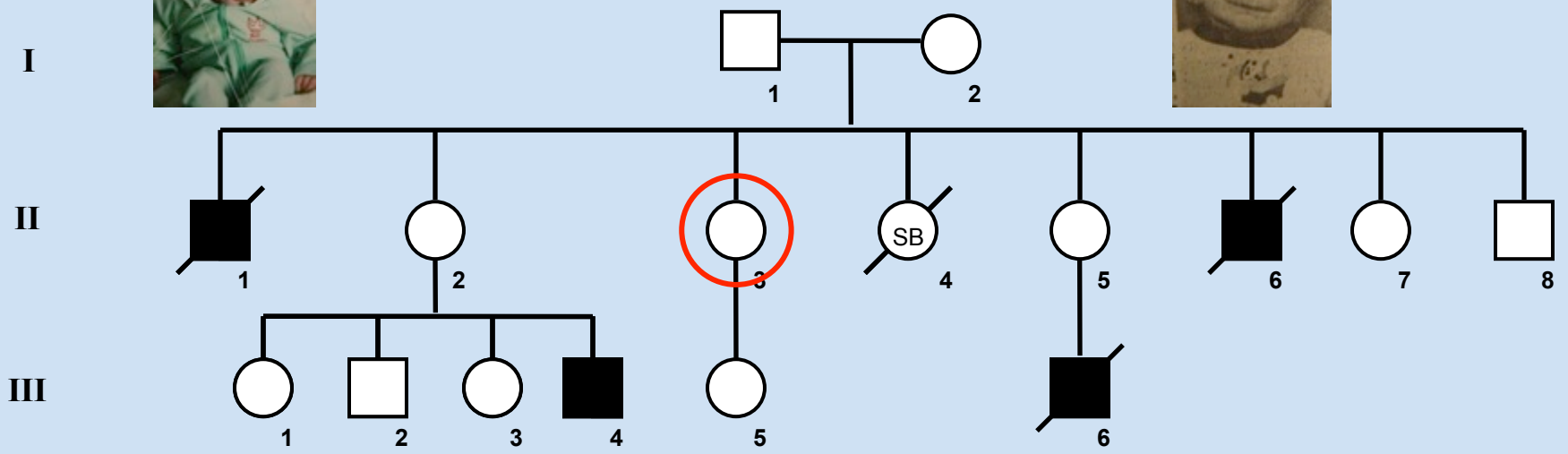
Proband

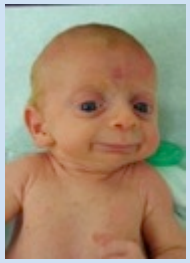
By November 2010, we had good functional data *in vitro* (bacterially expressed proteins) and *in vivo* (yeast, unpublished), leading me to believe we had identified the causative mutation.

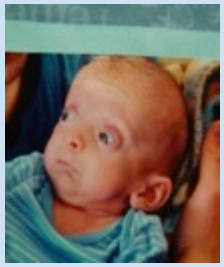
A new mother in the family informs me she is 4 months pregnant, with a boy!

The now pregnant mother-to-be is circled in red. Our Sanger Sequencing had shown her to be a carrier of the mutation.









BUT, as a researcher, I was naive and ignorant concerning the following question:

How do we give such research results back to research participants?

MAJOR ISSUES that I learned about

- I am a physician but not HER physician, therefore I had NOT entered into a “physician-patient contract” with her.

- This was not a “diagnostic test”. This was research, and not “CLIA-certified”. All Clinical Diagnostic Tests are regulated in America with the Clinical Laboratory Improvement Amendments act.

- How does one return research results to participants without breaking the law or doing something that might inadvertently harm the person?

- Please remember that CLIA was implemented to prevent people from being given wrong test results (due to poor quality).

Societal Issues

- Test tube babies are a success because the first baby born, Louise Brown, was fine and free of genetic defects.

- Gene Therapy was set back by >10 years due to the death of Jesse Geisinger and a disregard of rules and regulation by certain researchers.

- We have Nicholas Volcker as a shining example of success with WGS.

- But, we don't want to screw this up with some research lab giving back **incorrect** results to someone, leading to some calamitous outcome, such as someone thinking they have Huntington's mutation when they don't, and committing suicide.

Bring clinical standards to human–genetics research

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

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

"I suggest that we change the way we collect and process samples for human-genetics research. We should create a formalized protocol akin to the rigorous process that doctors and other health-care workers go through during any clinical lab test, which practically eliminates the chances of mistakes and mix-ups."

Technically, clinical grade DNA testing currently means the following:

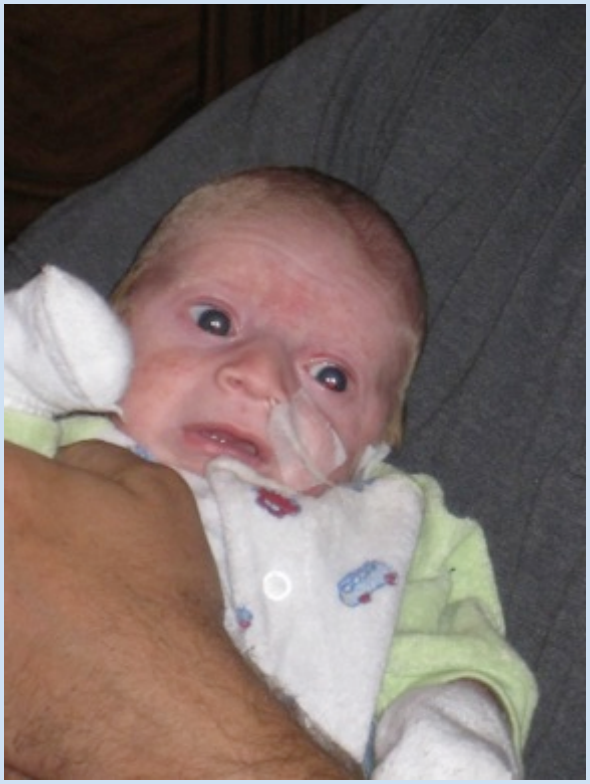
1) Blood or saliva collected with rigorous, automated sample tracking.

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.

This is what should happen with any sample with possible return of results!!!



Many barriers in the way of developing a test at ARUP (genetic laboratory I was using in Utah) – in retrospect, should have tried GeneDx.

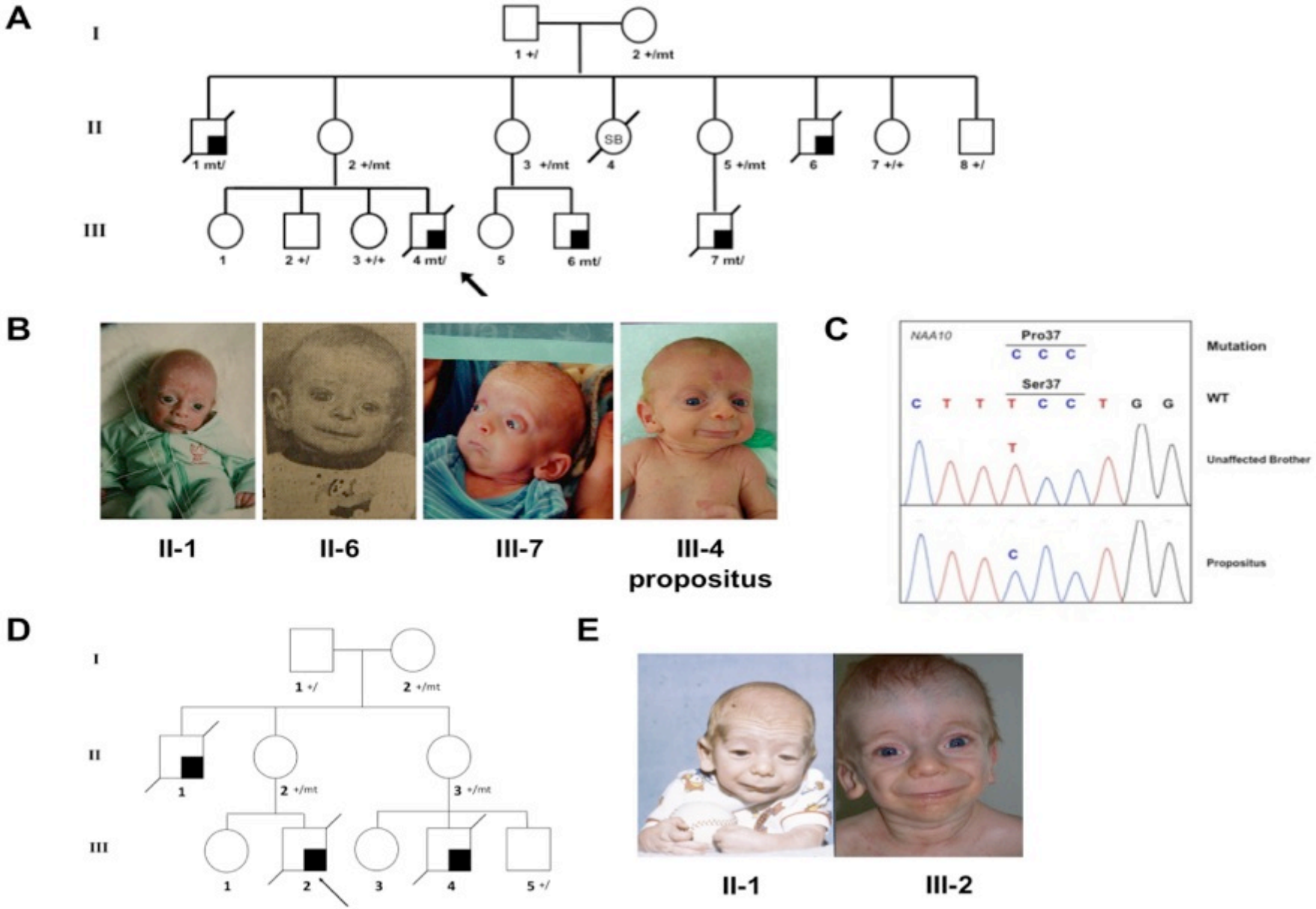
Mother four months pregnant Nov 2010

Baby born March 2011.

Affected with Disease.

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

For several reasons, it is not clear anything would have changed even if she had received the result during pregnancy.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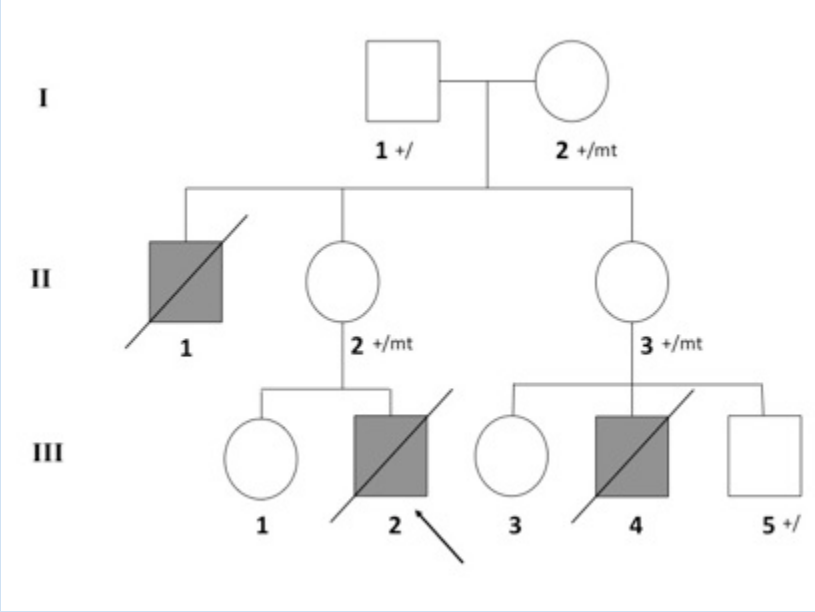
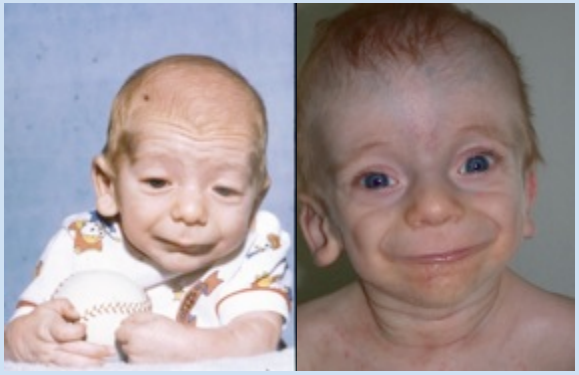
Proving Causality – MAJOR Problem with this "gold rush" of singleton mutations

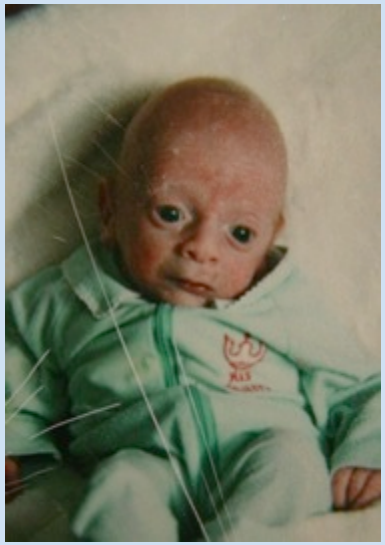
- Need to find the EXACT same mutation in another unrelated family, i.e. in a different genetic background and environment.
- This is because we do not have breeding or introgression in human genetics!
- How did we find the second family?

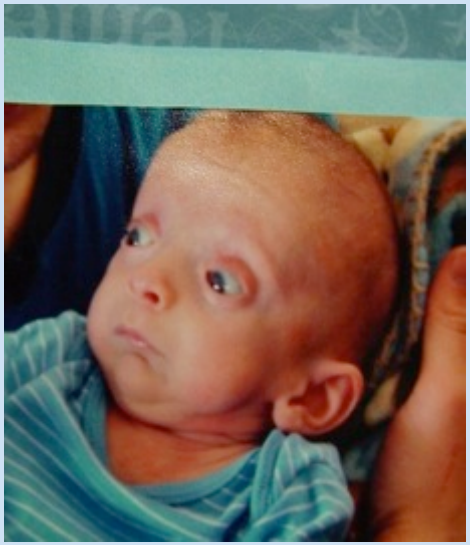
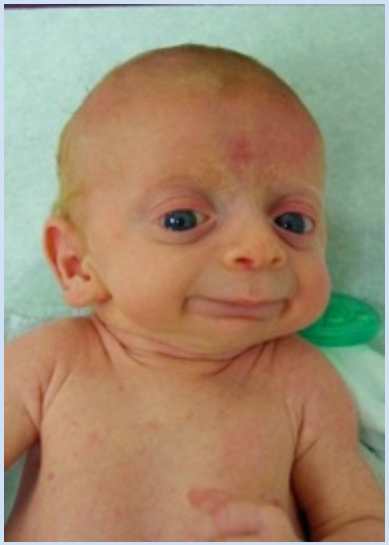
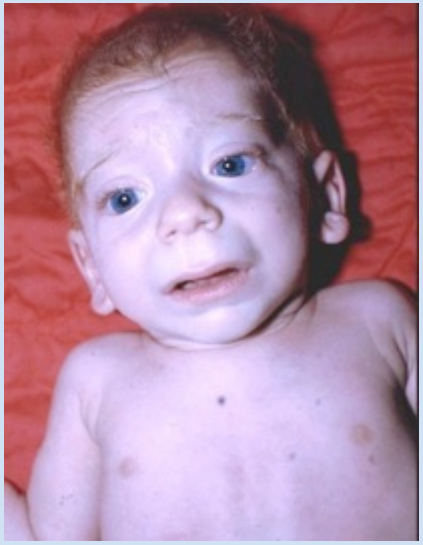
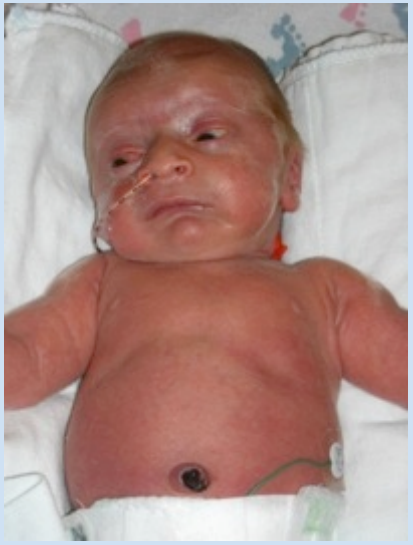
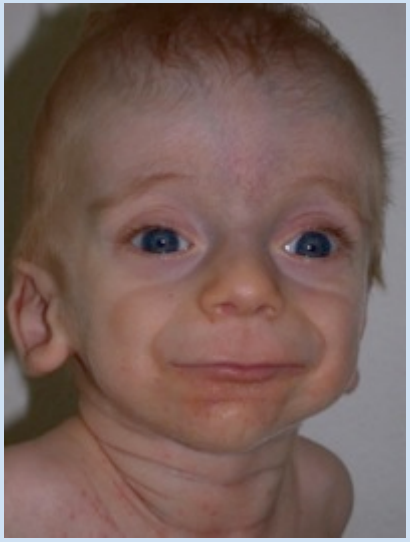




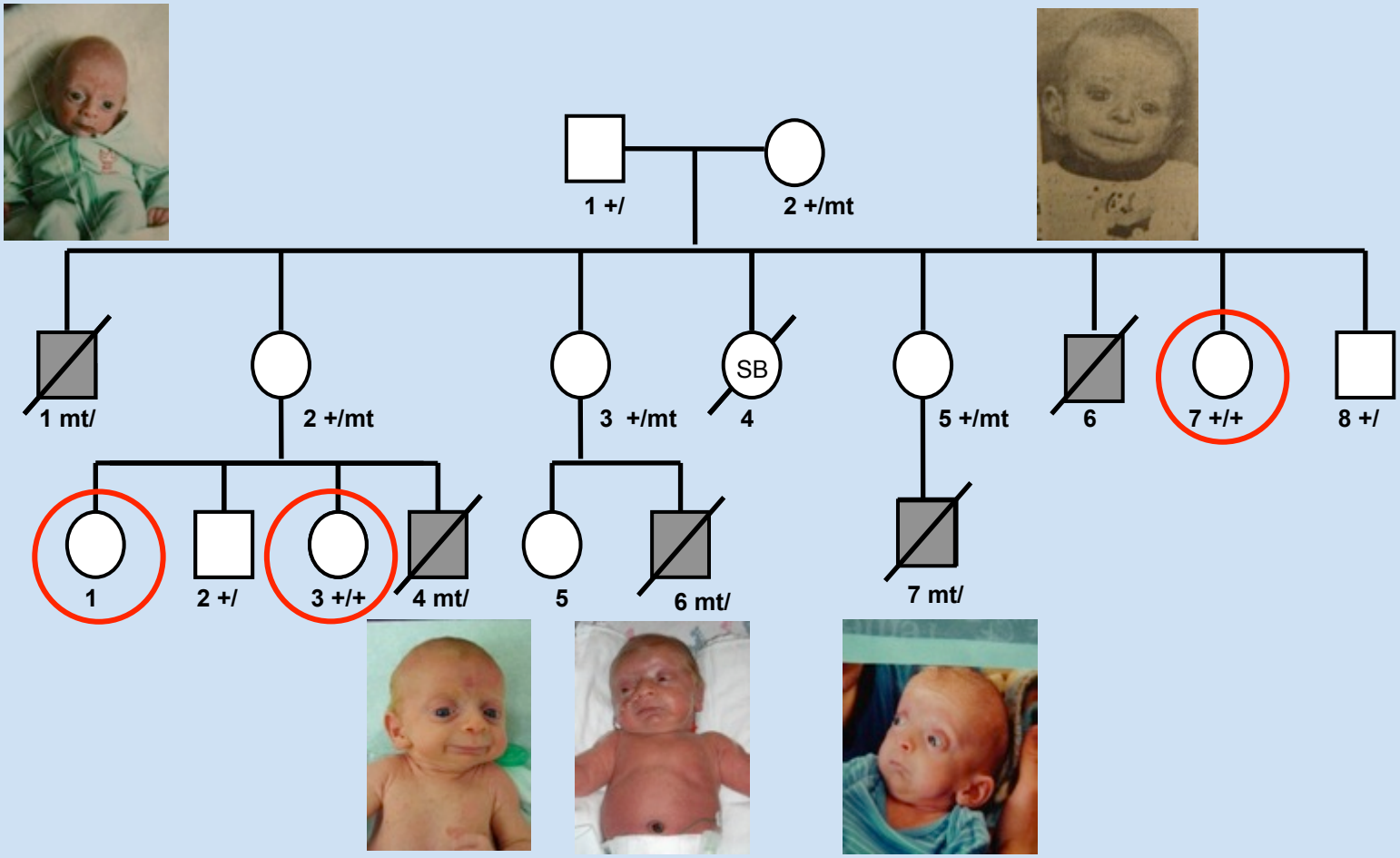
II-1 III-2

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



What about the other women in the family? Are they carriers? Once again, this was “research”.



Results from Next Gen Seq requires both Analytic and Clinical Validity

- Analytical Validity: the test is accurate with high sensitivity and specificity.

- Clinical Validity: Given an accurate test result, what impact and/or outcome does this have on the person?

Analytical Validity of Exome and WGS?

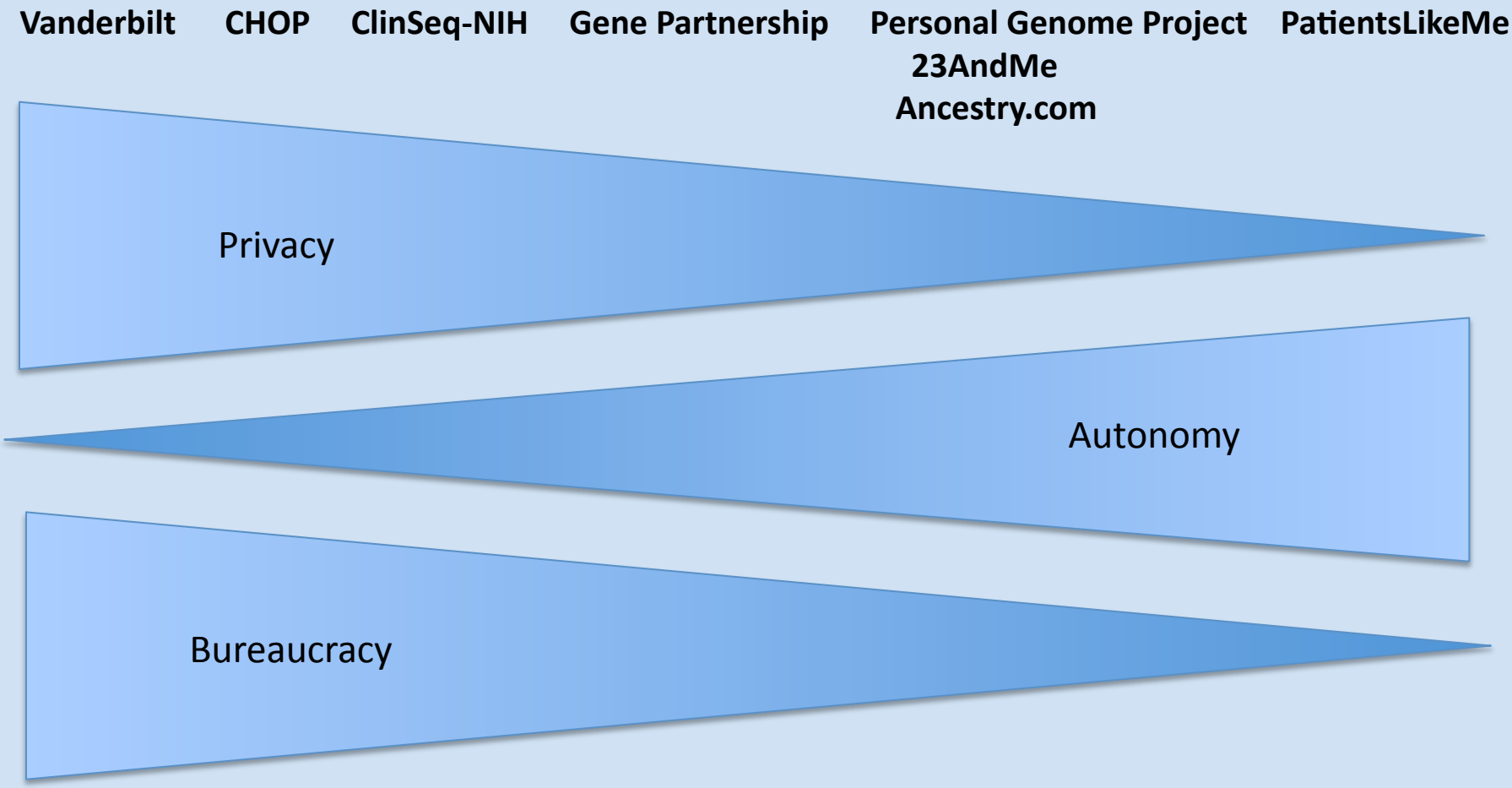
- Minimal Standard: exomes and genomes ought to be performed in a CLIA-certified environment for germline genomic DNA from live humans .
- Easier said than done in academia, but some companies offer this now: Illumina, 23andMe, Ambry Genetics, and some academic places do offer this now: UCLS, Baylor and WashU for exomes.
- I do NOT think the FDA should get involved to regulate this, nor do the results have to go through a physician, i.e. DTC is fine as long as CLIA-certified.

Why can UCLA implement CLIA-certified exome sequencing so quickly, whereas most have not or cannot?

- Others doing this are: Baylor, WashU-St.Louis, Ambry Genetics, 23andMe.

- For CLIA-certified WGS, there is Wisconsin, Baylor, WashU-St.Louis.

Autonomy vs. Privacy vs. Bureaucracy



Mapping clinical phenotype data elements to standardized metadata repositories and controlled terminologies: the eMERGE Network experience

Jyotishman Pathak,[1] Janey Wang,[2] Sudha Kashyap,[2] Melissa Basford,[2] Rongling Li,[3] Daniel R Masys,[2] Christopher G Chute[1]

J Am Med Inform Assoc 2011;**18**:376–386.

Phase I sites

1. Vanderbilt University
2. Marshfield Clinic
3. Mayo Clinic
4. Northwestern University
5. Group Health Cooperative-UW

Additional Sites in Phase II

1. Geisinger
2. Mount Sinai School of Medicine
3. The eMERGE Coordinating Center at Vanderbilt

Additional Pediatric Sites in Phase II

1. Children's Hospital of Philadelphia
2. Cincinnati Children's Hospital Medical Center & Boston Children's Hospital

Table 5 Snapshot of Northwestern University's type 2 diabetes data dictionary

Variable	Description	Type	Units	Permissible values
Subject_ID	Deidentified subject's ID	Integer		
Enrollment_age	Age at enrollment in DNA biorepository	Integer		
Case_control	Is the subject a case or a control?	Encoded		0=Case; 1=Control
Sex	Subject's gender	Encoded		M=Male; F=Female; U=Unknown
Race	Subject's race	Encoded		0=African American; 1=American Indian; 2=Asian; 3=White; 4=Native Hawaiian; 5=Other; 6=Unknown; 7=Missing
Weight	Subject's weight in kilograms	Decimal	kg	
Height	Subject's height in centimeters	Decimal	cm	
Glucose_measurement	Subject's random glucose level	Decimal	mg/dl	

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.

Genotype First, Phenotype Second AND Longitudinally

- Intellectual underpinnings of this seems to emerge from the work of Evan Eichler & many others, regarding CNVs and developmental delay.

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

VAAST shows that probabilistic ranking will be very useful going forward

- But, VAAST is currently dependent on the variant lists provided to it, as there is still a heuristic threshold with input of variant data, i.e. no probabilistic weighting of SNV or indel “true positive likelihood”.

- Therefore, need to optimize variant-calling to make sure variants provided are correct. Plus, VAAST chokes if background genomes are full of false positives.

- Thus, focused now on comprehensive comparison of NGS variant-calling on deep exome sequencing data

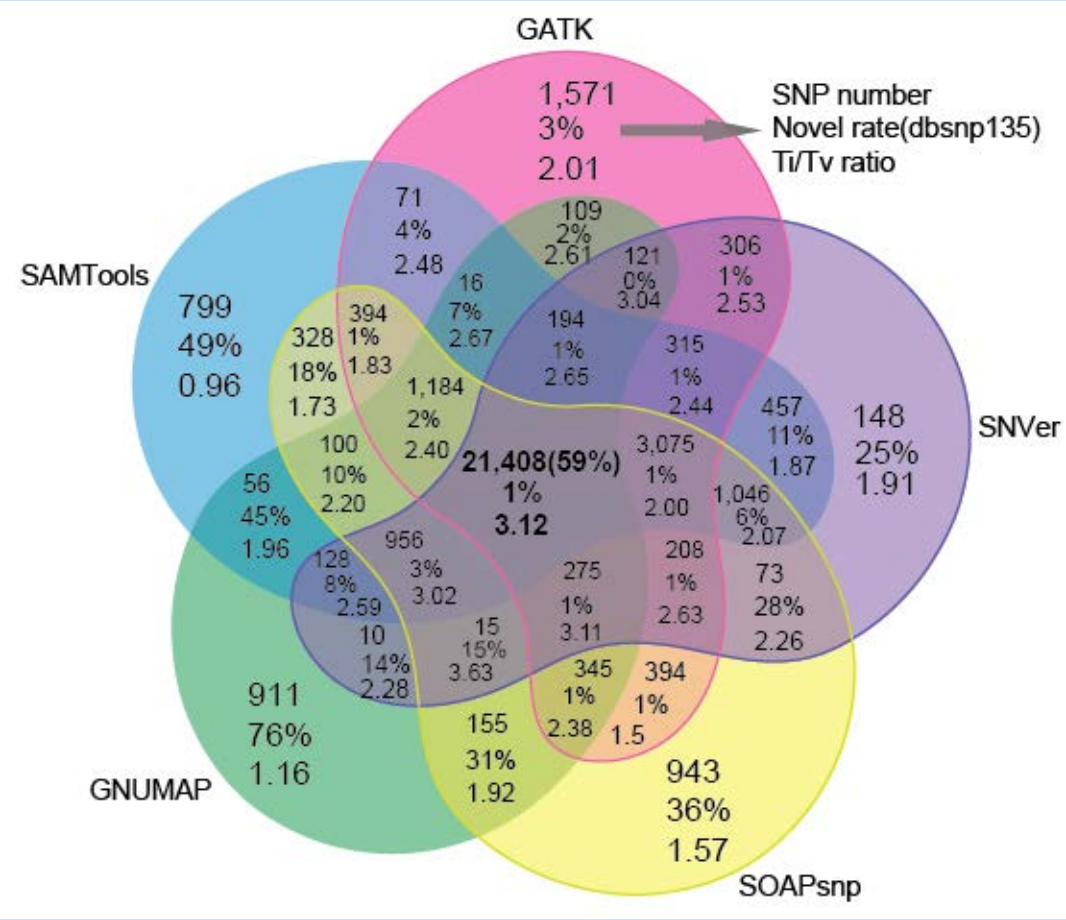
CLIA-certified exomes and WGS

- The CLIA-certified pipelines attempt to minimize false positives with increased stringency, but this results in many no-calls and other areas of uncertainty, which should be reported as No-Call Regions.
- BUT, this is ok, as minimizing false positives is very important in clinical medicine.

Optimizing Variant Calling in Exomes

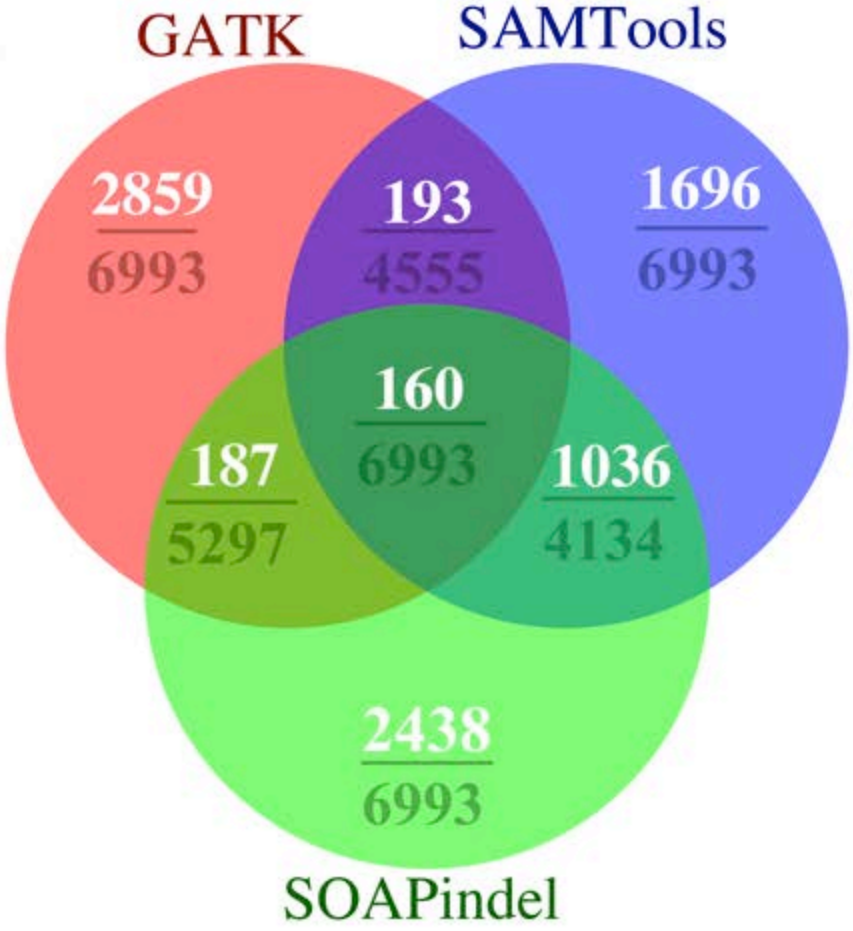
- Agilent v2 44 MB exome kit
- Illumina Hi-Seq for sequencing.
- Average coverage ~100-150x.
- Depth of sequencing of >80% of the target region with >20 reads or more per base pair.
- Comparing various pipelines for alignment and variant-calling.

SNV venn plot

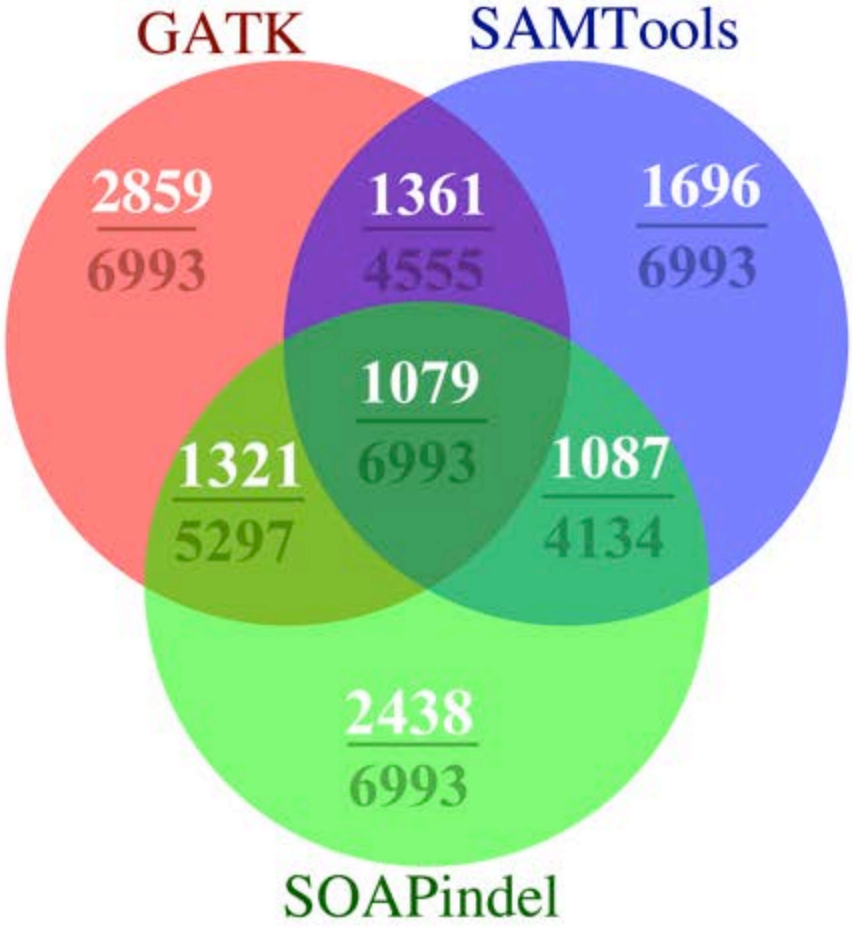


Total SNP

Comparing indel overlap by matching **base position** as well as **length and composition**.



Comparing indel overlap by matching **only base position**.



A total of **6993** indels were called between the three pipelines.

Genomic Dark Matter: The reliability of short read mapping illustrated by the Genome Mappability Score

Hayan Lee[1,2*] and Michael C. Schatz [1,2]

[1]Department of Computer Science, Stony Brook University, Stony Brook, NY
[2]Simons Center for Quantitive Biology, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Bioinformatics Advance Access published June 4, 2012

- Genome Mappability Score (GMS) -- measure of the complexity of resequencing a genome = a weighted probability that any read could be unambiguously mapped to a given position, and thus measures the overall composition of the genome itself.
- Genome Mappability Analyzer (GMA) -- to compute the GMS of every position in a genome. Helps identify the 5-14% of the human, mouse, fly, and yeast genomes that are difficult to analyze with short reads.
- With BWA/SAMtools polymorphism discovery pipeline, discovery errors are dominated by false negatives, especially in regions with poor GMS. These errors are fundamental to the mapping process and cannot be overcome by increasing coverage.
- The GMS should be considered in every resequencing project to pinpoint the dark matter of the genome, including of known clinically relevant variations in these regions.

Complete Genomics – LFR technology

Accurate whole-genome sequencing and haplotyping from 10 to 20 human cells

Brock A. Peters[1]*, Bahram G. Kermani[1]*, Andrew B. Sparks[1]†, Oleg Alferov[1], Peter Hong[1], Andrei Alexeev[1], Yuan Jiang[1], Fredrik Dahl[1]†, Y. Tom Tang[1], Juergen Haas[1], Kimberly Robasky[2,3], Alexander Wait Zaranek[2], Je-Hyuk Lee[2,4], Madeleine Price Ball[2], Joseph E. Peterson[1], Helena Perazich[1], George Yeung[1], Jia Liu[1], Linsu Chen[1], Michael I. Kennemer[1], Kaliprasad Pothuraju[1], Karel Konvicka[1], Mike Tsoupko-Sitnikov[1], Krishna P. Pant[1], Jessica C. Ebert[1], Geoffrey B. Nilsen[1], Jonathan Baccash[1], Aaron L. Halpern[1], George M. Church[2] & Radoje Drmanac[1]

NATURE | VOL 487 | 12 JULY 2012

"Substantial error rates (1 single nucleotide variants (SNV) in 100–1,000 called kilobases) are a common attribute of all current massively parallelized sequencing technologies2–10,12. These rates are probably too high for diagnostic use and complicate many studies searching for new mutations."

Much Higher Accuracy with LFR data

"To test LFR reproducibility we compared haplotype data between the two NA19240 replicate libraries. In general, the libraries were very concordant, with only 64 differences per library in 2.2 million heterozygous SNPs phased by both libraries or **1 of this error type in 44 Mb**."

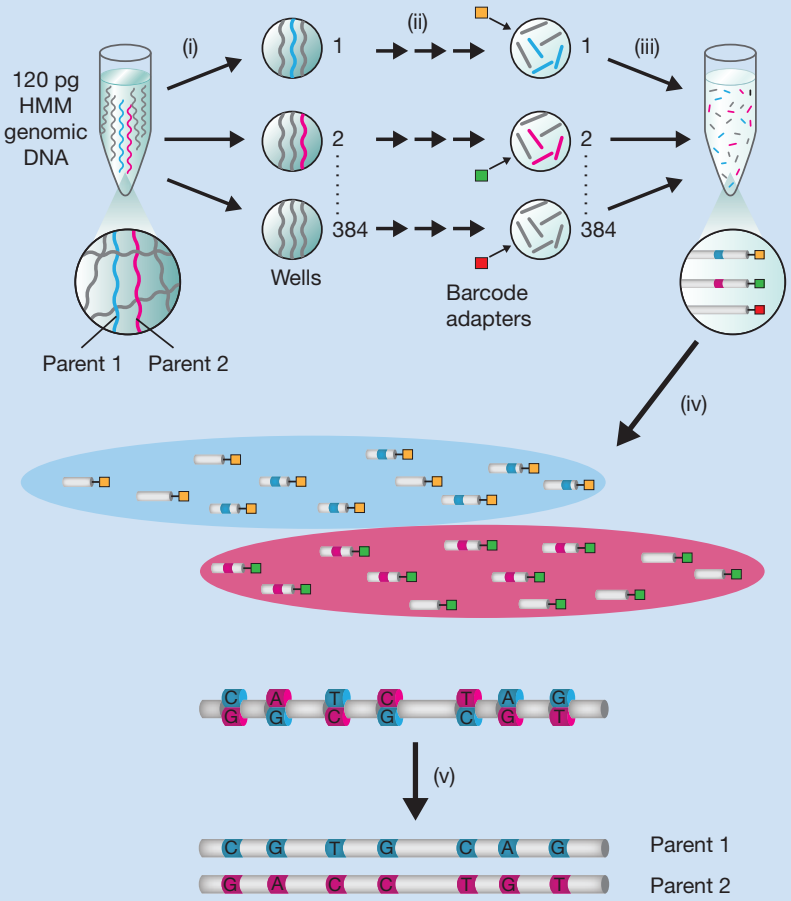


Figure 1 | The LFR technology. An overview of the LFR technology and controlled random enzymatic fragmenting is shown. (i) First, 100–130 pg of high molecular mass (HMM) DNA is physically separated into 384 distinct wells; (ii) through several steps, all within the same well without intervening purifications, the genomic DNA is amplified, fragmented and ligated to unique barcode adapters; (iii) all 384 wells are combined, purified and introduced into the sequencing platform of Complete Genomics[10]; (iv) mate-paired reads are mapped to the genome using a custom alignment program and barcode sequences are used to group tags into haplotype contigs; and (v) the final result is a diploid genome sequence.

Whole Genome Sequencing in a centralized facility 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.

Systemic Barriers

- Sometimes just simple lack of communication between researchers and the physicians and genetic counselors.
- We only have 1500 medical geneticists and 2000 certified genetic counselors for 310 million people in America!
- Insurance will often deny coverage of genetic testing.

Systemic Barriers cont….

- Physicians and health care system woefully uneducated regarding genetics.
- Most current sequencing (exomes and whole genomes) being sequenced in random laboratories with no clinical standards in place.

Quote from Clifford Reid at Complete Genomics

- “We have higher quality. But in the research market that doesn’t matter very much. Researchers can work with lower quality data. That’s really been a startling revelation to us that despite the community saying quality is everything, quality really isn’t everything.”

--- http://www.forbes.com/sites/matthewherper/2012/07/13/can-complete-genomics-escape-the-valley-of-death/

GENOMICS

Noninvasive Whole-Genome Sequencing of a Human Fetus

Jacob O. Kitzman,[1]* Matthew W. Snyder,[1] Mario Ventura,[1,2] Alexandra P. Lewis,[1] Ruolan Qiu,[1] LaVone E. Simmons,[3] Hilary S. Gammill,[3,4] Craig E. Rubens,[5,6] Donna A. Santillan,[7] Jeffrey C. Murray,[8] Holly K. Tabor,[5,9] Michael J. Bamshad,[1,5] Evan E. Eichler,[1,10] Jay Shendure[1]*

Science Translational Medicine 2012

- "The ability to noninvasively sequence a fetal genome to high accuracy and completeness will undoubtedly have profound implications for the future of prenatal genetic diagnostics."

- Yes, but this is NOT that study!

Actual Data

- “we found 2.5×10^7 candidate de novo sites, including 39 of the 44 true de novo sites. At baseline, this corresponds to sensitivity of 88.6% with a signal- to-noise ratio of 1-to-640,000”
- With other filters, they reduce number of “total positives” to 3884, of which 17 are true positives, from total known true positives of 44), so sensitivity= 38.6%.
- This is nowhere near accurate, or anything remotely close to a clinical grade test!

Rare Variants – CNVs, SNVs, indels, etc… in Rare AND Common diseases

High Frequencies of De Novo CNVs in Bipolar Disorder and Schizophrenia

Dheeraj Malhotra,[1,2,22] Shane McCarthy,[22] Jacob J. Michaelson,[1,2] Vladimir Vacic,[15,22] Katherine E. Burdick,[23] Seungtai Yoon,[5,22] Sven Cichon,[10,11,12] Aiden Corvin,[17] Sydney Gary,[22] Elliot S. Gershon,[21] Michael Gill,[17] Maria Karayiorgou,[18] John R. Kelsoe,[2,4,20] Olga Krastoshevsky,[19] Verena Krause,[19] Ellen Leibenluft,[7] Deborah L. Levy,[19] Vladimir Makarov,[5,22] Abhishek Bhandari,[1,2,22] Anil K. Malhotra,[6] Francis J. McMahon,[14] Markus M. Nöthen,[10,11,16] James B. Potash,[8] Marcella Rietschel,[13] Thomas G. Schulze,[9] and Jonathan Sebat[1,2,3,4,22,*]

Deep resequencing of GWAS loci identifies independent rare variants associated with inflammatory bowel disease

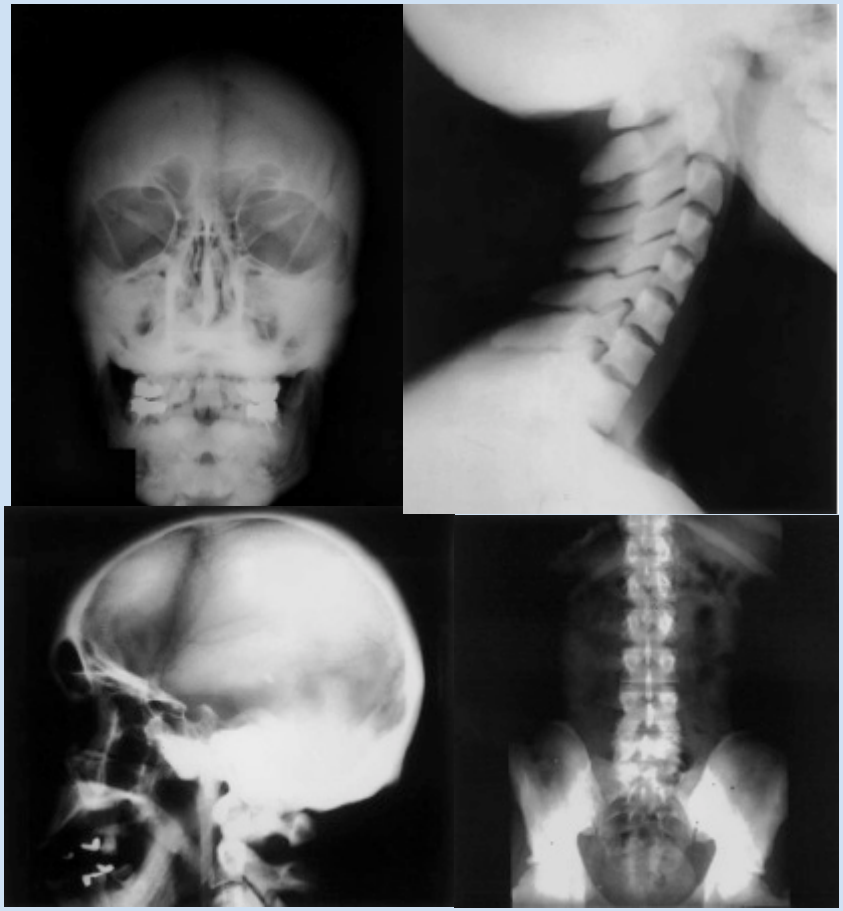
Manuel A Rivas[1–3], Mélissa Beaudoin[4,23], Agnes Gardet[5,23], Christine Stevens[2,23], Yashoda Sharma[6], Clarence K Zhang[6], Gabrielle Boucher[4], Stephan Ripke[1,2], David Ellinghaus[7], Noel Burtt[2], Tim Fennell[2], Andrew Kirby[1,2], Anna Latiano[8], Philippe Goyette[4], Todd Green[2], Jonas Halfvarson[9], Talin Haritunians[10], Joshua M Korn[2], Finny Kuruvilla[2,11], Caroline Lagacé[4], Benjamin Neale[1,2], Ken Sin Lo[4], Phil Schumm[12], Leif Törkvist[13], National Institute of Diabetes and Digestive Kidney Diseases Inflammatory Bowel Disease Genetics Consortium (NIDDK IBDGC)[14], United Kingdom Inflammatory Bowel Disease Genetics Consortium[14], International Inflammatory Bowel Disease Genetics Consortium[14], Marla C Dubinsky[15], Steven R Brant[16,17], Mark S Silverberg[18], Richard H Duerr[19,20], David Altshuler[1,2], Stacey Gabriel[2], Guillaume Lettre[4], Andre Franke[7], Mauro D'Amato[21], Dermot P B McGovern[10,22], Judy H Cho[6], John D Rioux[4], Ramnik J Xavier[1,2,5] & Mark J Daly[1,2]

Evolution and Functional Impact of Rare Coding Variation from Deep Sequencing of Human Exomes

Jacob A. Tennessen,[1*] Abigail W. Bigham,[2*†] Timothy D. O'Connor,[1*] Wenqing Fu,[1] Eimear E. Kenny,[3] Simon Gravel,[3] Sean McGee,[1] Ron Do,[4,5] Xiaoming Liu,[6] Goo Jun,[7] Hyun Min Kang,[7] Daniel Jordan,[8] Suzanne M. Leal,[9] Stacey Gabriel,[4] Mark J. Rieder,[1] Goncalo Abecasis,[7] David Altshuler,[4] Deborah A. Nickerson,[1] Eric Boerwinkle,[6,10] Shamil Sunyaev,[4,8] Carlos D. Bustamante,[3] Michael J. Bamshad,[1,2‡] Joshua M. Akey,[1‡] Broad GO, Seattle GO, on behalf of the NHLBI Exome Sequencing Project

“Superpower” mutations???

Myostatin mutation
Exon 2 allele P198A



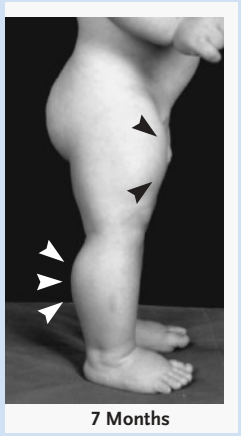
LRP5 mutation
D111Y, G171R, A214T, A214V, A242T, and T253I

**Thanks to George Church for discussions on this.

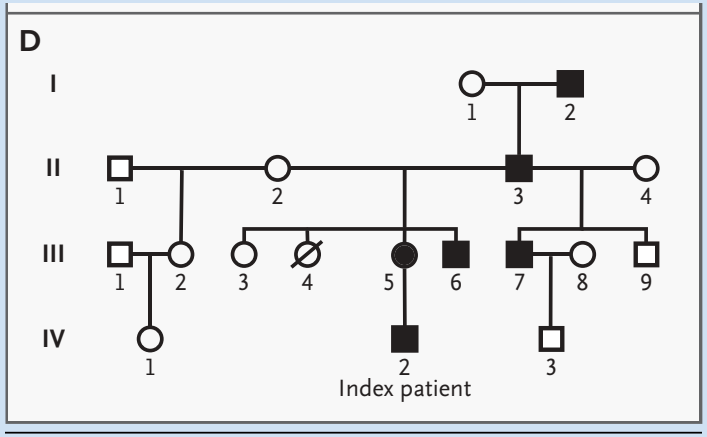
Myostatin Mutation Associated with Gross Muscle Hypertrophy in a Child

Markus Schuelke, M.D., Kathryn R. Wagner, M.D., Ph.D., Leslie E. Stolz, Ph.D., Christoph Hübner, M.D., Thomas Riebel, M.D., Wolfgang Kömen, M.D., Thomas Braun, M.D., Ph.D., James F. Tobin, Ph.D., and Se-Jin Lee, M.D., Ph.D.

N ENGL J MED 350;26 WWW.NEJM.ORG JUNE 24, 2004



7 Months



Liam is homozygous for the mutation.

Another example: Liam Hoekstra, known as the world's strongest toddler at age 3, has a condition called myostatin-related muscle hypertrophy which results in increased muscle mass and reduced body fat. Myostatin-related muscle hypertrophy, or muscle enlargement, is an extremely rare genetic condition. – How rare???

http://videos.disabled-world.com/video/159/liam-hoekstra-strongest-boy-in

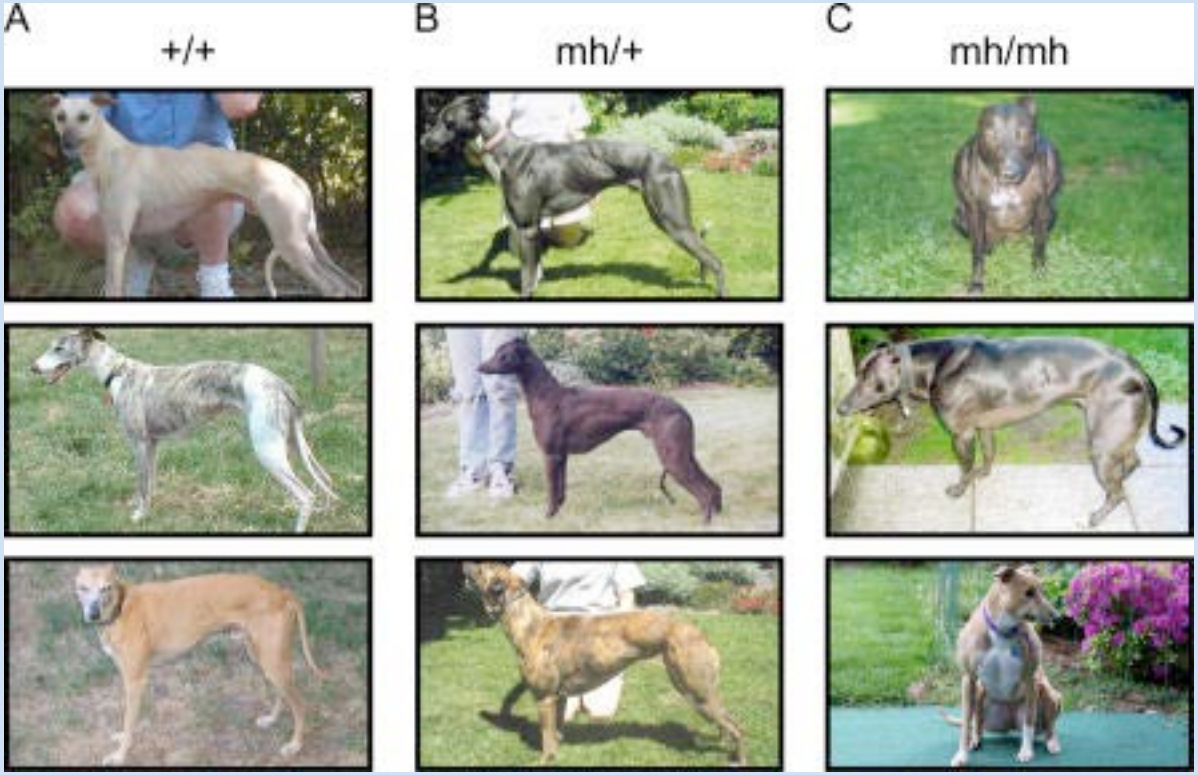
Belgian Blue is a breed of beef cattle from Belgium. The Belgian Blue has a natural mutation in the myostatin gene which codes for the protein, myostatin.

http://en.wikipedia.org/wiki/Belgian_Blue

A Mutation in the Myostatin Gene Increases Muscle Mass and Enhances Racing Performance in Heterozygote Dogs

Dana S. Mosher[1], Pascale Quignon[1], Carlos D. Bustamante[2], Nathan B. Sutter[1], Cathryn S. Mellersh[3], Heidi G. Parker[1], Elaine A. Ostrander[1*]

1 National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland, United States of America, **2** Department of Biological Statistics and Computational Biology, Cornell University, Ithaca, New York, United States of America, **3** Animal Health Trust, Center for Preventive Medicine, Newmarket, United Kingdom



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 people, nor categorized into ethnic classes, i.e. clans.

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

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.

Clinically Relevant Variants Resource, or CRVR

"The National Human Genome Research Institute is planning to fund the creation of a single resource that would house and disseminate data about potentially clinically relevant genetic variants that are being unearthed by genomics research projects."

-July 13, 2012, Genome Web Daily New

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 for 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

- Require that all **initial** germline exome and whole genome sequencing in live humans be performed in a CLIA-certified or other clinical-grade manner.
- I pray and hope that industry will collate and distribute 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

Need to change the Tenor of the debate

- Sequencing live humans without method in place to return results, perhaps no longer acceptable?
- I feel that it is ethically and morally unacceptable to sequence live humans only in research settings, particularly without genetic counseling and means for return of results in place.
- The **initial** germline exome and/or whole genome sequencing for each human should be performed in a clinical-grade (CLIA-certified) manner, including for those samples at the major genome sequencing centers involved in "complex disease" sequencing.
- "What kind of work deemed as accepted today will be denounced by future generations? The question is one that all researchers should bear in mind, because history may judge them more harshly than their peers do." -Nature editorial, February 9, 2012

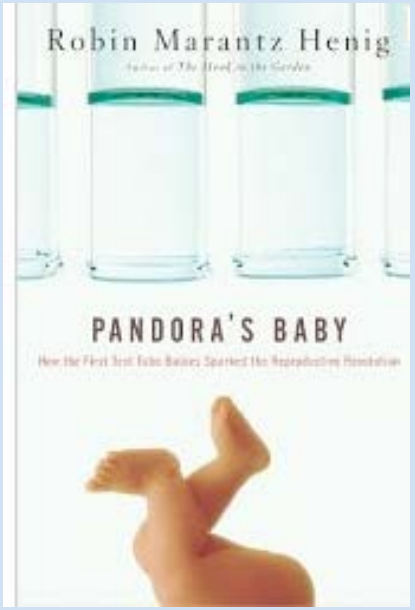
Need Minimal Regulatory Standards!

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.

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."



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Christa Schank
Sarah T. South
Jeffrey J Swensen
Jinchuan Xing
Mark Yandell

Reid Robison
Edwin Nyambi

Thomas Arnesen
Rune Evjenth
Johan R. Lillehaug

USC
Kai Wang

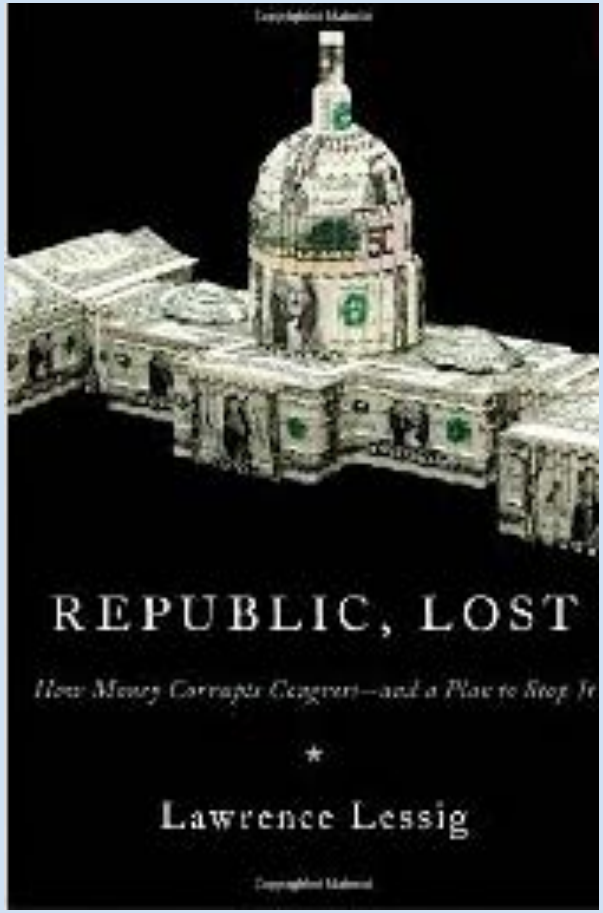
Jason O'Rawe
Michael Schatz
Giuseppe Narzisi

Zhi Wei
Lifeng Tian
Hakon Hakonarson

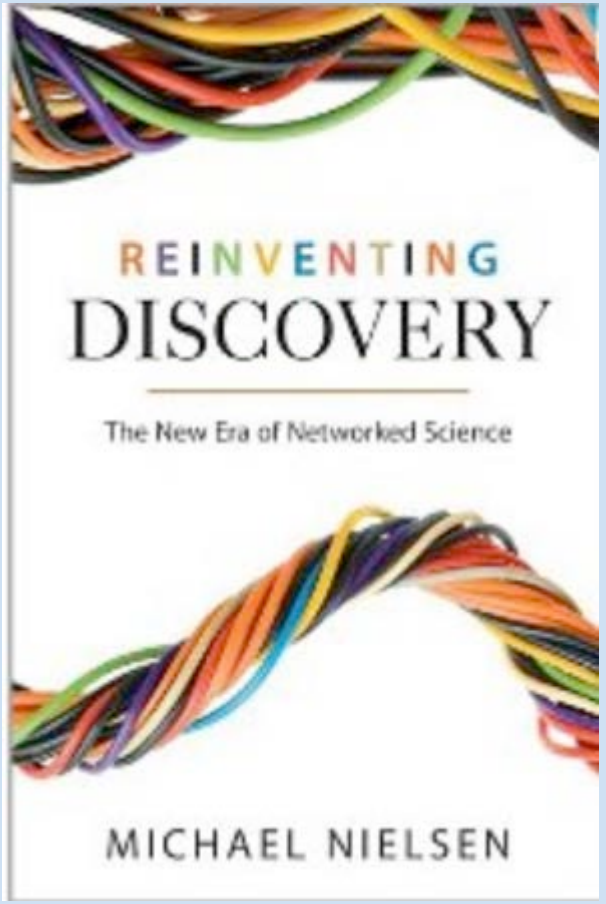
Tao Jiang
Jun Wang

our study families

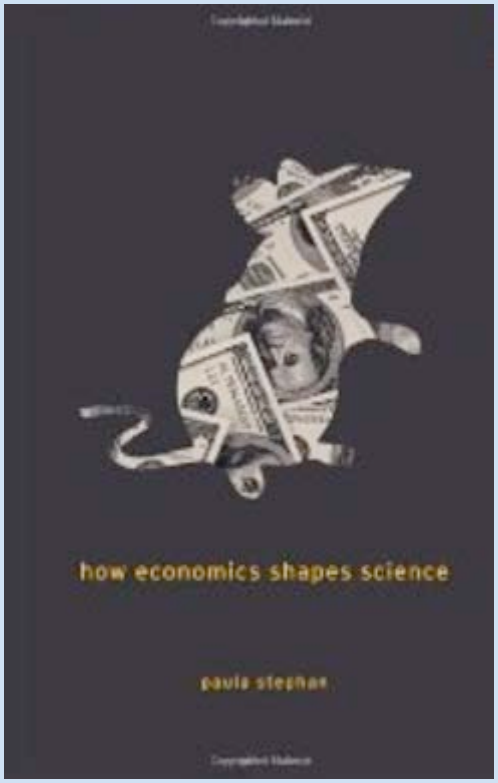
References

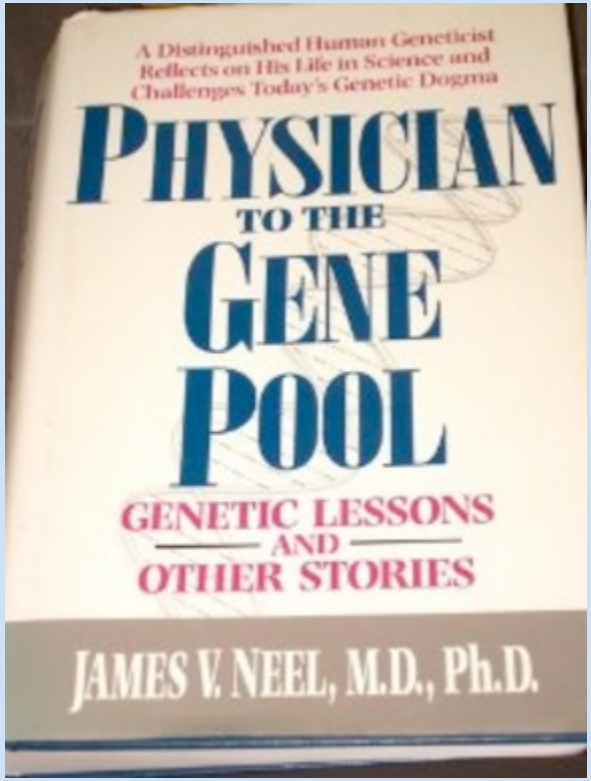

REPUBLIC, LOST
Lawrence Lessig

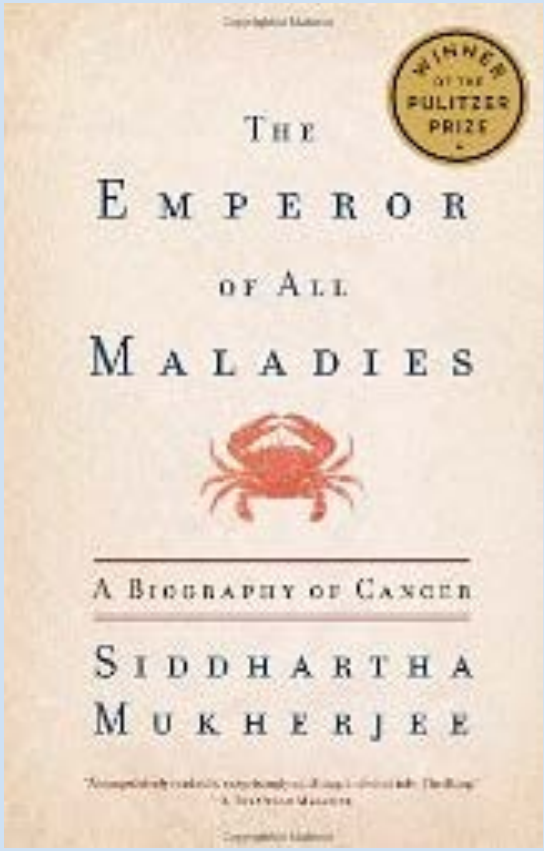

The Creative Destruction of
MEDICINE
HOW THE DIGITAL REVOLUTION WILL CREATE BETTER HEALTH CARE
ERIC TOPOL, M.D.


REINVENTING
DISCOVERY
The New Era of Networked Science
MICHAEL NIELSEN

References cont…







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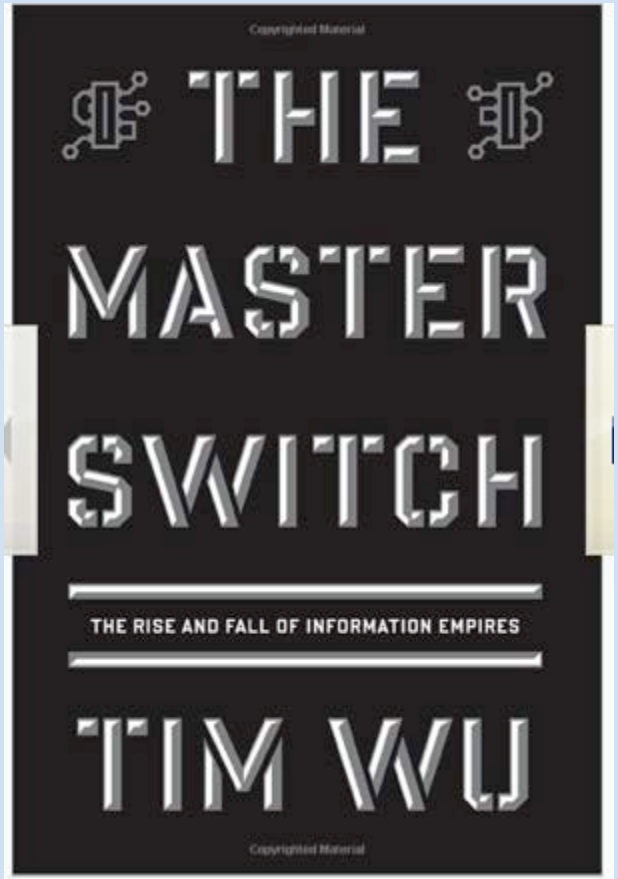

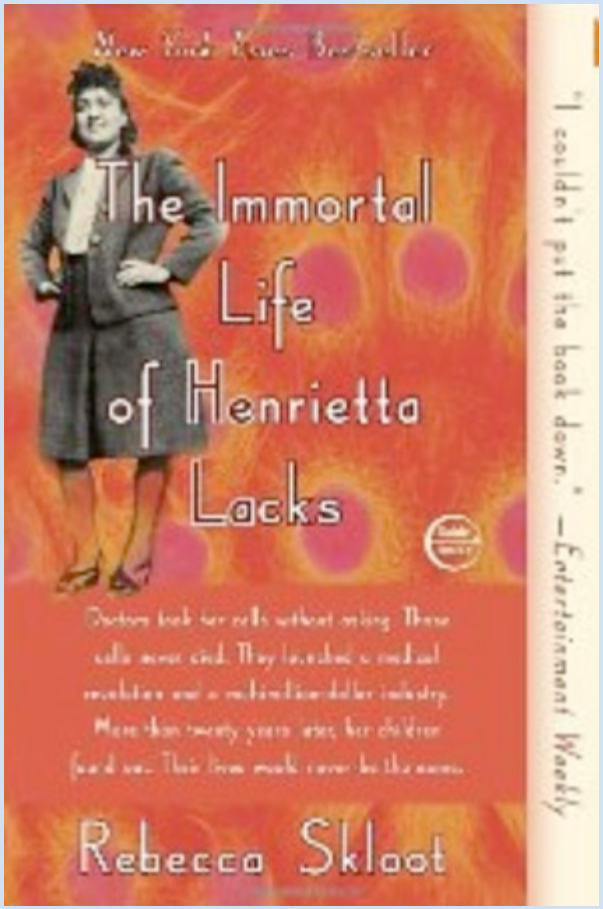

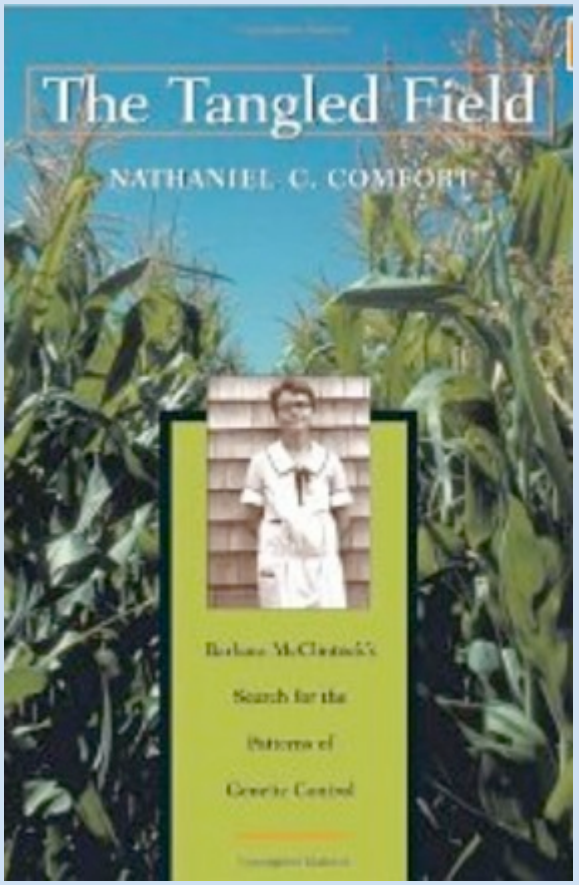

References

- **@Katy_Read**: Like many writers, I have rituals. Before writing, I pour some coffee, open the window by my desk, and attempt to read the entire internet.