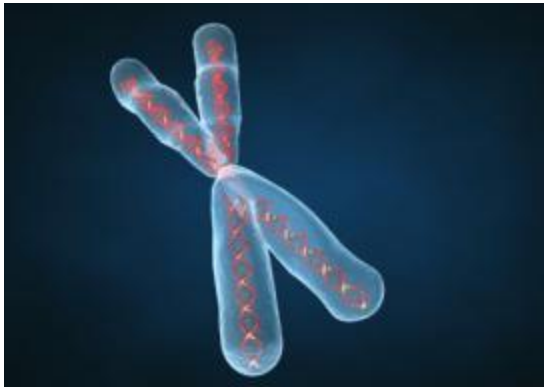


A case report in genomic medicine

Euan Ashley MRCP, DPhil, FACC, FAHA
Chief Futurist
Director, Center for Inherited Cardiovascular Disease
Stanford University School of Medicine

Disclosures
Founder, equity owner and consultant : Personalis Inc

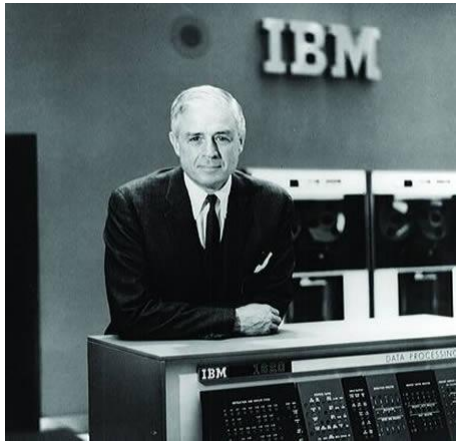
STANFORD CENTER FOR INHERITED CARDIOVASCULAR DISEASE

Futurist

A B C D E F G H I J K L M N
O P Q R S T U V W X Y Z
a b c d e f g h i j k l m n
o p q r s t u v w x y z
1 2 3 4 5 6 7 8 9 0
! @ # $ % ^ & * ( )

"*I think there is a world market for maybe five computers*.“

Thomas Watson, chairman of IBM, 1943



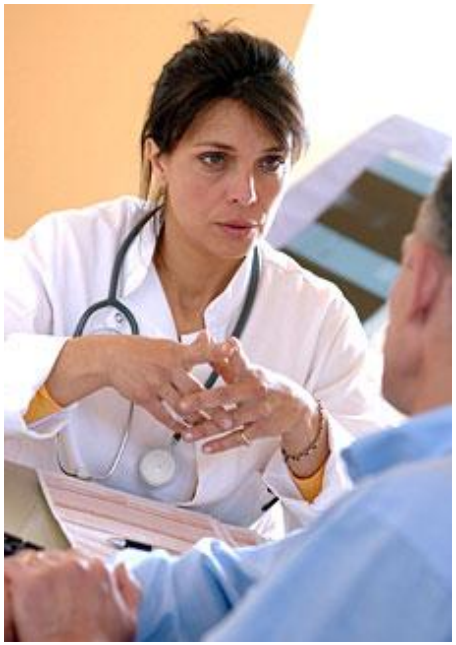
"*The concept is interesting and well-formed, but in order to earn better than a 'C,' the idea must be feasible*."

A Yale University management professor in response to Fred Smith's paper proposing reliable overnight delivery service. (Smith went on to found Federal Express Corp.)

"*We don't like their sound, and guitar music is on the way out”*

Decca Recording Co. rejecting the Beatles, 1962.

What if everybody's genome was available in their medical record?



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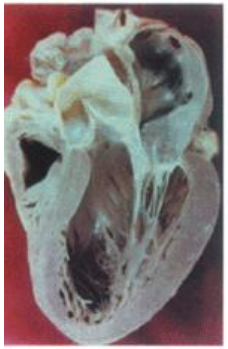
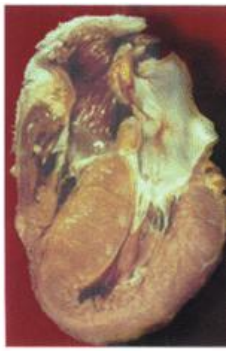
Parsing 6,000,000,000 data points

When one base pair change can turn this



into this



STANFORD CENTER FOR INHERITED CARDIOVASCULAR DISEASE

The Stanford Genome Interpretation team

Technology & Structural variation

Mike Snyder

Ancestry & admixture

Carlos Bustamante

Sequence, assembly & error

Steve Quake

Rare, Mendelian & non-coding variation

Euan Ashley

Common variation

Atul Butte

Pharmaco-genomic variation

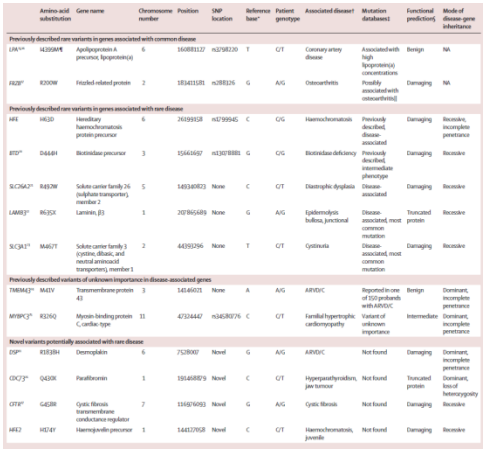
Russ Altman

Ethics & Consent

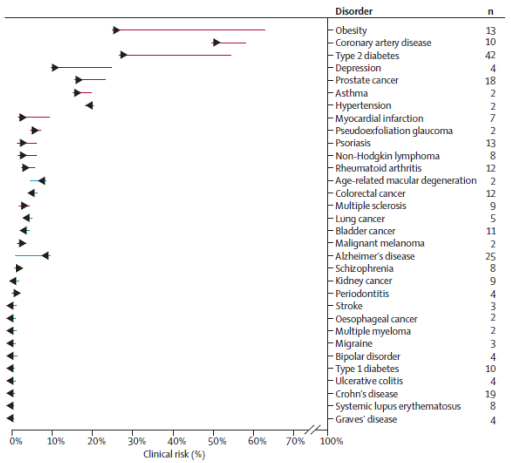
Hank Greely

Clinical assessment incorporating a personal genome

Euan A Ashley, Atul J Butte, Matthew T Wheeler, Rong Chen, Teri E Klein, Frederick E Dewey, Joel T Dudley, Kelly E Ormond, Aleksandra Pavlovic, Alexander A Morgan, Dmitry Pushkarev, Norma F Neff, Louanne Hudgins, Li Gong, Laura M Hodges, Dorit S Berlin, Caroline F Thorn, Katrin Sangkuhl, Joan M Hebert, Mark Woon, Hersh Sagreiya, Ryan Whaley, Joshua W Knowles, Michael F Chou, Joseph V Thakuria, Abraham M Rosenbaum, Alexander Wait Zaranek, George M Church, Henry T Greely, Stephen R Quake, Russ B Altman



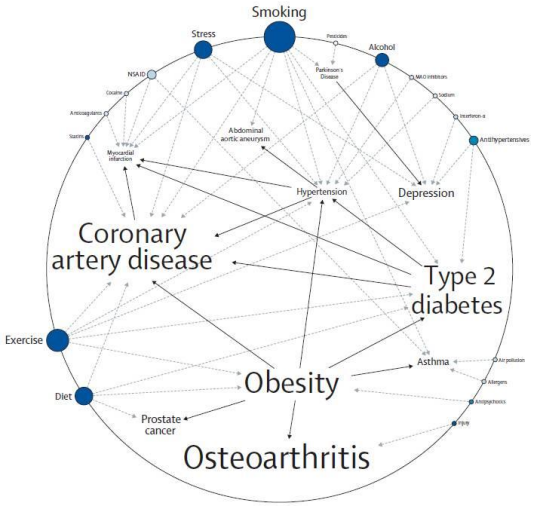
	Amino acid substitution	Gene name	Chromosome number	Position	SNP location	Reference base*	Patient genotype	Associated disease†	Mutation database‡	Functional prediction§	Mode of disease-gene inheritance
Previously described rare variants in genes associated with common disease											
LPA[a,b]	V4389M¶	Apolipoprotein A precursor, lipoprotein(a)	6	160881122	rs3798220	T	C/T	Coronary artery disease	Associated with high lipoprotein(a) concentrations	Benign	NA
FBZB*	R200W	Frizzled-related protein	2	181411581	rs288326	G	A/G	Osteoarthritis	Possibly associated with osteoarthritis‖	Damaging	NA
Previously described rare variants in genes associated with rare disease											
HFE	H63D	Hereditary haemochromatosis protein precursor	6	26159358	rs1799945	C	C/G	Haemochromatosis	Previously described, disease-associated	Damaging	Recessive, incomplete penetrance
BTD**	D444H	Biotinidase precursor	3	15661697	rs13078881	G	C/G	Biotinidase deficiency	Previously described, intermediate phenotype	Damaging	Recessive
SLC26A2**	R459W	Solute carrier family 26 (sulphate transporter), member 2	5	149340823	None	C	C/T	Diastrophic dysplasia	Disease-associated	Damaging	Recessive
LAMB3**	R635X	Laminin, β3	1	207865689	None	G	A/G	Epidermolysis bullosa, junctional	Disease-associated, most common mutation	Truncated protein	Recessive
SLC3A1**	M467T	Solute carrier family 3 (cystine, dibasic, and neutral aminoacid transporters), member 1	2	44393296	None	T	C/T	Cystinuria	Disease-associated, most common mutation	Damaging	Recessive
Previously described variants of unknown importance in disease-associated genes											
TMBIM4[a]**	M41V	Transmembrane protein 43	3	14146021	None	A	A/G	ARVD/C	Reported in one of 310 probands with ARVD/C	Benign	Dominant, incomplete penetrance
MYBPC3**	R326Q	Myosin-binding protein C, cardiac-type	11	47324447	rs1458076	C	C/T	Familial hypertrophic cardiomyopathy	Variant of unknown importance	Intermediate	Dominant, incomplete penetrance
Novel variants potentially associated with rare disease											
DSP**	R1838H	Desmoplakin	6	7528007	Novel	G	A/G	ARVD/C	Not found	Damaging	Dominant, incomplete penetrance
CDC73**	Q430X	Parafibromin	1	191468879	Novel	C	C/T	Hyperparathyroidism, jaw tumour	Not found	Truncated protein	Dominant, loss of heterozygosity
CFTR**	G458R	Cystic fibrosis transmembrane conductance regulator	7	116976093	Novel	G	A/G	Cystic fibrosis	Not found	Damaging	Recessive
HFE2	H374Y	Haemojuvelin precursor	1	144372958	Novel	C	C/T	Haemochromatosis, juvenile	Not found	Damaging	Recessive



	Gene name	SNP location	Patient genotype	Drug(s) affected	Summary of effects	Level of evidence
SLCO1B1	Solute carrier organic anion transporter family, member 1B1	rs4149056	T/T	HMG-CoA reductase inhibitors (statins)	No increased risk of myopathy	High[26,28]
CYP2C19	Cytochrome P450, family 2, subfamily C, polypeptide 19	rs4244285	A/G	Clopidogrel and CYP2C19 substrates	CYP2C19 poor metaboliser; many drugs might need adjustment	High[29]
VKORC1	Vitamin K epoxide reductase complex, subunit 1	rs9923231	C/T	Warfarin	Reduced dose needed	High[30]
CYP4F2	Cytochrome P450, family 4, subfamily F, polypeptide 2	rs2108622	C/C	Warfarin	Reduced dose needed	High[31]
ADRB1	β1 adrenergic receptor	rs1801252	A/A	Atenolol, metoprolol	Might be preferable to calcium-channel blockers	High[32,33]
SLCO1B1	Solute carrier organic anion transporter family, member 1B1	rs11045819	A/C	Fluvastatin	Good response	Medium[26]
HMGCR	HMG-CoA reductase	rs12238540	T/T	Pravastatin	Patient might have good response	Medium
HMGCR	HMG-CoA reductase	rs12444841	A/A	Pravastatin, simvastatin	No reduced efficacy	Medium
ADRB2	β2 adrenergic receptor, surface	rs1042713	A/G	β blockers	Other treatment options might be preferable	Medium[34]
ADRB2	β2 adrenergic receptor, surface	rs1042714	C/C	β blockers	Other treatment options might be preferable	Medium[34,35]
CYP2D6	Cytochrome P450, family 2, subfamily D, polypeptide 6	rs3892097 rs1800716	C/C	Metoprolol and other CYP2D6 substrates	Normal CYP2D6 metaboliser	Medium[36]
CDKN2A/B	Cyclin-dependent kinase inhibitor 2A/2B	rs10811661	T/T	Metformin	Reduced likelihood of response	Medium[38]
CDKN2A/B	Cyclin-dependent kinase inhibitor 2A/2B	rs10811661	T/T	Troglitazone	Reduced likelihood of response	Medium[38]

SNP=single nucleotide polymorphism. HMG-CoA=3-hydroxy-3-methylglutaryl-coenzyme A.

Table 3: Pharmacogenomic variants with summary of effects and level of evidence



	Gene name	SNP location	Patient genotype	Drug(s) affected	Effect type	Coding change
NOD2	Nucleotide-binding oligomerisation domain containing 2	16:49303700	A/G	Infliximab	Pharmacodynamic	V793M
NOD2	Nucleotide-binding oligomerisation domain containing 2	16:49302935	C/T	Infliximab	Pharmacodynamic	5435L
SLC15A1	Solute carrier family 15 (oligopeptide transporter), member 1	13:98176691	C/T	Atorvastatin, fluvastatin, HMG-CoA reductase inhibitors, lovastatin, pravastatin, rosuvastatin, simvastatin	Pharmacokinetic	Y25C
HLA-DRB1	MHC class II, DR beta 1	6:32593811	T/T	Clozapine	Pharmacodynamic	T262K
MTCA	MHC class I polypeptide-related sequence A	6:31484467	C/T	Mercaptopurine, methotrexate	Pharmacodynamic	I147
SLC22A8	Solute carrier family 22 (organic anion transporter), member 8	11:62517376	C/T	Cimetidine, estrone, anti-inflammatory and antirheumatic products, non-steroids, ibuprofen, indometacin, ketoprofen, methotrexate, phenylbutazone, piroxicam, probenecid, atorvastatin, fluvastatin, HMG-CoA reductase inhibitors, lovastatin, pravastatin, rosuvastatin, simvastatin, adefovir dipivoxil, tenofovir, antineoplastic agents, cyanocobalamin, folic acid, folinic acid, pyridoxine	Pharmacokinetic	R534Q

SNP=single nucleotide polymorphism. HMG-CoA=3-hydroxy-3-methylglutaryl-coenzyme A. *Predicted to be damaging by PHD-SNP algorithm.*

Table 4: Pharmacogenomic rare and novel non-synonymous damaging variants*

Lancet 2010; 375: 1525–35

THE WALL STREET JOURNAL.
WSJ.com

HEALTH INDUSTRY | SEPTEMBER 16, 2011

Family Pioneers in Exploration of the Genome

By AMY DOCKSER MARCUS

A group of researchers said that by examining the whole genome of a family of four, they were able to make unusually specific findings, including the daughter's risk of blood clots, and suggestions for preventive care.

Channing Johnson for The Wall Street Journal

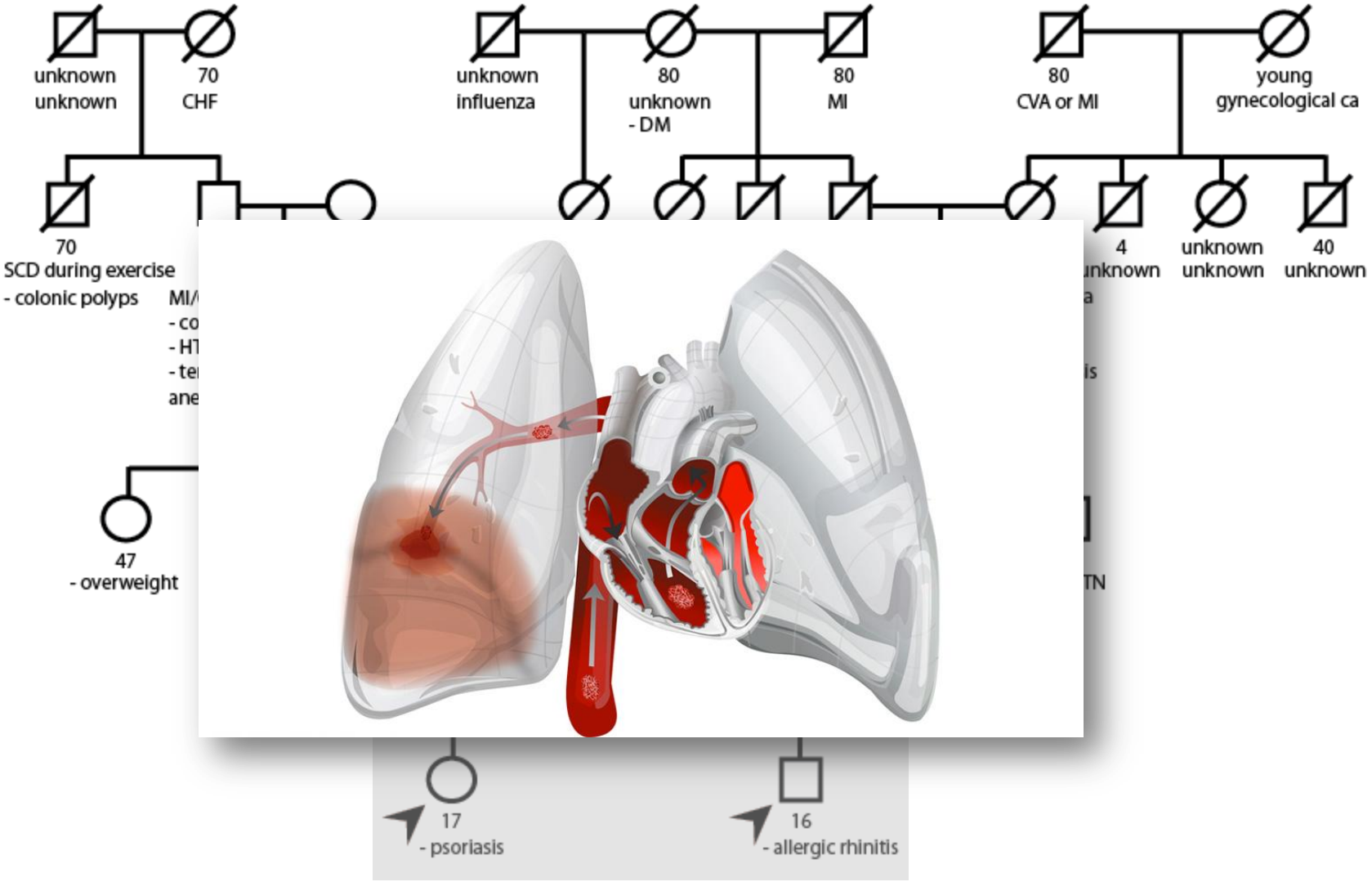
Wellesley College freshman Anne West.

The study, published Thursday in the journal PLoS Genetics, was led by researchers at Stanford University School of Medicine in Palo Alto, Calif., but also listed as co-authors John and Anne West, a father and daughter who were researching their own genetic make-up at home in Silicon Valley and met the Stanford team in the process. The research is part of scientists' continuing quest to extract truly useful information from the genome, a person's complete genetic code.

This is the second time a paper has been published about a family's whole genome. In the earlier paper, published last year in Science Express by a different group of researchers, the two children in the family had rare genetic conditions, and researchers were searching for the genes that caused them. The goal in the current study was to better predict the disease risk of a family and how family members might respond to medications.

Many people have turned to genetics companies like 23andme that offer information on specific traits for under $400. But genome sequencing of the six billion letters of a person's genetic code has been prohibitively expensive. Now, the price of the technology is rapidly plummeting. Illumina, which charged $48,000 to do individual genomes in 2009, now charges $9,500, or less for patients with life-threatening diseases.

But extracting useful clinical information from the data is still challenging. For one thing, "at this point, we are still not sure exactly what most genes predict about disease," said Lynn Jorde of the University of Utah School of Medicine, a co-author of the earlier family paper.



PLoS Genet 7(9): e1002280

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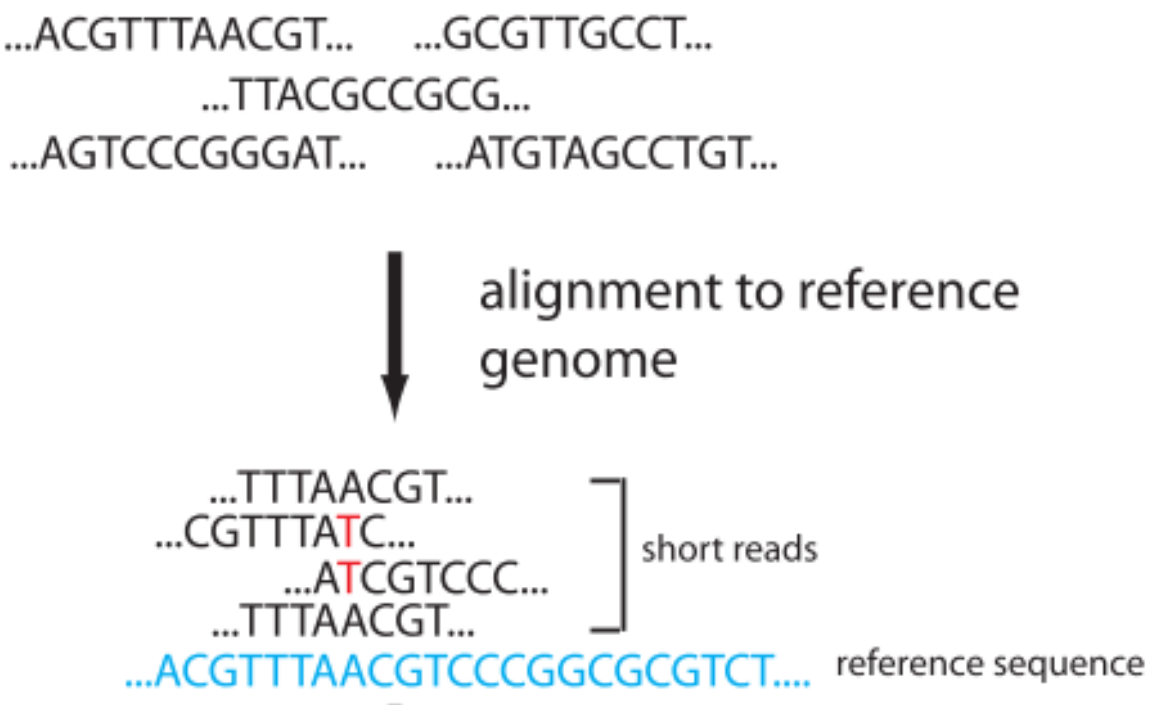
The problem(s) with short reads



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The human reference sequence

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CARDIOVASCULAR DISEASE

The human reference sequence contains highly validated risk alleles

Disease/trait	dbSNP	symbol	Type	Risk	P value	Odds Ratio	Pop
Blood pressure	653178	ATXN2	intron	C	3.00E-18		ALL, CHB/JPT, YRI
Celiac disease	653178	ATXN2	intron	C	7.15E-21	1.20	ALL, CHB/JPT, YRI
Celiac disease	3184504	SH2B3	missense	T	1.23E-12	1.21	ALL, CHB/JPT, YRI
Coronary artery disease	3184504	SH2B3	missense	T	4.23E-11	1.14	CHB/JPT, YRI
End-Stage renal disease	2032487	MYH9	intron	C	1.98E-15	2.61	CHB/JPT
End-Stage renal disease	4821480	MYH9	intron	G	8.48E-17	2.29	CHB/JPT
End-Stage renal disease	4821481	MYH9	intron	C	2.28E-16	2.62	CHB/JPT
End-Stage renal disease	5756152	MYH9	intron	A	5.84E-11	2.82	ALL, CHB/JPT
Focal segmental glomerulosclerosis	4821481	MYH9	intron	C	1.28E-09		CHB/JPT
Ige levels	1295685	IL13	UTR-3	A	2.00E-07	1.61	YRI
Rheumatoid arthritis	1217413		intergenic	G	4.00E-08	1.38	YRI
Rheumatoid arthritis	2476601	PTPN22	missense	A	2.30E-98	1.96	ALL, CHB/JPT, YRI
Systemic lupus erythematosus	2476601	PTPN22	missense	A	3.4E-12	1.57	ALL, CHB/JPT, YRI
Type 1 diabetes	2476601	PTPN22	missense	A	2.11E-87	2.25	ALL, CHB/JPT, YRI
Type 1 diabetes	3184504	SH2B3	missense	T	5.62E-31	1.24	ALL, CHB/JPT, YRI
Type 2 diabetes	5219	KCNJ11	missense	T	6.7E-11	1.26	YRI
Type 2 diabetes	2970847	PPARGC1A	cds-synon	T	1.59e-18	2.10	YRI
Vitiligo	2476601	PTPN22	missense	A	6.79E-09	1.82	ALL, CHB/JPT, YRI

PLoS Genet 7(9): e1002280

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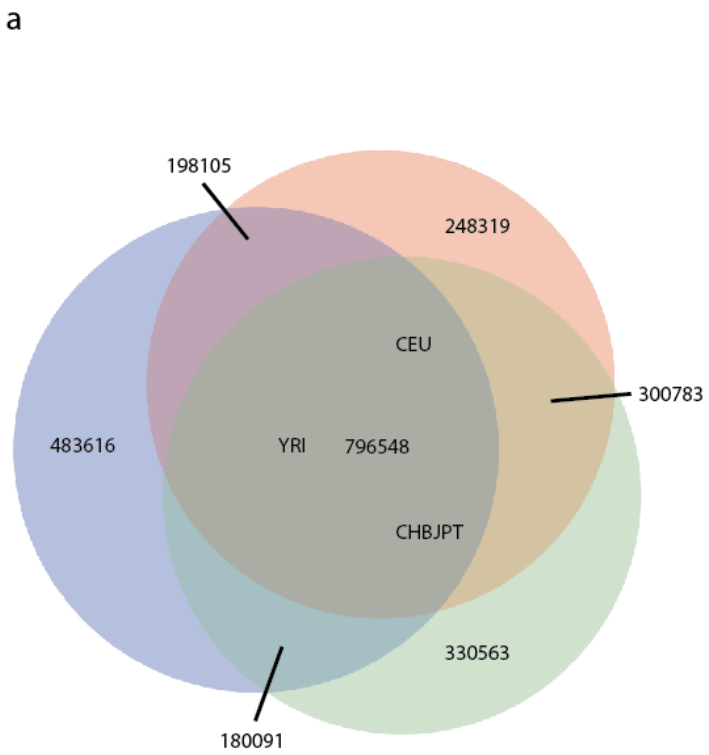
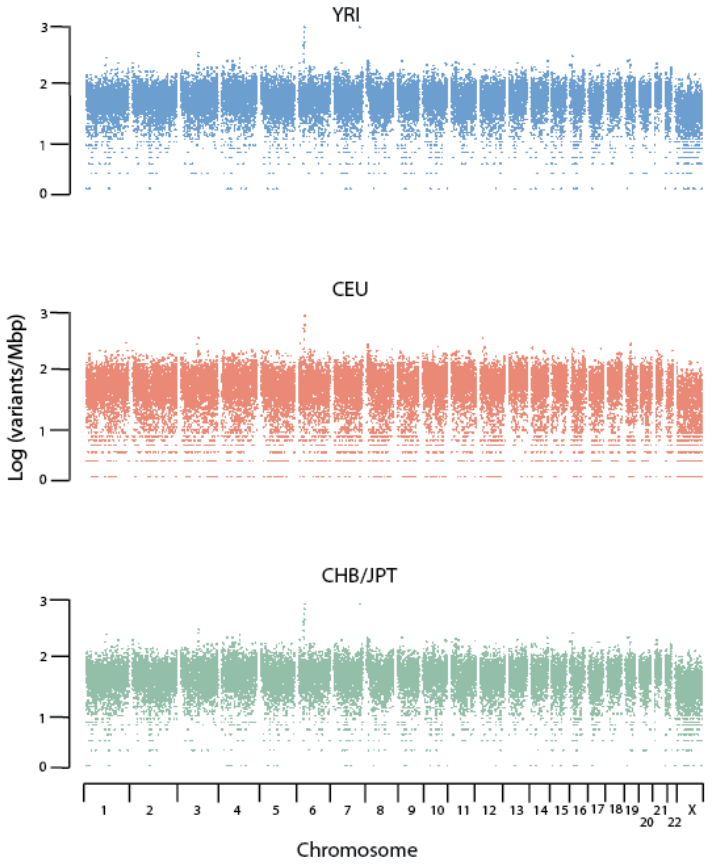
Creating synthetic reference sequences





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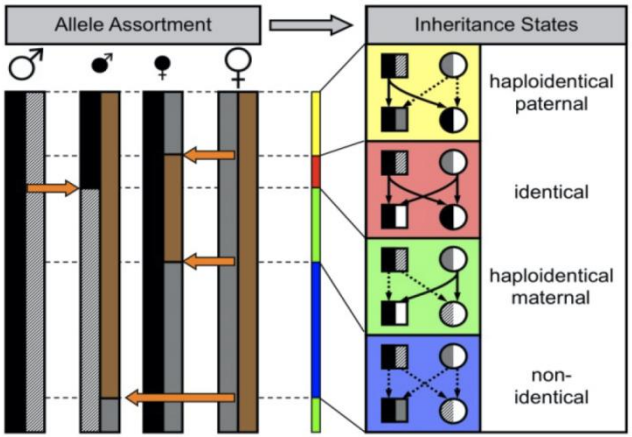
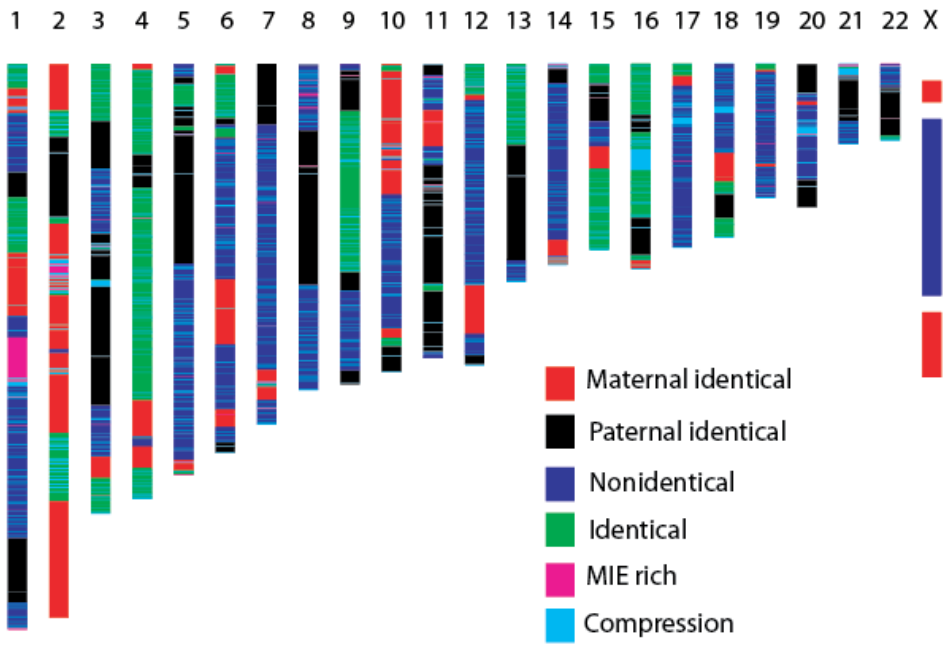
Family based care: inheritance state analysis





Science **328**, 636 (2010)
PLoS Genet 7(9): e1002280

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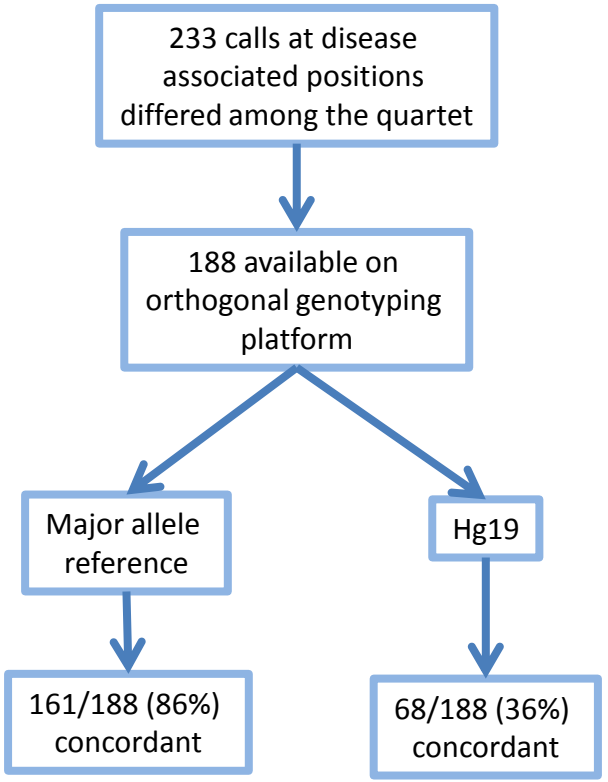
Towards a medical grade genome - reducing error

- HMM is a powerful tool
- For novel reference, greatest where it matters most
  - Some improvement in alignment
  - Significant improvement in MIE
  - Significant improvement in variant calling
- New approaches
  - NHLBI Exome upgrade
  - Null
  - indels
  - Alternate allele aware assembly
    - Lookup vs ‘aware’



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Inheritance of potential risk alleles for thrombophilia

Chromosome	Gene	rsid	Affected family members	Disease	Inheritance	Onset-earliest	Onset-median	Severity	Actionability	Lifetime risk	Variant pathogenicity
12	*VWF*	rs61750615	M, S, D	Von Willebrand disease	Incomplete dominant	1	1	5	5	variable	7
10	*HABP2*	rs7080536	M, S, D	Carotid stenosis, thrombophilia	AD	4	4	1	5	variable	7
19	*SLC7A9*	rs79389353	M, D	Cysteinuria – kidney stones	AR	1	1	3	5	7	7
1	*F5*	rs6025	F, D	Thrombophilia	Incomplete dominant	4	4	4	5	2	7
1	*MTHFR*	rs1801133	F, D	Hyperhomocystein-emia	AR	1	1	1	6	2	7

Key: Father, mother, son, daughter = F, M, S, D. Abbreviations: AD, autosomal dominant; AR, autosomal recessive. Variants were scored according to disease phenotype features and variant pathogenicty as outlined in Table S4.

doi:10.1371/journal.pgen.1002280.t002

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PLoS GENETICS

Phased Whole-Genome Genetic Risk in a Family Quartet Using a Major Allele Reference Sequence

Frederick E. Dewey[1], Rong Chen[2], Sergio P. Cordero[3], Kelly E. Ormond[4,5], Colleen Caleshu[1], Konrad J. Karczewski[3,4], Michelle Whirl-Carrillo[4], Matthew T. Wheeler[1], Joel T. Dudley[2,3], Jake K. Byrnes[4], Omar E. Cornejo[4], Joshua W. Knowles[1], Mark Woon[4], Katrin Sangkuhl[4], Li Gong[4], Caroline F. Thorn[4], Joan M. Hebert[4], Emidio Capriotti[4], Sean P. David[4], Aleksandra Pavlovic[1], Anne West[6], Joseph V. Thakuria[7], Madeleine P. Ball[8], Alexander W. Zaranek[8], Heidi L. Rehm[9], George M. Church[8], John S. West[10], Carlos D. Bustamante[4], Michael Snyder[4], Russ B. Altman[4,11], Teri E. Klein[4], Atul J. Butte[2], Euan A. Ashley[1]*

SNP Location	Drug(s)	Drug(s) More Likely to Work	Drug(s) Less Likely to Work	Drug(s) More Likely to Cause Side Effect	Drug(s) Less Likely to Cause Side Effect	Drug Dose(s) Above Average	Drug Dose(s) Below Average	Drug Dose(s) Average	No PGx Action/ Phenotype Unknown	Confidence Level
rs9934438	warfarin	·	·	·	·	·	·	■○■○	·	High
rs1954787	citalopram	■○○	■	·	·	·	·	·	·	High
rs776746	cyclosporine	·	·	·	·	·	■○■○	·	·	High
rs1800460	thiopurines	·	·	·	·	·	·	·	·	High
rs2108622	warfarin	·	·	·	·	·	■○■○	·	·	Medium
rs4680	morphine	·	·	·	·	■○■○	·	·	·	Medium
rs5443	statins	■○	■○	·	·	·	·	·	·	Medium
rs4253778	beta blocking agents	○	■○■	·	·	·	·	·	·	Medium
rs622342	metformin	○■	■○	·	·	·	·	·	·	Medium
rs7569963	citalopram	·	·	■	■	·	·	·	○○	Medium
rs8012552	ACE inhibitors	·	·	·	■○■○	·	·	·	·	Low
rs11209716	ACE inhibitors	·	·	·	■■○	·	·	·	○	Low
Key: Father, Mother, Brother, Sister =	■○■○									

*All family members are homozygous for the most common CYP2C9 allele (CYP2C9*1) associated with normal warfarin pharmacokinetics, and heterozygous for the allele at VKORC1- 1639 (rs9923231) associated with therapeutic prolongation of the international normalized ratio at low doses of warfarin. We used this variant data and clinical data to predict the father's exact empirically-determined dose of warfarin (5 mg) using the International Warfarin Pharmacogenetics Consortium dosing algorithm .*

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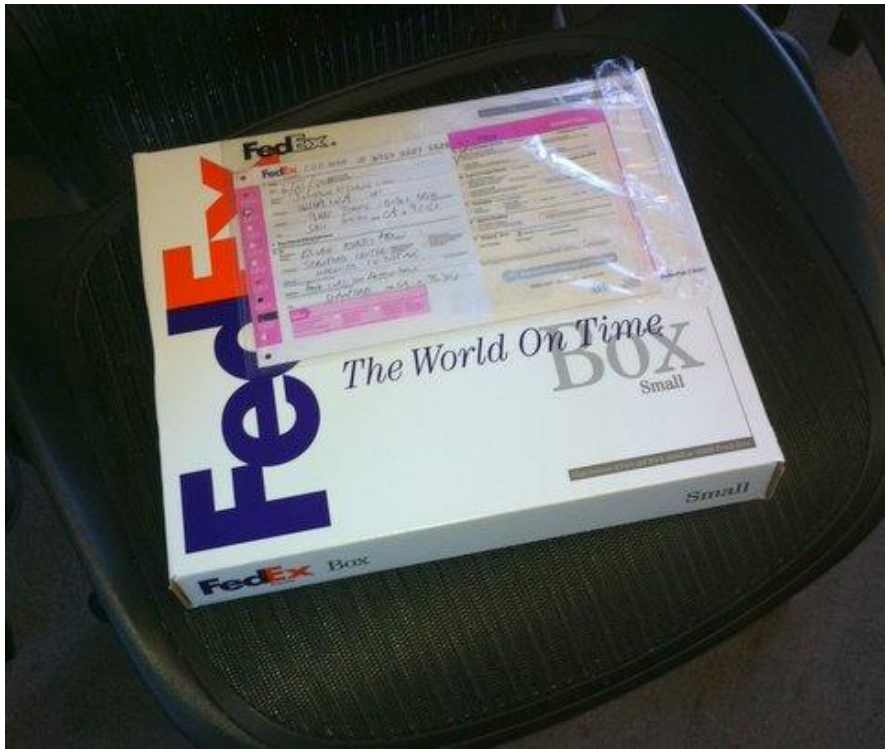
The genome has arrived



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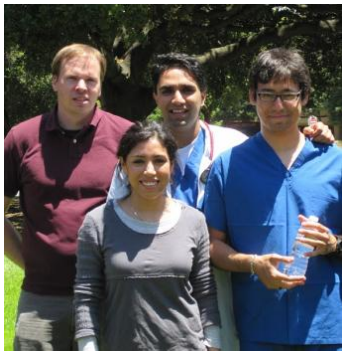

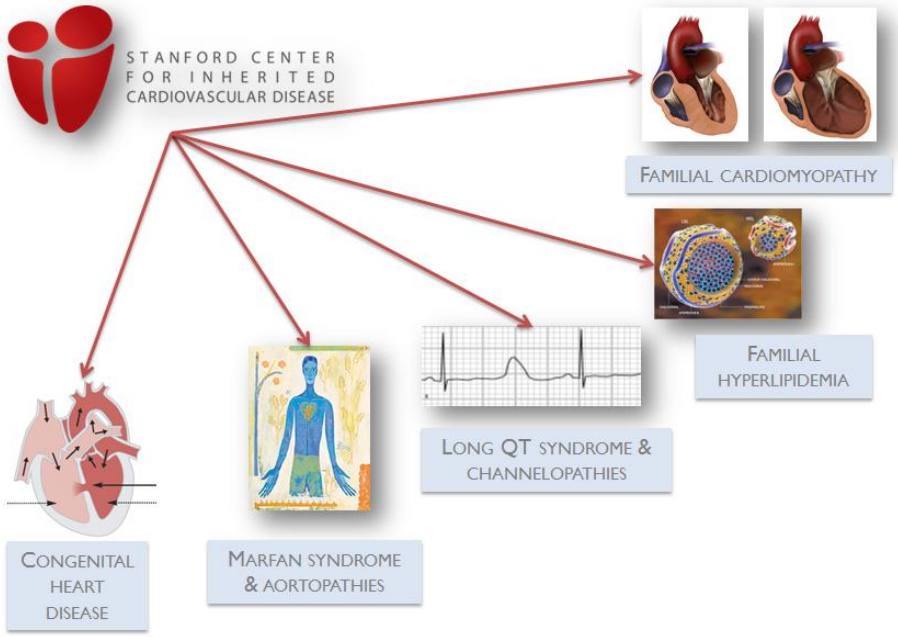

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NIH UL1RR029890
NIH HL094274 (T32)
Breetwor Foundation
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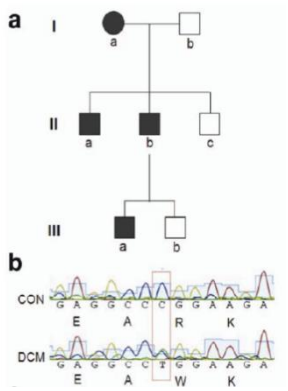
Conclusion

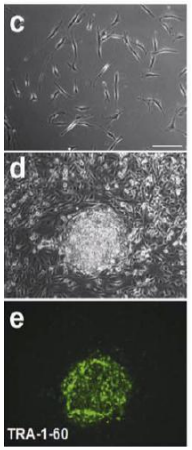
- In the future, we will not be limited by the availability of genetic information
- We need to decide how to use this information for the improvement of our patients lives

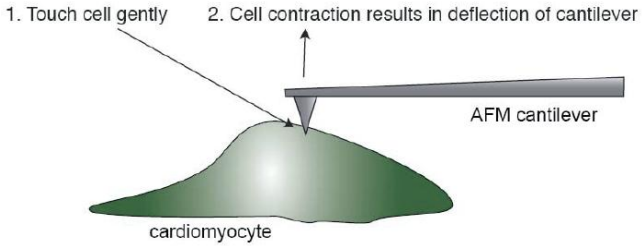
Patient-Specific Induced Pluripotent Stem Cells as a Model for Familial Dilated Cardiomyopathy

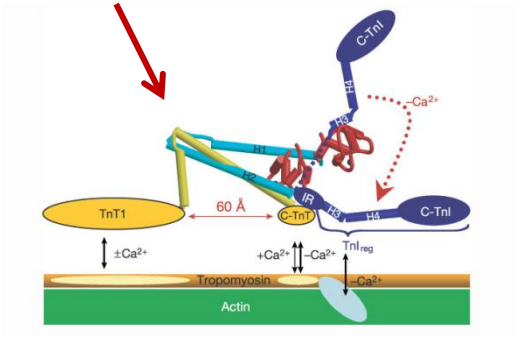
Ning Sun,[1,2,3]* Masayuki Yazawa,[4]* Jianwei Liu,[5] Leng Han,[1,2] Veronica Sanchez-Freire,[1,2] Oscar J. Abilez,[6] Enrique G. Navarrete,[2] Shijun Hu,[1,2] Li Wang,[1,2,3] Andrew Lee,[1,2,3] Aleksandra Pavlovic,[1] Shin Lin,[1] Rui Chen,[7] Roger J. Hajjar,[8] Michael P. Snyder,[7] Ricardo E. Dolmetsch,[4] Manish J. Butte,[5] Euan A. Ashley,[1] Michael T. Longaker,[3,9] Robert C. Robbins,[10] Joseph C. Wu[1,2,3,10†]

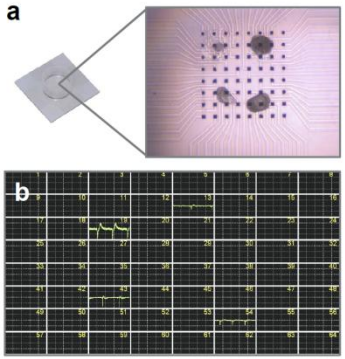


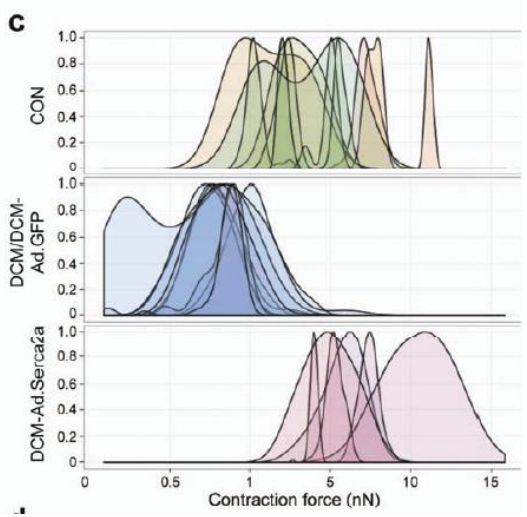
Troponin T (R173W)











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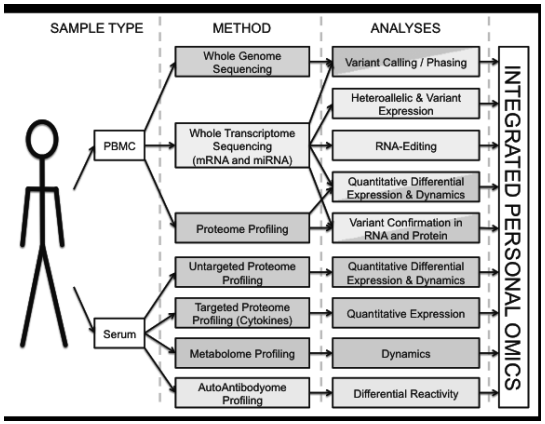
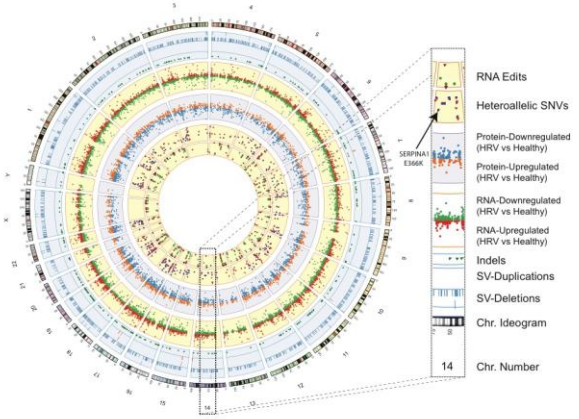
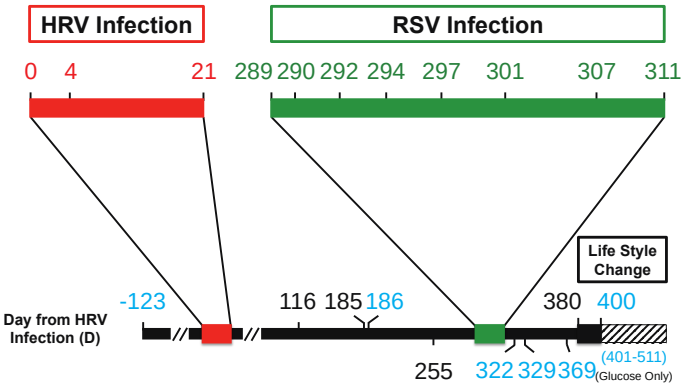
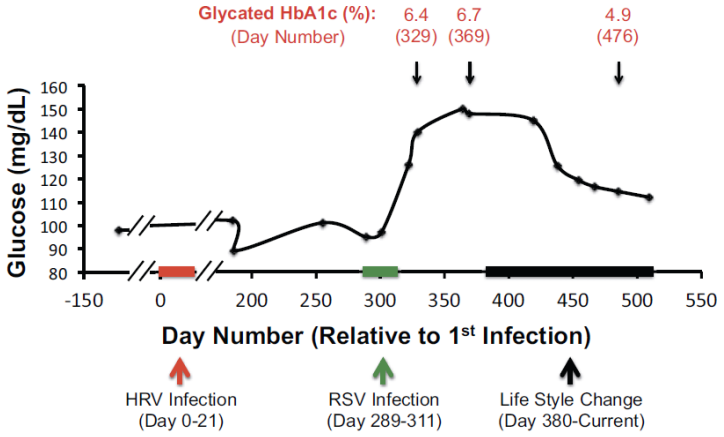
Personal Omics Profiling Reveals Dynamic Molecular and Medical Phenotypes

Rui Chen,[1,11] George I. Mias,[1,11] Jennifer Li-Pook-Than,[1,11] Lihua Jiang,[1,11] Hugo Y.K. Lam,[1,12] Rong Chen,[2,12] Elana Miriami,[1] Konrad J. Karczewski,[1] Manoj Hariharan,[1] Frederick E. Dewey,[3] Yong Cheng,[1] Michael J. Clark,[1] Hogune Im,[1] Lukas Habegger,[6,7] Suganthi Balasubramanian,[6,7] Maeve O'Huallachain,[1] Joel T. Dudley,[2] Sara Hillenmeyer,[1] Rajini Haraksingh,[1] Donald Sharon,[1] Ghia Euskirchen,[1] Phil Lacroute,[1] Keith Bettinger,[1] Alan P. Boyle,[1] Maya Kasowski,[1] Fabian Grubert,[1] Scott Seki,[2] Marco Garcia,[2] Michelle Whirl-Carrillo,[1] Mercedes Gallardo,[9,10] Maria A. Blasco,[9] Peter L. Greenberg,[4] Phyllis Snyder,[1] Teri E. Klein,[1] Russ B. Altman,[1,5] Atul J. Butte,[2] Euan A. Ashley,[3] Mark Gerstein,[6,7,8] Kari C. Nadeau,[2] Hua Tang,[1] and Michael Snyder[1,*]









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