# Patient zero

and the new world of genomic medicine

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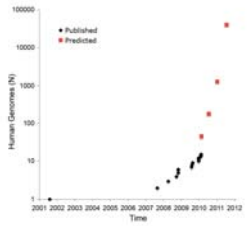
## The question

- 10 years since draft HGP
- 2 years since the "Year of the GWAS"
- Very little impact on clinical medicine
- But, sequencing is getting cheaper
- The number of genomes is set to rise
- What does a consultation look like in 5 years?

| Year | Cost estimate | Technology |
|---|---|---|
| 2001 | $300,000,000 | Sanger (ABI) |
| 2001 | $100,000,000 | Sanger (ABI) |
| 2007 | $10,000,000 | Sanger (ABI) |
| 2008 | $2,000,000 | Roche (454) |
| 2008 | $1,000,000 | Illumina |
| 2008 | $500,000 | Illumina |
| 2008 | $250,000 | Illumina |
| 2009 | $48,000 | Helicos |

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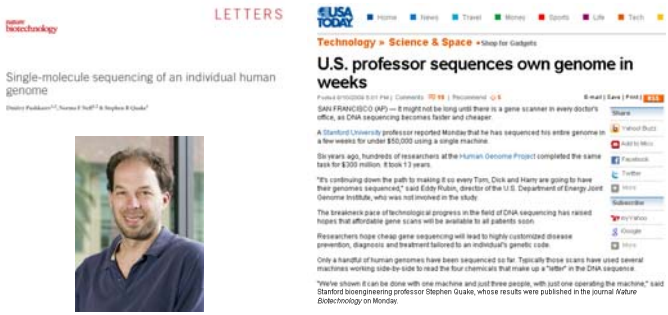
## The idea

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

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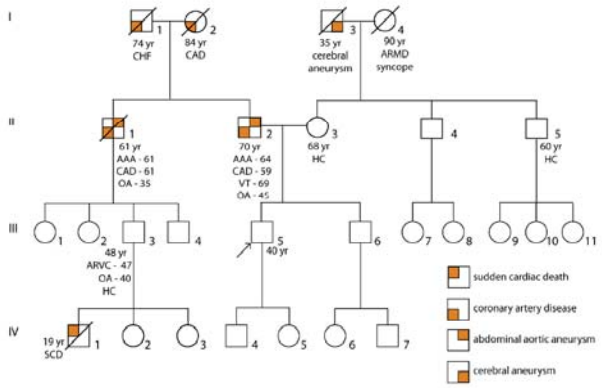
## Patient zero

- 40 year old male in good health presents to his doctor with his whole genome
- No symptoms
- Exercises regularly
- Takes no medication
- Family history of aortic aneurysm
- Family history of sudden death

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## Clinical examination

- Normal appearing male
- Comfortable at rest
- HS 1,2+0
- No murmurs, rubs or gallops
- Chest clear, abdomen nad
- Musculoskeletal, neuropsych examinations grossly normal
- Afebrile
- HR 60pm, BP 128/80

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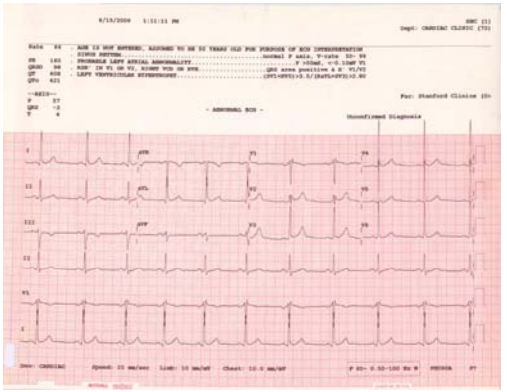
## Electrocardiogram



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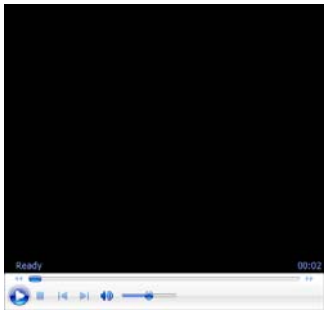
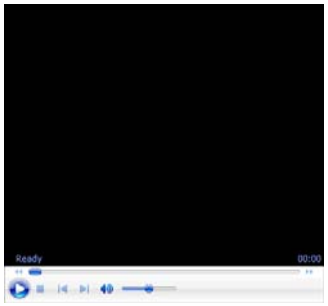
## Echocardiography




## Exercise test

| | | | | |
|---|---|---|---|---|
| Test Date: | 09/04/2009 | | | |
| Name (First Last) | | | MRN | 11581501 |
| Referring MD | Esau Ashley | | Diagnosis | Health care maintenance |
| Age (years) | 40 | | Weight (lbs): 190 | Height (in): 71 |
| Exercise mode | Bike | | Protocol | Ramp (Actual exercise time: 16 mins 9 secs) |
| | Rest | Max | | |
| Heart rate (bpm) | 76 | 191 | % pred HR | 106 |
| $VO_2$ (ml/kg/min) | 3.5 | 49.6 | % pred $VO_2$ max | 145 |
| Systolic blood pressure (mmHg) | 100 | 192 | | |
| $O_2$ saturation (%) | 99 | 99 | | |
| Respiratory exchange ratio | 0.93 | 1.13 | RER > 1.05 indicates adequate exercise challenge | |
| Perceived exertion | -- | 17 | Scale of 6 - 20 (13 - somewhat hard, 15 - hard, 17 - very hard) | |
| Ve/VCO2 slope | 26 | | <30 normal<br>> 34 is an adverse prognostic marker in the heart failure population | |
| Ventilatory threshold (%) | 42 | | "Anaerobic Threshold - generally > 40% of $VO_{2max}$ is normal | |
| Heart rate recovery at 1 minute (bpm) | 41 | | < 12 beat drop at 1 minute is abnormal (general, clinically-referred population)<br>< 6 beat drop at 1 minute is abnormal (heart failure) | |
| External work equivalent | 450Watts | | | |
| Breathing reserve | | | < 20% suggests possible pulmonary limitation to exercise | |
| ECG findings | Rhythm: Sinus<br>Arrhythmias*: None A 1.5 mm upsloping ST depression was seen in one region | | | |
| Notes | * Some artifact noted during exercise. 1.5mm upsloping ST depression noted in leads V4-V6, resolved immediately in recovery<br>Estimated cardiac output available separately. | | | |

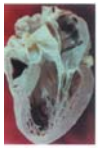
Musculature not to scale

## Lab tests panel

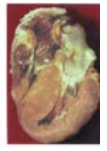
| | | | | |
|---|---|---|---|---|
| WBC | 4.9 | | Total bili | 0.5 |
| Hb | 15.7 | | AST | 25 |
| Platelets | 147 | | ALT | 33 |
| Na | 143 | | ALP | 93 |
| K | 4.0 | | Alb | 4.2 |
| BUN | 20 | | | |
| Cr | 1.2 | | Cholesterol | 218 |
| eGFR | | | LDL | 156 |
| Ca | 9.4 | | HDL | 48 |
| Fasting glucose | 93 | | TG | 68 |
| | | | hsCRP | <0.2 |
| | | | Lp(a) | 114 |

## Parsing 6,000,000,000 data points

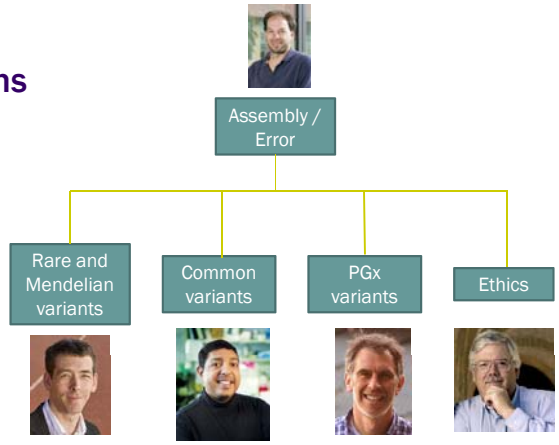
When one base pair change can turn this



into this



## The Teams

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## 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

Lancet 2010; 375: 1525-35



Technology Review
npr
BBC
WebMD
USA TODAY
THE WALL STREET JOURNAL
NewScientist

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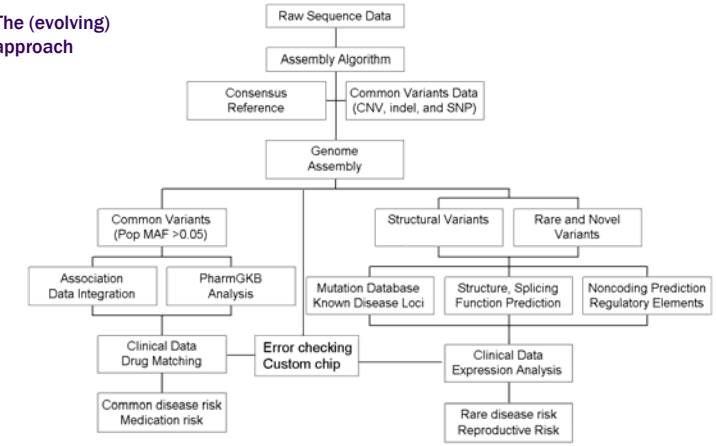
## The (evolving) approach

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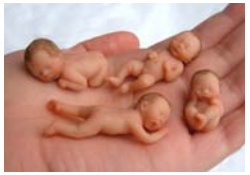

## Rare, novel and Mendelian variants

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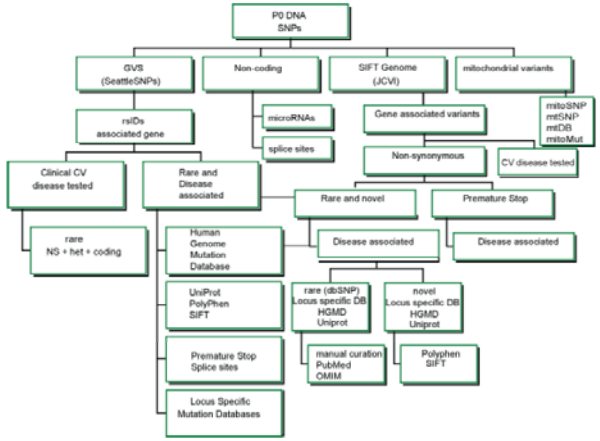
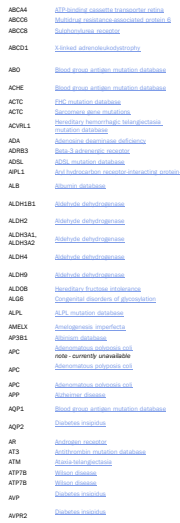
## Rare/novel algorithm

- What does it mean for a variant to be associated with disease?
  - Cosegregation in a large kindred?
  - Early stop in key gene
    - in one proband? in several individuals?
  - Splice site mutation?
  - Novel mutation?
  - Not seen in ?how many ?controls
    - What's a control?


## Rare variant databases

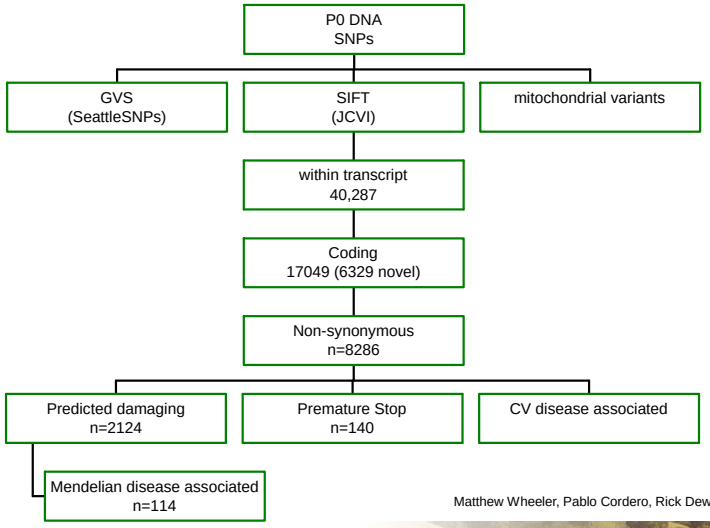
- Human Genome Mutation database
  - Public/professional
- Human Variome project
- Human genome variation database
- Private databases



| | | |
|---|---|---|
| ABCA4 | ATP-binding cassette transporter retina | Retina International |
| ABCC6 | Multidrug resistance-associated protein 6 | Retina International |
| ABCC8 | Sulphonylurea receptor | Hôpital Necker-Enfants Malades (Paris), France |
| ABCD1 | X-linked adrenoleukodystrophy | Academic Medical Center, Amsterdam, Holland and Kennedy Krieger Institute, Baltimore MD, USA |
| ABO | Blood group antigen mutation database | Albert Einstein College of Medicine, New York, USA |
| ACHE | Blood group antigen mutation database | Albert Einstein College of Medicine, New York, USA |
| ACTC | FHC mutation database | Australian National Genomic Information Service |
| ACTC | Sarcomere gene mutations | Harvard University, USA |
| ACVRL1 | Hereditary hemorrhagic telangiectasia mutation database | Heriot-Watt University, Edinburgh, UK |
| ADA | Adenosine deaminase deficiency | University of Tampere, Finland |
| ADRB3 | Beta-3 adrenergic receptor | Tel-Aviv University, Israel |
| ADSL | ADSL mutation database | University of Louvain Medical School, Belgium |
| AIPL1 | Aryl hydrocarbon receptor-interacting protein-like 1 | Retina International |
| ALB | Albumin database | Mary Imogene Bassett Hospital Research Institute, New York, USA |
| ALDH1B1 | Aldehyde dehydrogenase | University of Colorado Health Sciences Centre, USA |
| ALDH2 | Aldehyde dehydrogenase | University of Colorado Health Sciences Centre, USA |
| ALDH3A1, ALDH3A2 | Aldehyde dehydrogenase | University of Colorado Health Sciences Centre, USA |
| ALDH4 | Aldehyde dehydrogenase | University of Colorado Health Sciences Centre, USA |
| ALDH9 | Aldehyde dehydrogenase | University of Colorado Health Sciences Centre, USA |
| ALDOB | Hereditary fructose intolerance | Boston University, USA |
| ALG6 | Congenital disorders of glycosylation | Leuven University, Belgium |
| ALPL | ALPL mutation database | University of Versailles-Saint Quentin en Yvelines, France |
| AMELX | Amelogenesis imperfecta | University of North Carolina, USA |
| AP3B1 | Albinism database | University of Minnesota, USA |
| APC | Adenomatous polyposis coli<br>*note - currently unavailable* | Mayo Clinic, USA |
| APC | Adenomatous polyposis coli | Institut Curie (Paris), France |
| APC | Adenomatous polyposis coli | Tel-Aviv University, Israel |
| APP | Alzheimer disease | Antwerp University, Belgium |
| AQP1 | Blood group antigen mutation database | Albert Einstein College of Medicine, New York, USA |
| AQP2 | Diabetes insipidus | McGill University (Quebec), Canada |
| AR | Androgen receptor | McGill University (Quebec), Canada |
| AT3 | Antithrombin mutation database | Imperial College School of Medicine, London, UK |
| ATM | Ataxia-telangiectasia | Virginia Mason Research Center (Seattle), USA |
| ATP7B | Wilson disease | University of Alberta, Canada |
| ATP7B | Wilson disease | Tel-Aviv University, Israel |
| AVP | Diabetes insipidus | McGill University (Quebec), Canada |
| AVPR2 | Diabetes insipidus | McGill University (Quebec), Canada |

## Private mutation databases

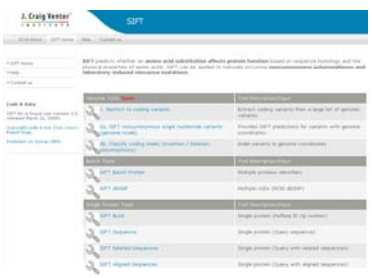
http://www.hgmd.cf.ac.uk/docs/oth_mut.html
Accessed 3/11/2010




Matthew Wheeler, Pablo Cordero, Rick Dewey



## Algorithms for entirely novel variants

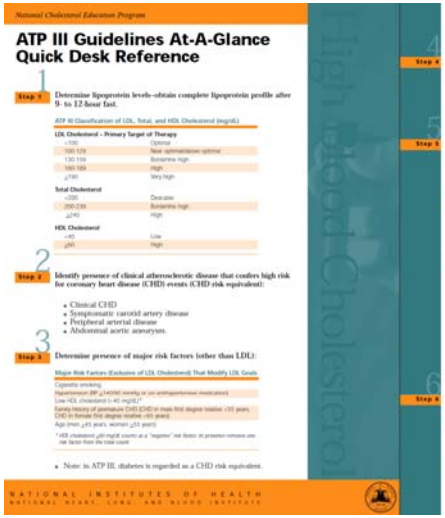


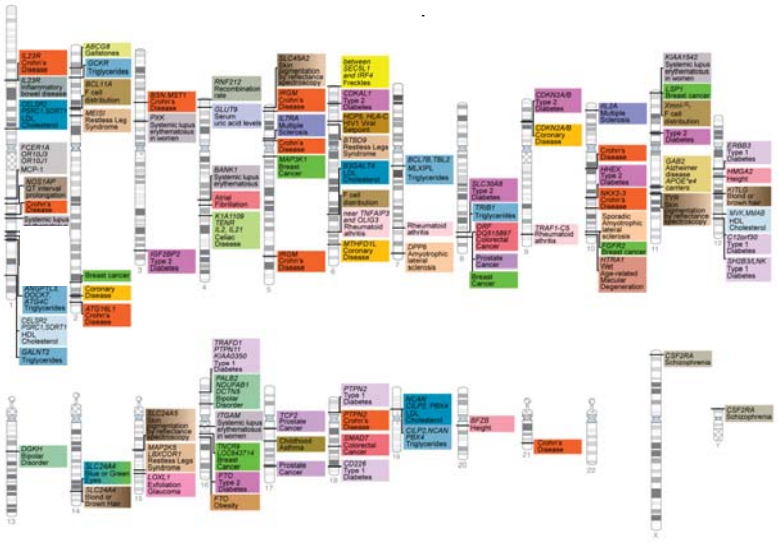




## Polygenic disease – what we have now

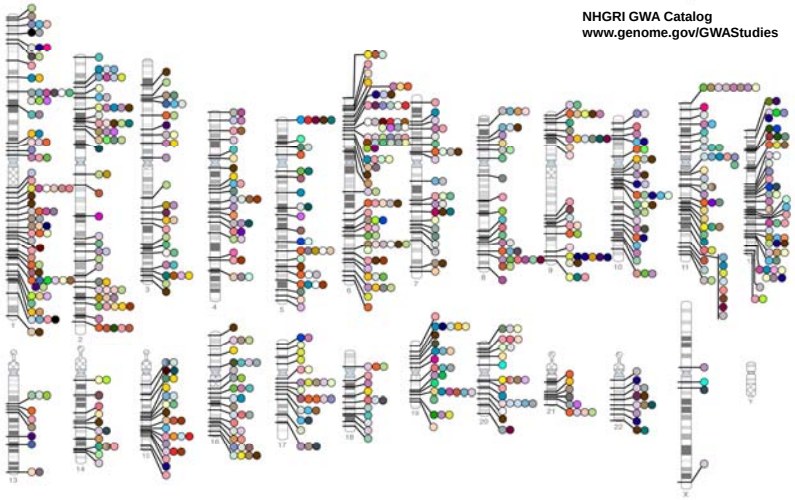






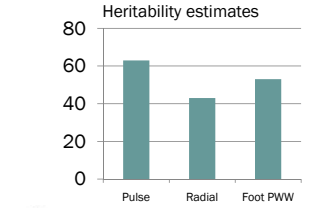
**Published Genome-Wide Associations through 3/2010,**
**779 published GWA at p≤5x10$^{-8}$ for 148 traits**

**NHGRI GWA Catalog**
**www.genome.gov/GWAStudies**

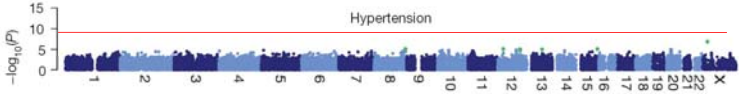


- Acute lymphoblastic leukemia
- Adhesion molecules
- Adiponectin levels
- Age-related macular degeneration
- AIDS progression
- Alcohol dependence
- Alzheimer disease
- Amyotrophic lateral sclerosis
- Angiotensin-converting enzyme activity
- Ankylosing spondylitis
- Arterial stiffness
- Asthma
- Atherosclerosis in HIV
- Atrial fibrillation
- Attention deficit hyperactivity disorder
- Autism
- Basal cell cancer
- Bipolar disorder
- Bilirubin
- Bladder cancer
- Blond or brown hair
- Blood pressure
- Blue or green eyes
- BMI, waist circumference
- Bone density
- Breast cancer
- C-reactive protein
- Cardiac structure/function
- Carnitine levels
- Carotenoid/tocopherol levels
- Celiac disease
- Chronic lymphocytic leukemia
- Cleft lip/palate
- Cognitive function
- Colorectal cancer
- Coronary disease
- Creutzfeldt-Jakob disease
- Crohn's disease
- Cutaneous nevi
- Dermatitis
- Drug-induced liver injury
- Eosinophil count
- Eosinophilic esophagitis
- Erythrocyte parameters
- Esophageal cancer
- Essential tremor
- Exfoliation glaucoma
- F cell distribution
- Fibrinogen levels
- Folate pathway vitamins
- Freckles and burning
- Gallstones
- Glioma
- Glycemic traits
- Hair color
- Hair morphology
- HDL cholesterol
- Heart rate
- Height
- Hemostasis parameters
- Hepatitis
- Hirschsprung's disease
- HIV-1 control
- Homocysteine levels
- Idiopathic pulmonary fibrosis
- IgE levels
- Inflammatory bowel disease
- Intracranial aneurysm
- Iris color
- Iron status markers
- Ischemic stroke
- Juvenile idiopathic arthritis
- Kidney stones
- LDL cholesterol
- Leprosy
- Leptin receptor levels
- Liver enzymes
- LP (a) levels
- Lung cancer
- Major mood disorders
- Malaria
- Male pattern baldness
- Matrix metalloproteinase levels
- MCP-1
- Melanoma
- Menarche & menopause
- Multiple sclerosis
- Myeloproliferative neoplasms
- Narcolepsy
- Nasopharyngeal cancer
- Neuroblastoma
- Nicotine dependence
- Obesity
- Open personality
- Osteoarthritis
- Osteoporosis
- Otosclerosis
- Other metabolic traits
- Ovarian cancer
- Pain
- Pancreatic cancer
- Panic disorder
- Parkinson's disease
- Periodontitis
- Peripheral arterial disease
- Phosphatidylcholine levels
- Platelet count
- Primary biliary cirrhosis
- PR interval
- Prostate cancer
- Protein levels
- Psoriasis
- Pulmonary funct. COPD
- QRS interval
- QT interval
- Quantitative traits
- Recombination rate
- Red vs.non-red hair
- Renal function
- Response to antipsychotic therapy
- Response to hepatitis C treatment
- Response to statin therapy
- Restless legs syndrome
- Rheumatoid arthritis
- Schizophrenia
- Serum metabolites
- Skin pigmentation
- Speech perception
- Sphingolipid levels
- Statin-induced myopathy
- Stroke
- Systemic lupus erythematosus
- Telomere length
- Testicular germ cell tumor
- Thyroid cancer
- Tooth development
- Total cholesterol
- Triglycerides
- Type 1 diabetes
- Type 2 diabetes
- Ulcerative colitis
- Urate
- Venous thromboembolism
- Vitamin B12 levels
- Warfarin dose
- Weight
- White cell count
- YKL-40 levels

## 2008: the year of the GWAS – time for celebration?








J Hypertens. 2004 Sep;22(9):1717-21.
Am J Hypertens. 2007 Oct;20(10):1065-72

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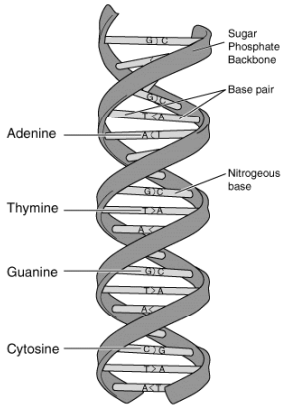
## Missing heritability

- Rare variants
- Structural variants
- Epigenetic phenomena
- Over-zealous bounding of FWER
- G-G interaction
- G-E interaction
- G-G-E interaction






# Can we apply this to individual genomes?

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## One approach


ORIGINAL ARTICLE

Polymorphisms Associated with Cholesterol and Risk of Cardiovascular Events

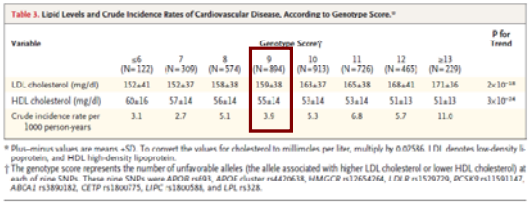
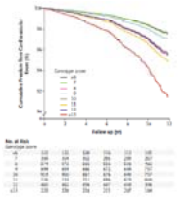


Table 3. Lipid Levels and Crude Incidence Rates of Cardiovascular Disease, According to Genotype Score.*

| Variable | Genotype Score† | | | | | | | | P for Trend |
|---|---|---|---|---|---|---|---|---|---|
| | ≤6 (N=122) | 7 (N=309) | 8 (N=574) | 9 (N=894) | 10 (N=913) | 11 (N=726) | 12 (N=465) | ≥13 (N=229) | |
| LDL cholesterol (mg/dl) | 152±41 | 152±37 | 158±38 | 159±38 | 163±37 | 165±38 | 168±41 | 171±36 | $2\times10^{-18}$ |
| HDL cholesterol (mg/dl) | 60±16 | 57±14 | 56±14 | 55±14 | 53±14 | 53±14 | 51±13 | 51±13 | $3\times10^{-24}$ |
| Crude incidence rate per 1000 person-years | 3.1 | 2.7 | 5.1 | 3.9 | 5.3 | 6.8 | 5.7 | 11.6 | |

* Plus–minus values are means ±SD. To convert the values for cholesterol to millimoles per liter, multiply by 0.02586. LDL denotes low-density lipoprotein, and HDL high-density lipoprotein.
† The genotype score represents the number of unfavorable alleles (the allele associated with higher LDL cholesterol or lower HDL cholesterol) at each of nine SNPs. These nine SNPs were *APOB* rs693, *APOE* cluster rs4420638, *HMGCR* rs12654264, *LDLR* rs1529730, *PCSK9* rs11591147, *ABCA1* rs3890182, *CETP* rs1800775, *LIPC* −514C→T rs1800588, and *LPL* rs328.



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## Challenges in applying results of GWAS to individual genomes

- Theoretical
  - Not enough variance explained
- Practical
  - Most NCBI databases are catalogs
  - Although sharing and making data publicly available (despite ethical concerns) remains routine, journals have not traditionally insisted on sufficient data for genome interpretation (standard is 'reproduce the expt' but even that often not met)
  - Even the GWAS catalogs do not contain sufficient data
    - Genotype frequencies
    - Strand direction variable, rarely reported
    - Chromosomal position changes with each genome build

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## Existing SNP databases are limited in resource and content

- NHGRI GWAS Catalog
  - 2,387 SNPs → 321 diseases, curated from 509 PubMed
  - Odds Ratio, but no genotypes
- NHLBI GWAS Catalog
  - 52,546 SNPs → 87 diseases, curated from 119 PubMed
  - p_value, no OR

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## Stanford genetic variation database


| Field name | Description |
|---|---|
| Broad Phenotype | The general disease or phenotypic condition under study |
| Narrow Phenotype | Detailed description of the studied phenotype |
| Is_it_disease | Diseases or phenotypic trait? |
| MESH heading | MESH heading of the studied disease |
| UMLS CUI | Manually curated UMLS CUI for the disease |
| dbSNP ID | Identifier used in dbSNP build 130, or rsID |
| Significance | Whether the association was reported as significant in the literature |
| Study ID | An internal identifier to distinguish multiple studies in one literature |
| P-value | P-value of the association |
| Model | The genetic model used to calculate the p-value, such as additive, multiplicative, recessive, or dominant |
| Odds Ratio | The odds ratio, relative risk, or hazards ratio of disease association between two comparing genotypes or alleles |
| 95% CI | 95% confidence interval of the odds ratio |
| Comparison | Two genotypes or alleles used to calculate the odds ratio |
| Total sample size | Sum of patients in the case and control groups or the cohort size |
| Cases/Affected | Description of the patients in the case group |
| Controls/Unaffected | Description of the patients in the control group |
| Cohort | Description of the patients in the cohort |
| Gender | The gender of the studied patients |
| Population | The ethic group of the studied patients |
| Major/minor alleles | The major/minor alleles of the SNP |
| Strand direction | The strand direction was determined by comparing the major/minor alleles in the literature with the major/minor alleles in a similar population in the Hapmap project |
| Risk allele | The allele susceptible to diseases |
| Single SNP/haplotype | Was the association studied for single SNP or haplotype? |
| Interaction | Was the association studied for gene-environmental interaction? |
| GWAS | GWAS or candidate gene/SNP study |
| PubMed | PubMed ID of the publication |
| Method | Genotyping technology, such as Taqman or Affymetrix 6.0 |
| Comment | Comments from curators |
| Status | Review status of the entry |

Rong Chen,
Atul Butte

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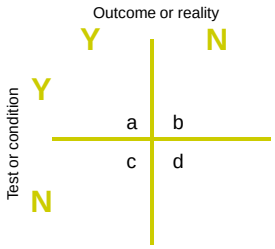
## Ways to apply this for genomic medicine




b= type 1 error
c= type 2 error

| Parameter | expression |
|---|---|
| Sensitivity | a/a+c |
| Specificity | d/d+b |
| Prevalence | a+b+c+d |
| NPV | d/d+c |
| PPV | a/a+b |
| OR | ad/cb |
| OR | (a/b ) / (c/d) |
| RR | (a/a+b ) / (c/c+d) |
| LR+ | sen/1-spec |
| LR- | 1-sen/spec |

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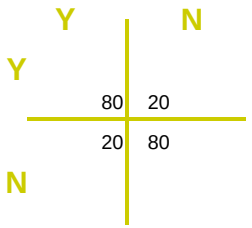
## Odds are....the effect will be exaggerated

- Two groups (n=100), two conditions
- First group Y=80, N=20
- Second group Y=20, N=80
- First group is 4x more likely to be Y
- However, OR=(80/20)/(20/80) = 16
- This can be even more extreme
  - eg (90/10)/(10/90), OR=81!
- Remember that for GWAS, most OR are in the range 1.3-1.6

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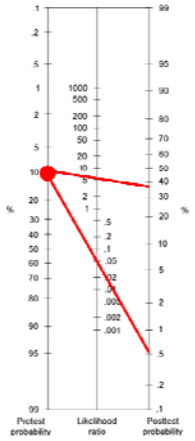
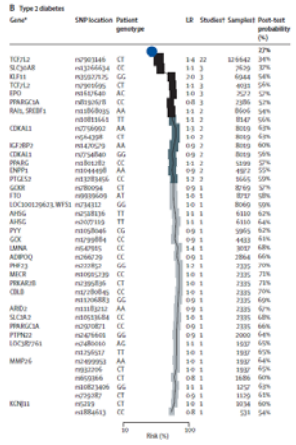
## The Likelihood is . . . you will at least account for test characteristics

- The LR is easily overlaid on the pre-probability to provide a post-test probability
- This helps with the "relative risk" problem

| Parameter | Expression |
|---|---|
| Pre test probability | Prevalence |
| Pre test odds | Prev/1-prev |
| Post test odds | Pre-test odds x LR |
| Post test probability | Post test odds / post test odds +1 |



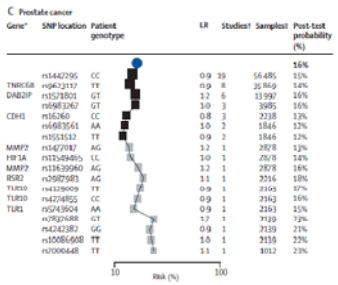



Fagan TJ. Nomogram for Bayes theorem. *N Engl J Med.* 1975 31;293(5): 257.



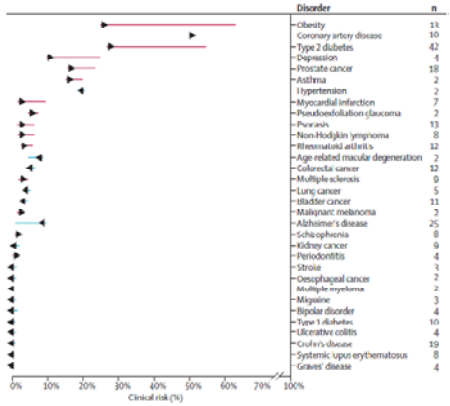
## Riskogram methods and figure

- Pre test prob from various sources
  - Prevalence usually (matched to age, sex, ethnicity if possible)
  - Lifetime risk occasionally
- Mean LR when multiple studies for same SNP
  - Weighted mean (square root of sample size)
- Only one SNP per haplotype block (largest LR)
- Pre test odds multiplied by LRs cumulatively
  - Presented in decreasing order of studies, then sample size



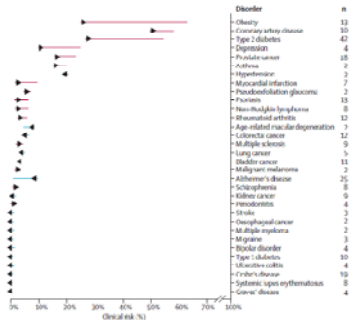


## Report card



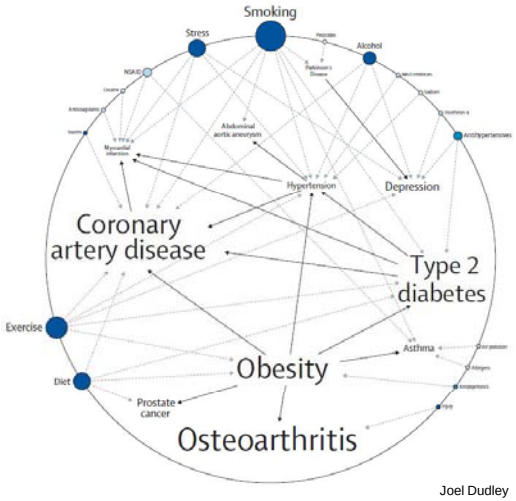
## Challenges

- Calls were made vs human reference sequence
  - Risk alleles in human reference sequence
- Winner's curse
  - Literature bias towards positive results
- Negative studies need to be included in algorithm
- Data for LR only available for 40% papers






## Gene environment interaction




Joel Dudley

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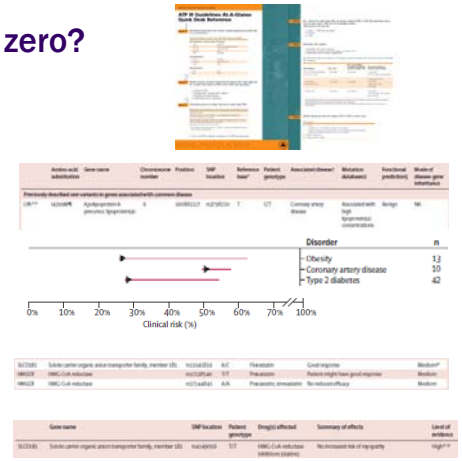
## What of "patient" zero?

- SQ feedback
  - PGx information welcome
  - Approach to personal and family screening
- Medical advice
  - Personal and family screening
  - CAD risk
    - ATP3+LPA+LR+PGx +clinical judgement
    - Rx statin

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## Conclusion

- In the future, we will not be limited by the availability of genetic information
- For medicine to become "personalized" we will need to learn how to parse this data

## Acknowledgements

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 E 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

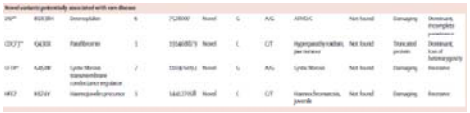
# Practical



## Check for rare variants

- Is it in dbSNP?
- e.g. Chr position 6: 160881127
- http://gvs.gs.washington.edu/GVS
- rs3798220 (LPA)
- Yes

- Is it in dbSNP?
- e.g. 6: 7528007
- No



- Go to Sift http://sift.jcvi.org/
- Choose 2a (nonsynonymous SNP genome scale), build 36
- Enter: 6,7528007,1,G/A
- Tick gene name
- Choose Proceed to Sift results page
- Choose complete set view results

| Coordinates | Codons | Transcript ID | Protein ID | Substitution | Region | dbSNP ID | SNP Type | Prediction | Score | Median Info | # Seqs at position | Gene Name |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 6,7528007,1,G/A | CGT-CaT | ENST00000379802 | ENSP00000369129 | R1838H | EXON CDS | novel | Nonsynonymous | DAMAGING *Warning! Low confidence | 0.05 | 3.37 | 27 | DSP |

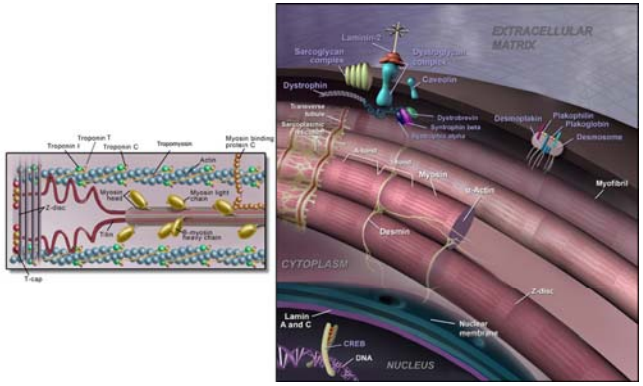
## Same mutation in PolyPhen – polymorphism phenotyping

- http://genetics.bwh.harvard.edu/pph/
- Desmoplakin
- Uniprot ID = P15924
- Position: 1838
- AA1= Arginine (arg, R), polar, +ve charge
- AA2=Histidine (his, H), polar, neutral charge

## Polyphen result


## Private database lookup

- Cardiogenomics
- http://genepath.med.harvard.edu/~seidman/cg3/index.html
- Myosin binding protein C
- R326Q

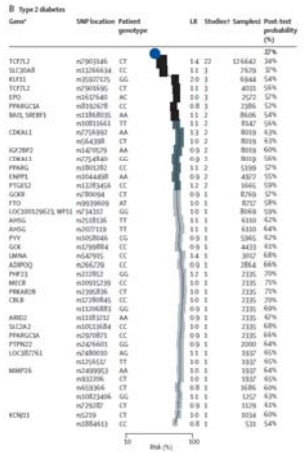




## Published data on this mutation


References and comments

1. Maron BJ, Niimura H, Casey SA, Soper MK, Wright GB, Seidman JG, Seidman CE. Development of left ventricular hypertrophy in adults in hypertrophic cardiomyopathy caused by cardiac myosin-binding protein C gene mutations. J Am Coll Cardiol 2001 Aug;38(2):315-21. (PubMed:11499718)
2. Niimura H, Patton KK, McKenna WJ, Soults J, Maron BJ, Seidman JG, Seidman CE. Sarcomere protein gene mutations in hypertrophic cardiomyopathy of the elderly. Circulation 2002 Jan 29;105(4):446-51. (PubMed:11815426)
   - *Equivocal; R326Q was also found in 1 of 170 normal control samples*
3. Jaaskelainen P, Kuusisto J, Miettinen R, Kankkainen P, Kankkainen S, Heikkinen S, Peltola P, Pihlajamaki J, Vauhkonen I, Laakso M. Mutations in the cardiac myosin-binding protein C gene are the predominant cause of familial hypertrophic cardiomyopathy in eastern Finland. J Mol Med 2002 Jul;80(7):412-22. Epub 2002 Apr 11. (PubMed:12110947)
   - *R326Q was found in 7 of 111 normal control individuals, and in one unaffected sibling of a proband (LOD = -0.21 at 0%).*
4. Richard P, Charron P, Carrier L, Ledeuil C, Cheav T, Pichereau C, Benaiche A, Isnard R, Dubourg O, Burban M, Gueffet JP, Millaire A, Desnos M, Schwartz K, Hainque B, Komajda M. Hypertrophic cardiomyopathy: distribution of disease genes, spectrum of mutations, and implications for a molecular diagnosis strategy. Circulation 2003 May 6;107(17):2227-32. Epub 2003 Apr 21. (PubMed:12707239)
5. Morner S, Richard P, Kazzam E, Hellman U, Hainque B, Schwartz K, Waldenstrom A. Identification of the genotypes causing hypertrophic cardiomyopathy in northern Sweden. J Mol Cell Cardiol 2003 Jul;35(7):841-9. (PubMed:12818675)
   - *R326Q found in two affected brothers but not in 200 normal chromosomes*
6. Merk, Seidman et al., 2004 (this study)
7. Van Driest SL, Vasile VC, Ommen SR, Will ML, Tajik AJ, Gersh BJ, Ackerman MJ. Myosin binding protein C mutations and compound heterozygosity in hypertrophic cardiomyopathy. J Am Coll Cardiol 2004 Nov 2;44(9):1903-10. (PubMed:15519027)
   - *Called a polymorphism based on published reports.*
8. Ingles J, Doolan A, Chiu C, Seidman J, Seidman C, Semsarian C. Compound and double mutations in patients with hypertrophic cardiomyopathy: implications for genetic testing and counselling. J Med Genet 2005 Oct;42(10):e59. (PubMed:16199542)
   - *compound heterozygote, with MYBPC3 Gln1233ter*

## Likelihood ratios and the riskogram





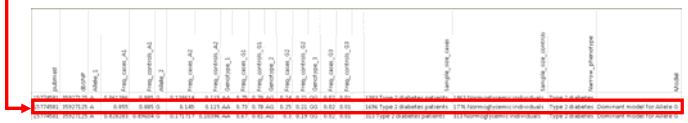
## Likelihood ratio example – KLF11

Table 2. Frequency of Gln62Arg *KLF11* in additional population samples

| Sample | NN | NM | MM | n | Minor allele frequency | OR (95% CI), $\chi^2$ *P* value: Allele frequency | Dominant model |
|---|---|---|---|---|---|---|---|
| Initial familial study | | | | | | | |
| Normoglycemic | 252 | 58 | 3 | 313 | 0.102 | 1.85 (1.33–2.57) | 2.00 (1.39–2.87) |
| T2DM subjects | 211 | 95 | 7 | 313 | 0.174 | 0.00025 | 0.00019 |
| Second set | | | | | | | |
| Normoglycemic | 1,138 | 307 | 18 | 1,463 | 0.112 | 1.18 (1.01–1.38) | 1.18 (1.00–1.40) |
| T2DM subjects | 1,032 | 326 | 25 | 1,383 | 0.136 | 0.034 | 0.057 |
| Overall | | | | | | | |
| Normoglycemic | 1,390 | 365 | 21 | 1,776 | 0.115 | 1.29 (1.12–1.49) | 1.32 (1.13–1.54) |
| T2DM subjects | 1,243 | 421 | 32 | 1,696 | 0.143 | 0.00033* | 0.00054* |

NN, homozygous wild-type allele carriers; NM, heterozygous allele carriers; MM, homozygous mutant allele carriers; OR, odds ratio.
*P value and combined OR for allelic effects calculated by a Mantel–Haenszel test (25).

Role of transcription factor KLF11 and its diabetes-associated gene variants in pancreatic beta cell function. PNAS 102 (13): 4807-4812





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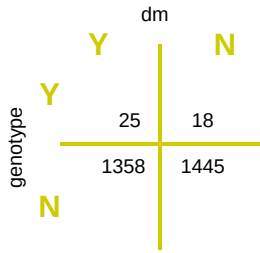


LR = sens / 1-spec
LR=(a/a+c) / (1 – (d/d+b))
LR=(25/25+1358) / (1-(1445/1445+18))
LR=0.02 / (1 - 0.99)
LR=0.02 / 0.01
LR=2