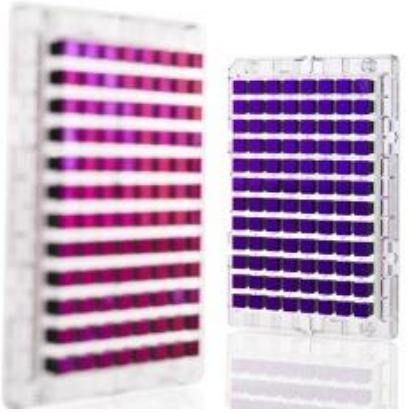
# Genome-wide association studies (GWAS)



# Further reading (not required)

- Nature reviews genetics:
http://www.nature.com/nrg/series/gwas/index.html
  - McCarthy et al. Genome-wide association studies for complex traits: consensus, uncertainty and challenges
  - Marchini and Howie. Genotype imputation for genome-wide association studies
  - Ott. Family-based designs for genome-wide association studies.
  - Gibson. Rare and common variants: twenty arguments.

# Outline for GWAS

- Review / Overview
- Design
- Analysis
  - QC
  - Prostate cancer example
  - Imputation
  - Replication & Meta-analysis
- Advanced analysis
  - Limitations & “missing heritability”
  - Gene/pathway tests
  - Polygenic models

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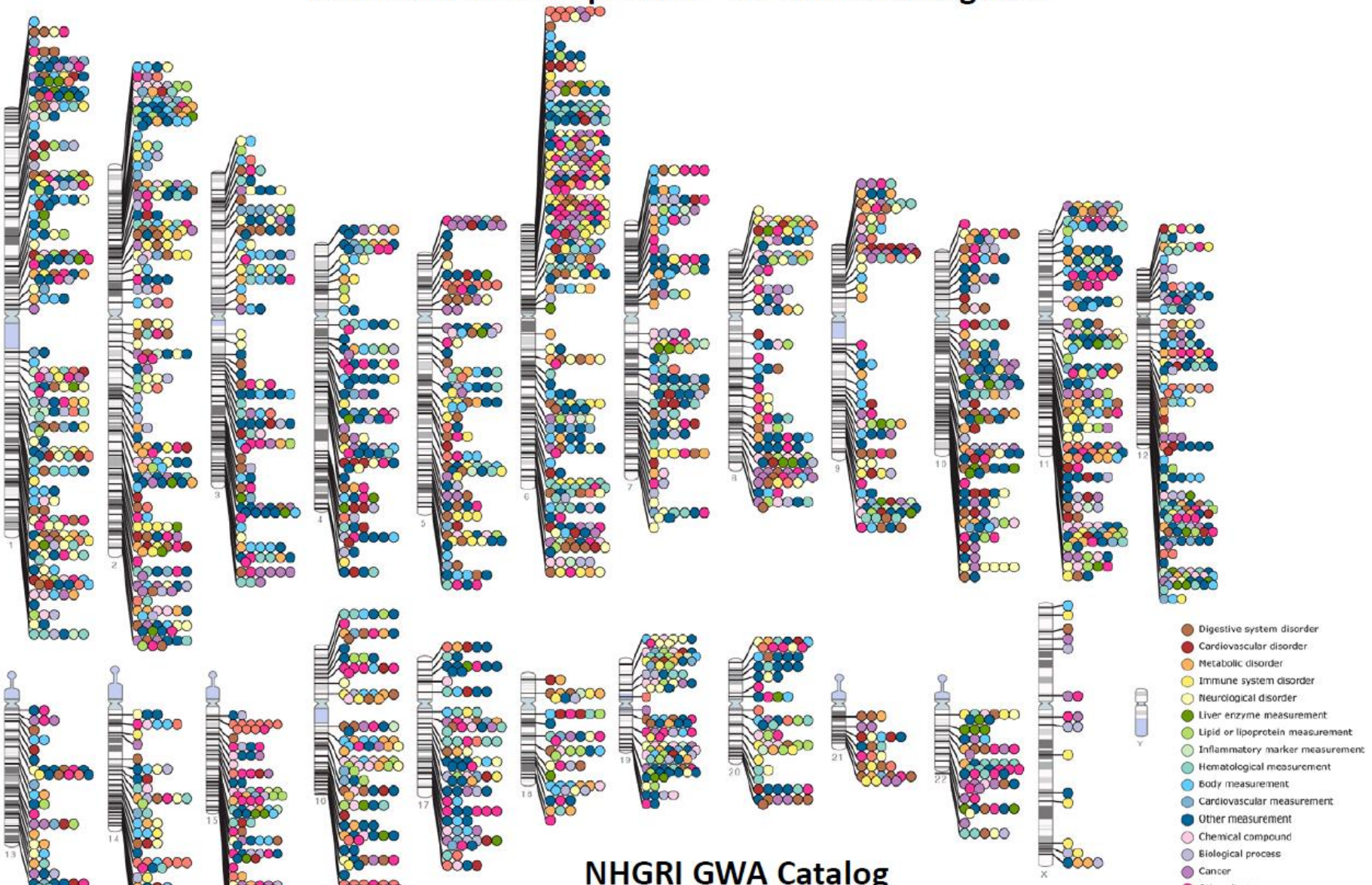
# Published Genome-Wide Associations through 07/2012

## Published GWA at $p \leq 5X10^{-8}$ for 18 trait categories




**NHGRI GWA Catalog**

**www.genome.gov/GWAStudies**

**www.ebi.ac.uk/fgpt/gwas/**

National Human Genome Research Institute

EMBL-EBI

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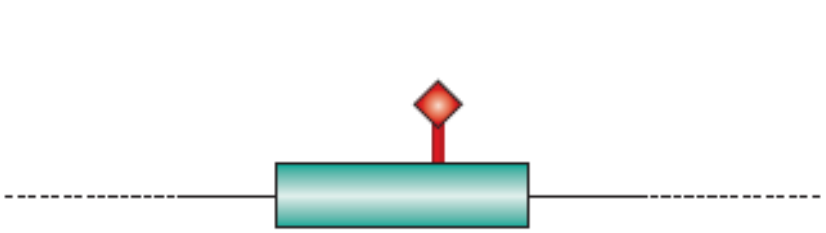
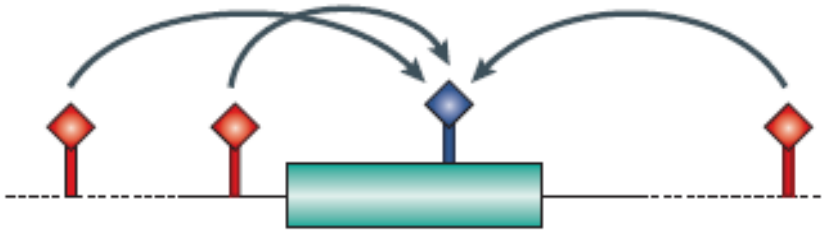
# Design 1: Microarray – Genotype a subset of markers available



Direct association

Disease locus directly typed



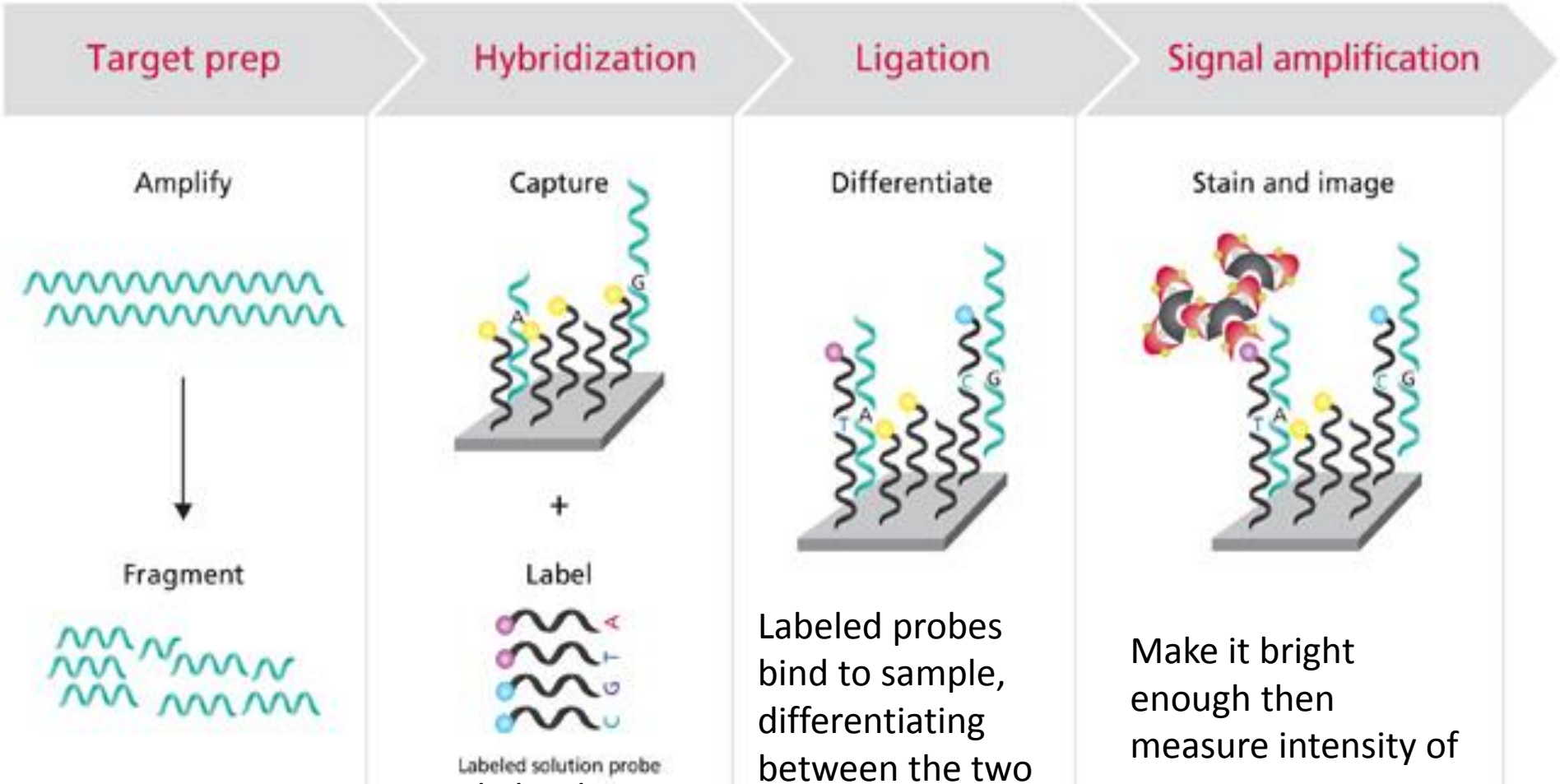
Indirect association (guilt by association)

Marker correlated with typed disease locus

- Previously we talked about using this to "tag" candidate genes (only Genotype a subset of the SNPs).
- Same principal applies to tagging the whole genome.

Hirschhorn & Daly, Nat Rev Genet 2005

# Technology behind a GWAS Microarray




Sample binds to array

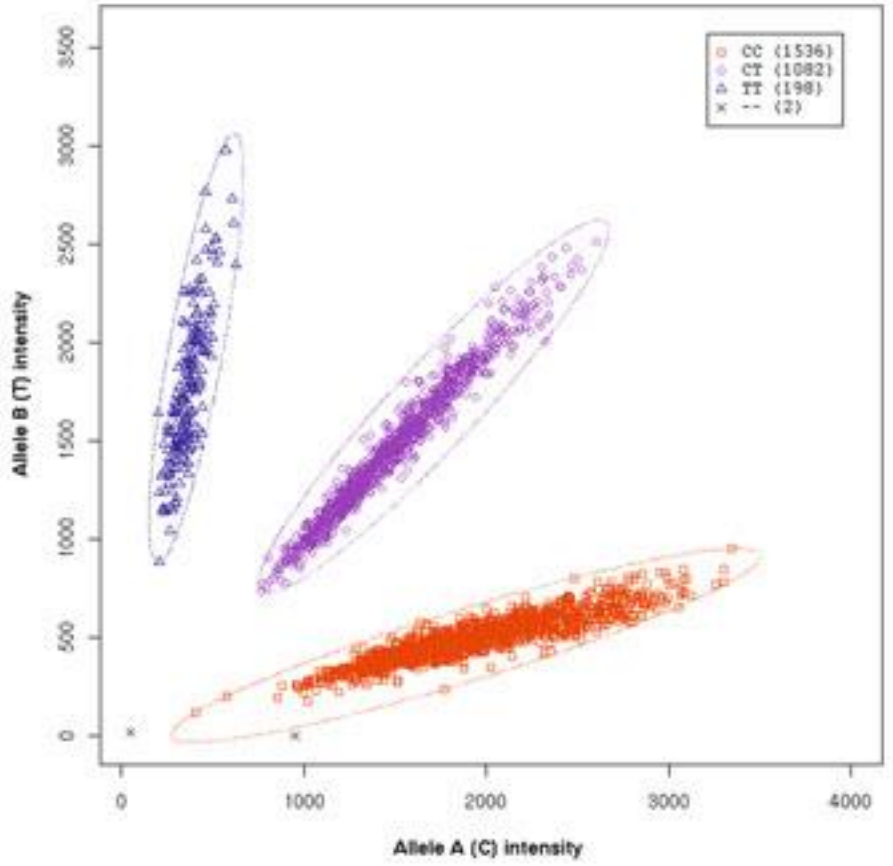
Labeled probes bind to sample, differentiating between the two alleles

Make it bright enough then measure intensity of array

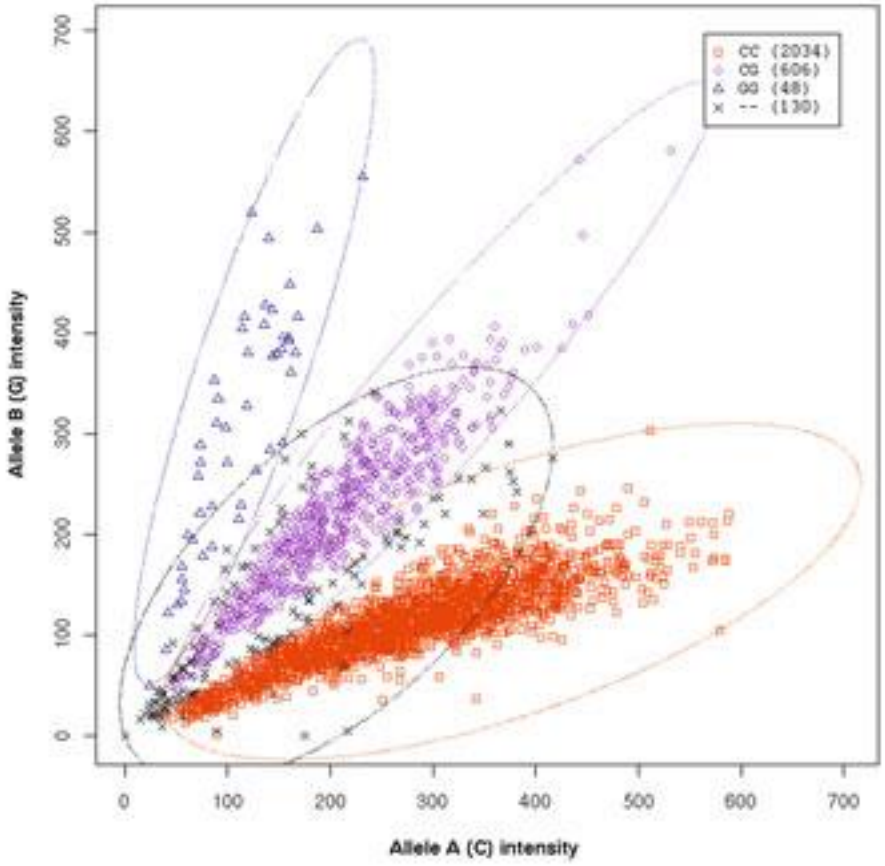
Assay ~ 0.7 - 5M SNPs (keeps increasing)

Affymetrix, http://www.affymetrix.com

# Raw microarray data: Genotype calls




Good calls!




Bad calls!

# **Class Exercise!**: How would you design a *general* GWAS microarray?

- It will be linked to an EHR, all diseases of interest
- Do you have to genotype *everything*?
- Regions/SNPs to prioritize?

Hoffmann et al., Next generation genome-wide association tool. Design and coverage of a high-throughput European-optimized SNP array. Genomics, 2012.
Hoffmann et al., Design and coverage of high throughput genotyping arrays optimized for individuals of East Asian, African American, and Latino race/ethnicity using imputation and a novel hybrid SNP selection algorithm. Genomics, 2012.

# **Class Exercise!**: How would you design a *general* GWAS microarray?

- It will be linked to an EHR, all diseases of interest
- Do you have to genotype *everything*? [tag SNPs (population?), minor allele frequency limit, old multistage design]
- Regions/SNPs to prioritize? [genes?, prev hits, functional significance?; some SNPs cost more; some SNPs work better]
- [Similar to candidate genes before, but grander scale]

Hoffmann et al., Next generation genome-wide association tool. Design and coverage of a high-throughput European-optimized SNP array. Genomics, 2012.
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# **Class Exercise!**: How would you design a *general* GWAS microarray?



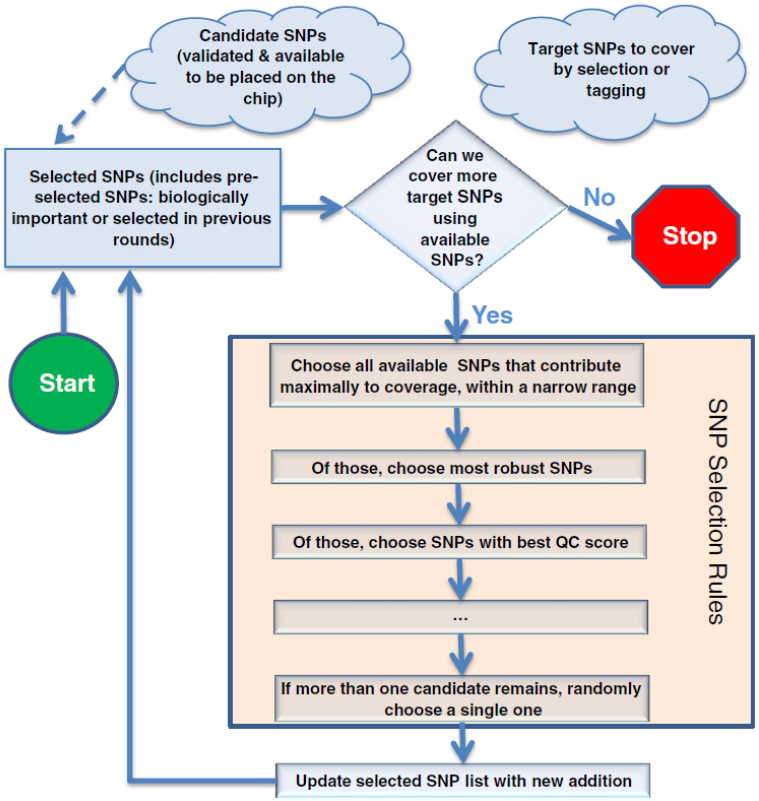
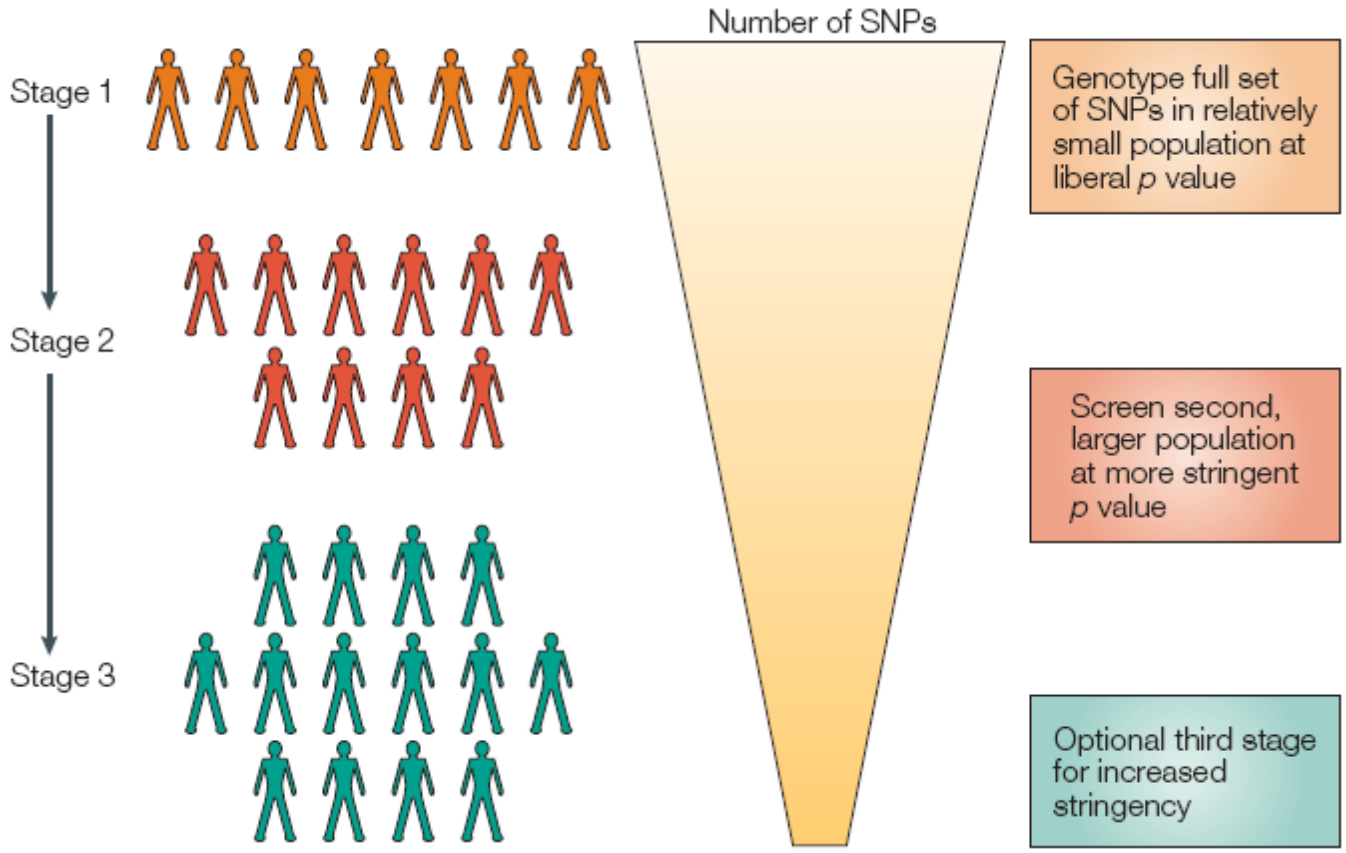


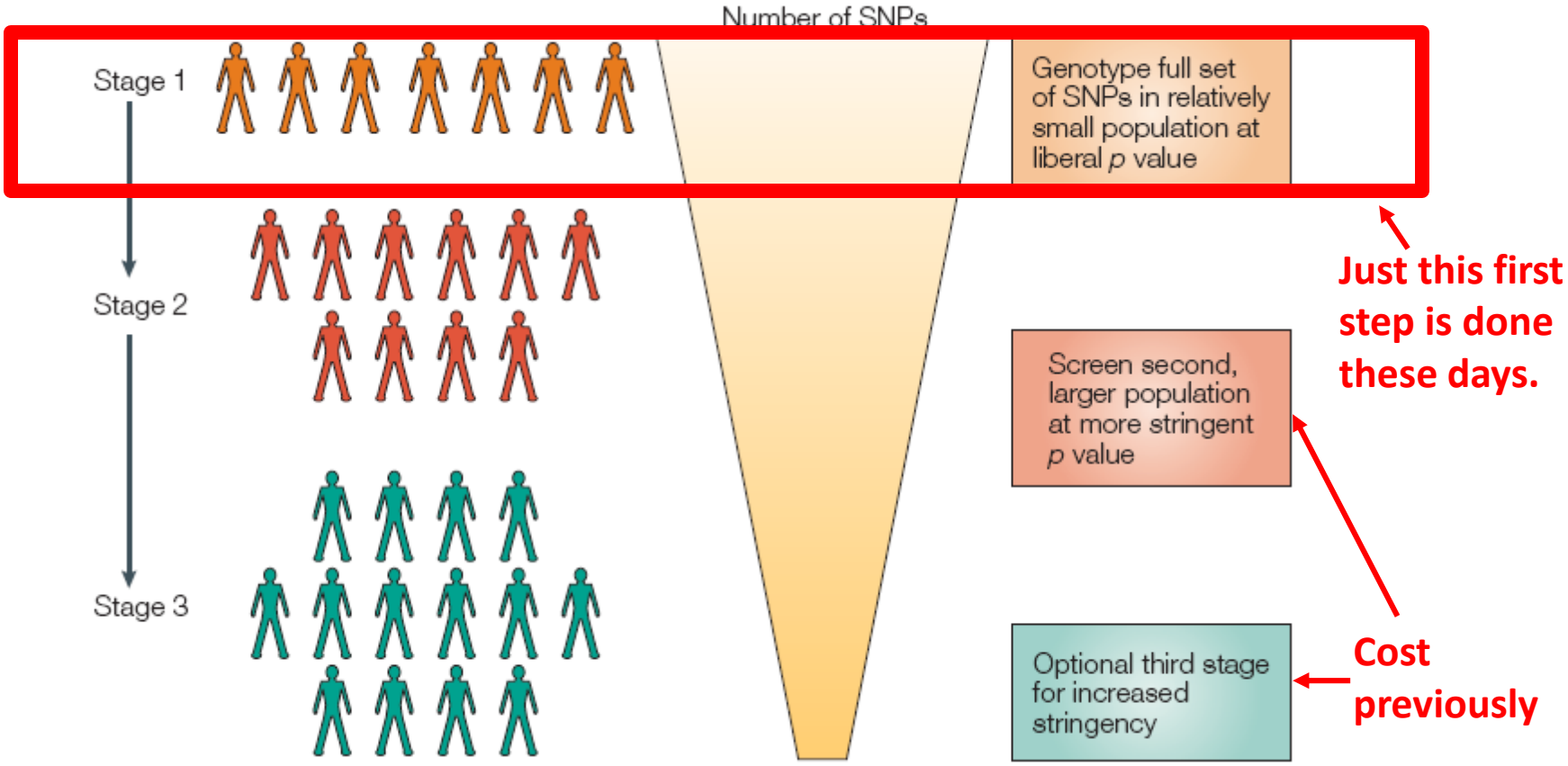

**Fig. 7.** Greedy SNP selection algorithm. A set of SNPs are chosen for reasons of biological importance, significance in published GWAS, etc., or as the result of previous rounds of greedy SNP selection. The "target" set of SNPs to be covered by tagging is established to fit the purpose of the current round of SNP selection, e.g., maximize coverage of SNPs in coding regions, or maximize general coverage of the genome. Then, SNPs which are available to be placed on the microarray are assessed for their ability to increase coverage of the target set. If coverage can be increased, a set of decision rules is applied to select the best single SNP to add to the selected list, as described in the text. This process continues until maximum coverage of the target set is achieved, or no space for additional SNPs on the microarray remains.

Hoffmann et al., Next generation genome-wide association tool. Design and coverage of a high-throughput European-optimized SNP array. Genomics, 2012.
Hoffmann et al., Design and coverage of high throughput genotyping arrays optimized for individuals of East Asian, African American, and Latino race/ethnicity using imputation and a novel hybrid SNP selection algorithm. Genomics, 2012.

# Genome-wide Assocation Studies (GWAS)



# Genome-wide Assocation Studies (GWAS)



# Design 2: Next generation sequencing

- Genotype essentially “all” SNPs in genome
  - A bunch of random reads all over the genome are assembled to produce the genome
  - Some regions of the genome have more reads than others; can choose how much you want to sequence (5x coverage 1000 Genomes, 60-80x Complete Genomics, …)
- More expensive
- Extreme phenotypes, since can't do everyone?
- More in “Next Generation Sequencing” lecture

# Design choices

- **GWAS Microarray**
  - Only assay SNPs designed into array (0.7-5 million)
  - Cheaper (so more subjects tradeoff)

**GWAS Sequencing**

"De novo" discovery (particularly good for rare variants)

More expensive (but costs are falling) (many less subjects)

Need much more expansive IT support

Lots of interesting interpretation problems (field rapidly evolving)

Can also do a hybrid design, more later in the lecture.

# Design choices

- **Exome Microarray**
  - Only assay SNPs designed into array (~300K+custom); in **exons only** and that could affect protein coding function
  - Cheapest (so many more subjects)

**Exome Sequencing**

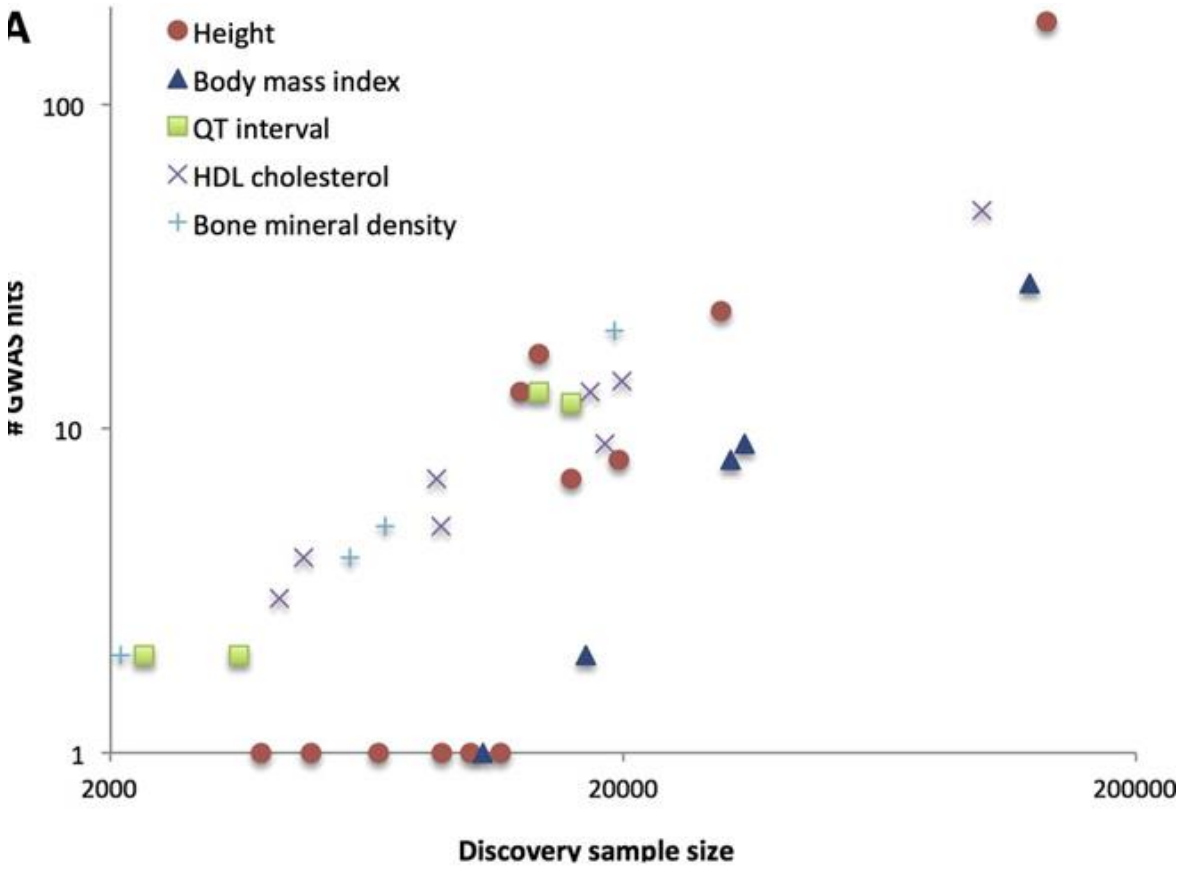
"De novo" discovery (particularly good for rare variants); **%age of exons only** (first step is a pull down that does not capture all exons)

More expensive than microarrays, less expensive than gwas sequencing

Need more expansive IT support, but not as much as whole genome

# Size matters

- Large # SNPs tested – multiple comparisons
- Very small effect sizes
- Tag SNP, rather than actual SNP
- Consortiums




Visscher, AJHG 2012

# Biggest studies...

- GWAS Microarray:
  - US: 100K people in the Kaiser RPGEH (Hoffmann et al., Genomics, 2011a&b)
  - UK: 500K people in process (150K current)
  - MVP: 1M people in process (400K soon)
- Sequencing: 1000 Genomes Project, UK10K
- Exome Sequencing: GO ESP (12,031 subjects, for exome microarray design)

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# QC Steps: Class exercise!

- Can you think about potential QC steps you might take at an individual and SNP level basis? Which one first?
- What tests have we talked about in this course?
- Special chromosomes?
- (Could families be problematic? useful?)

# QC Steps

- "garbage rises to the top"
- Filter SNPs and Individuals
  - MAF (e.g., 1%, but very sample size dependent!)
  - Low call rates (means genotype probably didn't work correctly)

•Test for HWE among controls & within ethnic groups. Use conservative alpha-level ($10^{-6}$, e.g., but opinions vary) (again, to remove artifacts)

•Check for relatedness.

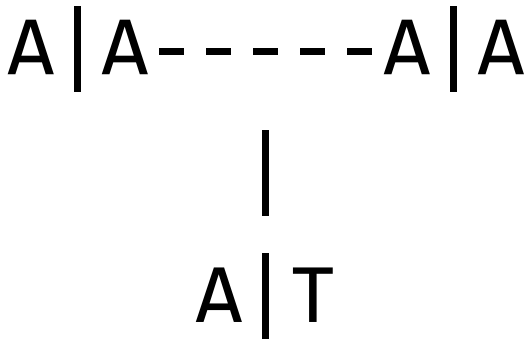
  - MZ twins, or accidentally genotyped same sample twice?
  - Remove first degree, or account for.

•Check genotype gender (mislabeled samples)

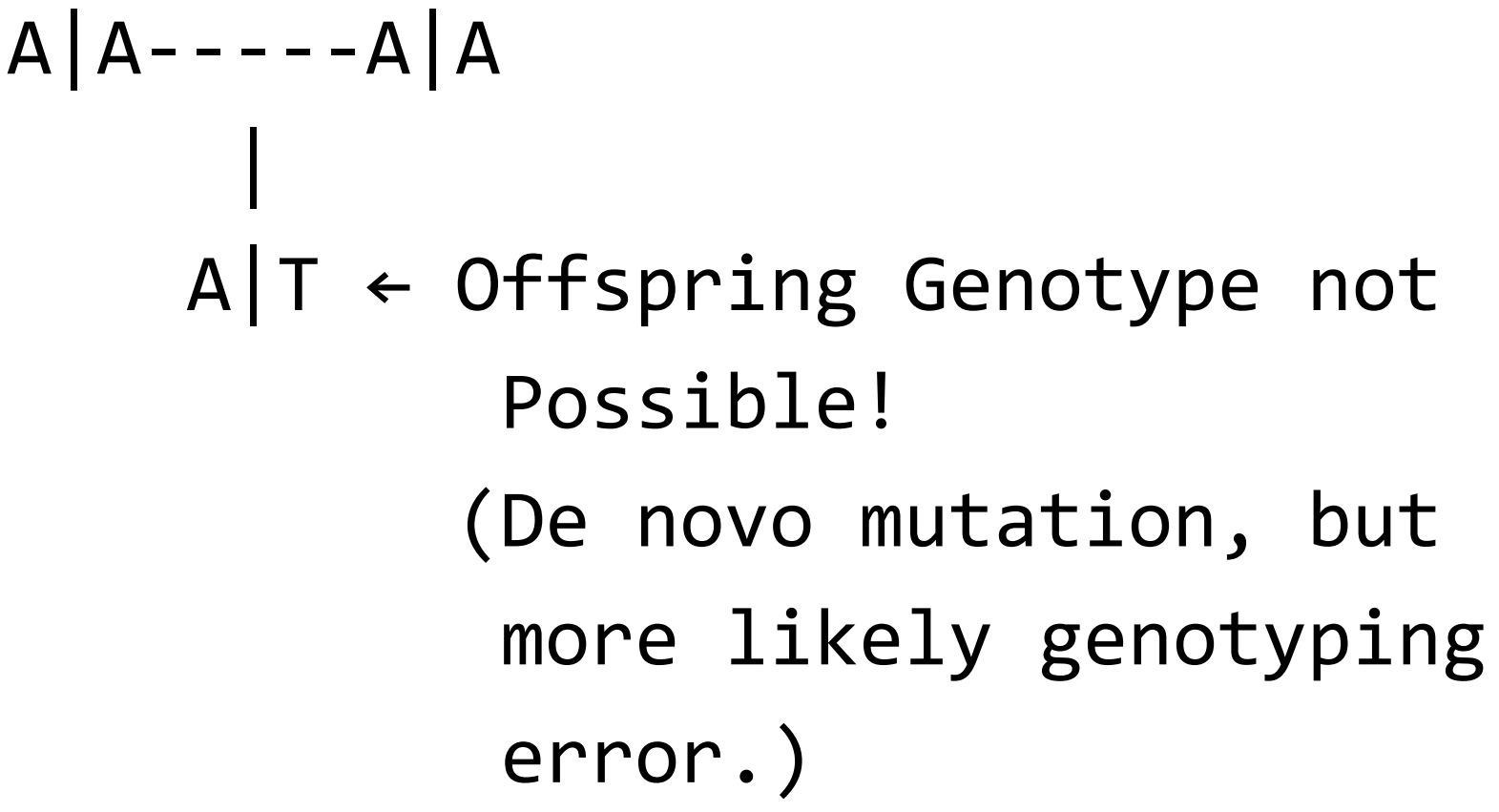
•Filter Mendelian inhertance (family-based, or potentially cryptics, if large enough sample)

•plink sofware: pngu.mgh.harvard.edu/~purcell/plink/reference.shtml

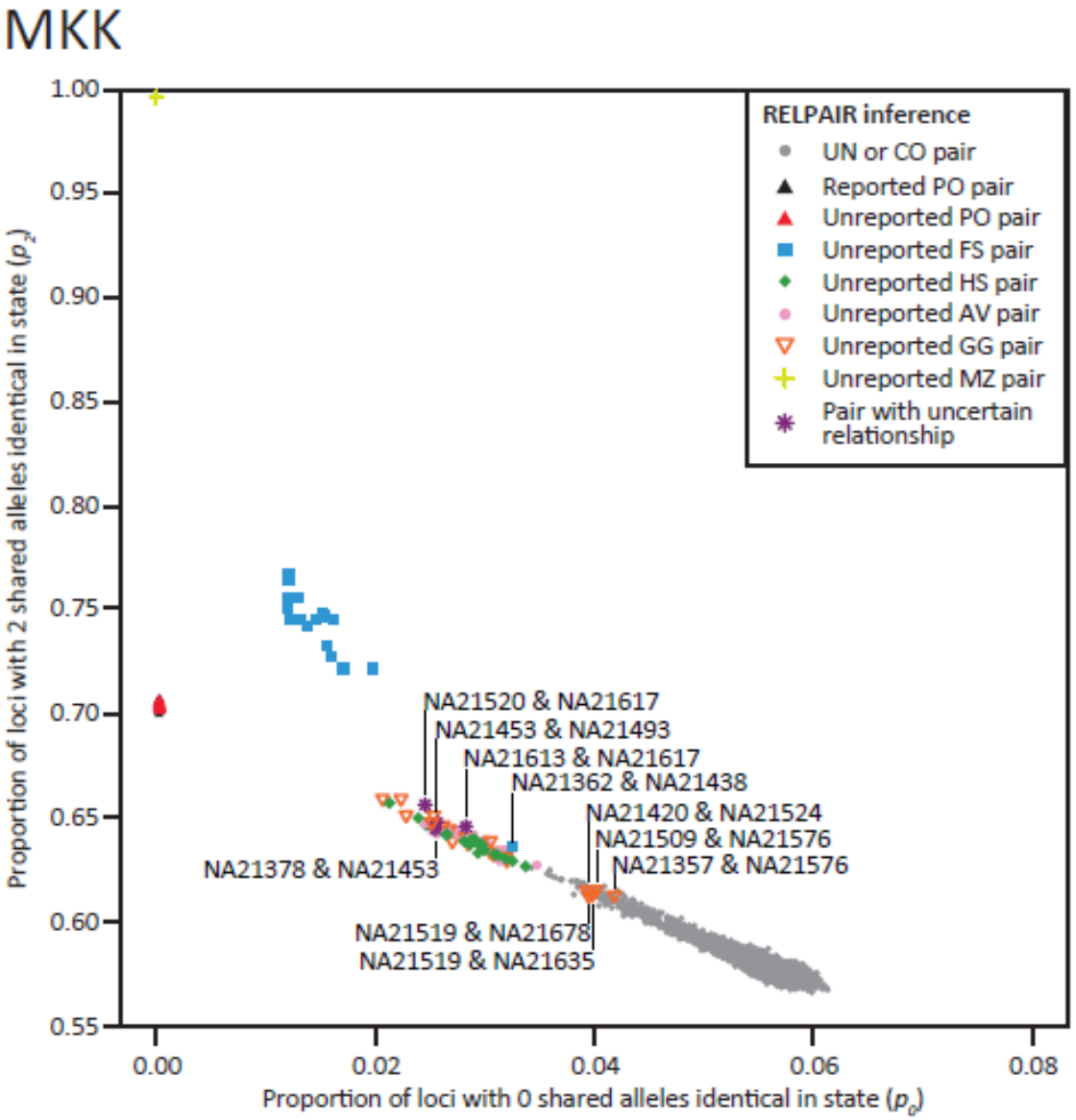
# Filter for Mendelian inheritance



# Filter for Mendelian inheritance



# Check for relatedness, e.g., HapMap




- Take overall average of all SNPs of how many alleles are shared.
- E.g., parent-offspring never shares zero alleles.
- HapMap was supposed to be unrelateds (this was a bit of a surprise!)

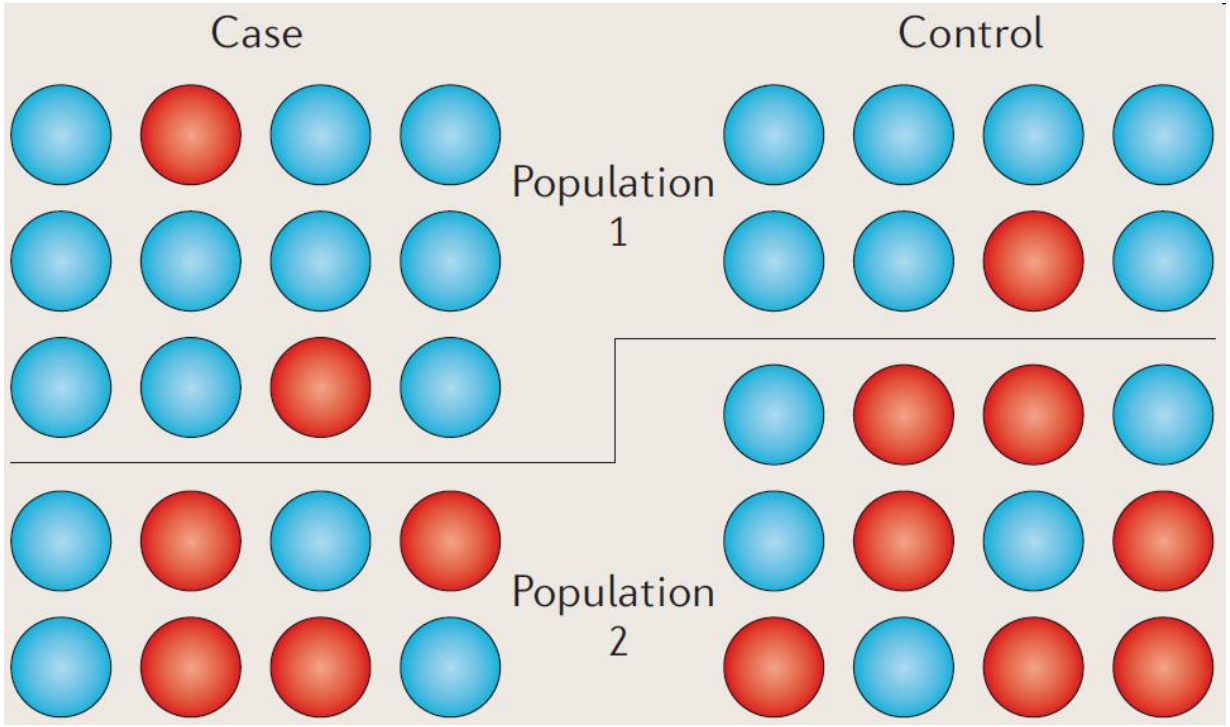
Pemberton et al., AJHG 2010

# GWAS analysis

- Most common approach: Model each SNP separately
- Additive coding of SNP most common, just a covariate in a regression framework
- Dichotomous phenotype: logistic regression
- Continuous phenotype: linear regression
- Correct for multiple comparisons
  - e.g., Bonferroni, 1 million gives $\alpha=5x10^{-8}$
  - [Often use $5x10^{-8}$ for larger GWAS arrays (or imputed SNPs, more later) also, SNPs are correlated, so Bonferroni is a little too conservative…]
- Adjust for potential population stratification (details next slides…)
  - principal components (PC's), on best performing SNPs
  - software usually does LD filter (e.g., Eigensoft)

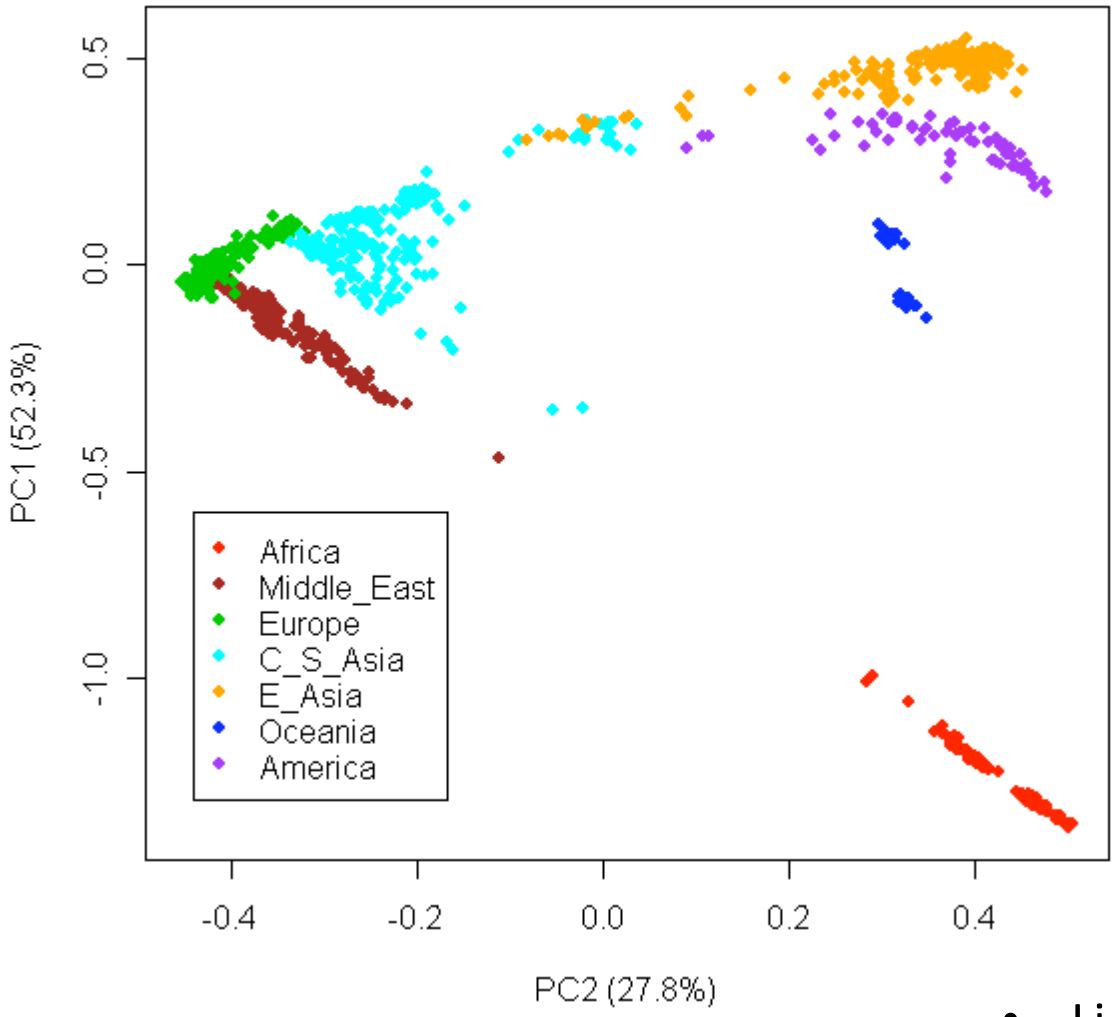
# Recap of population substructure

- Two populations have different disease frequency, and different allele frequency (Recall Pima & Papago example).
- Association picks up they are different populations!



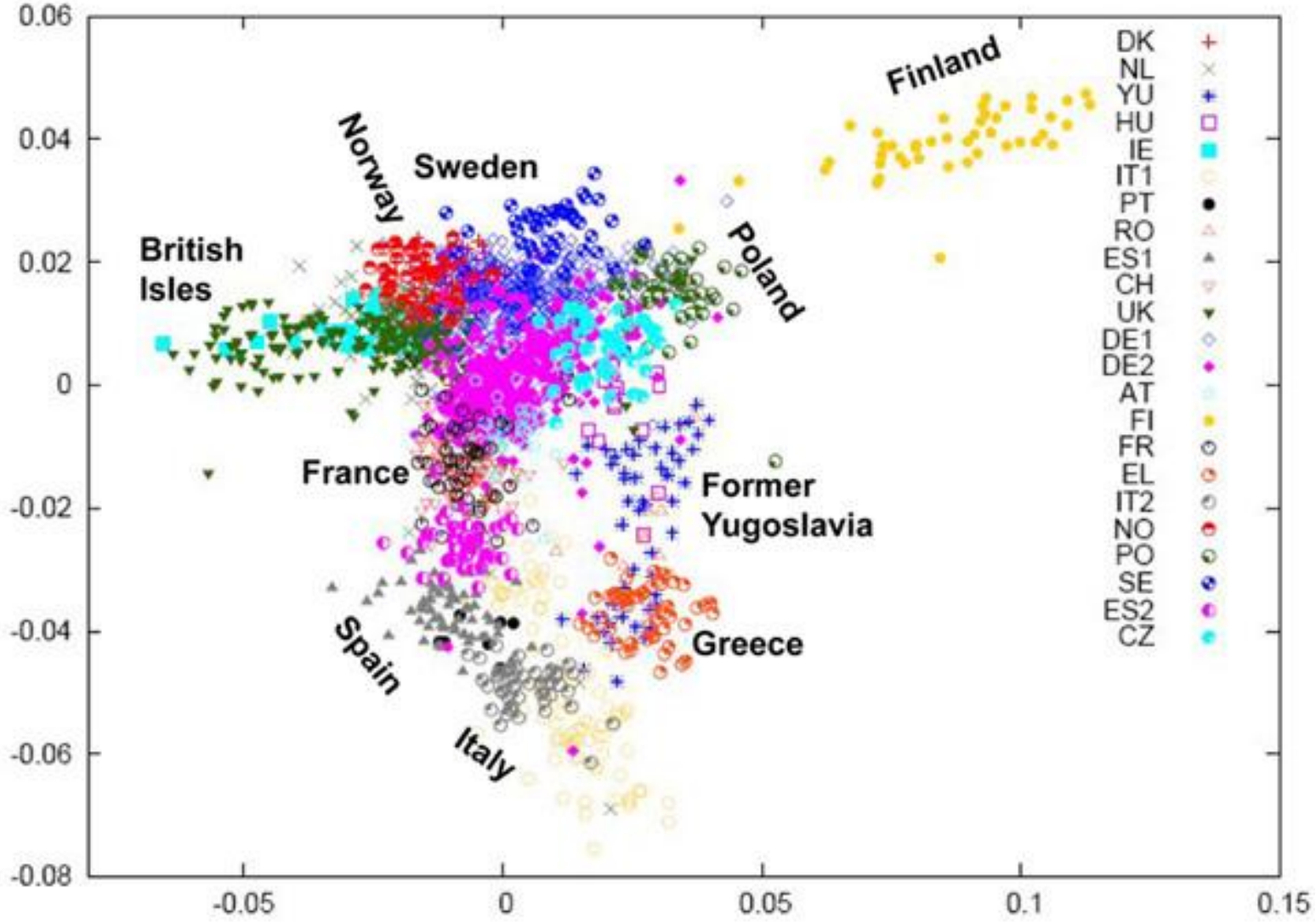

Balding, Nature Reviews Genetics 2010

# Control for race/ethnicity/ancestry: Principal Components (PCs) as covariates in regression model




- Li et al., Science 2008

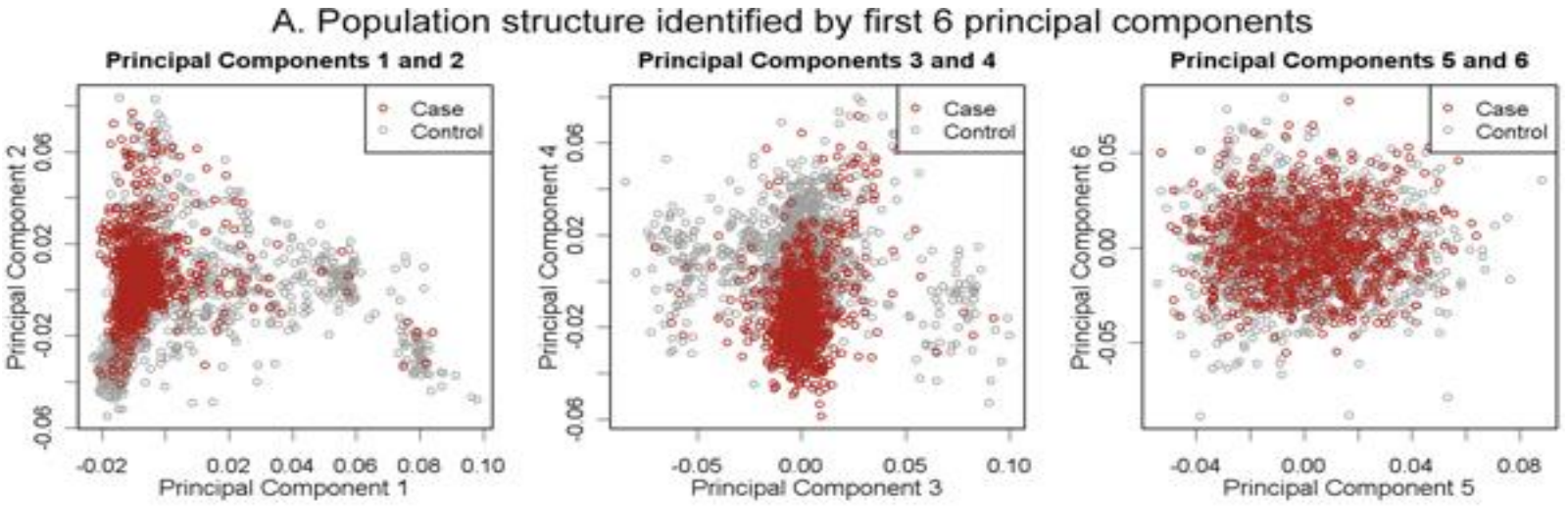
# PCs pick up fine population structure

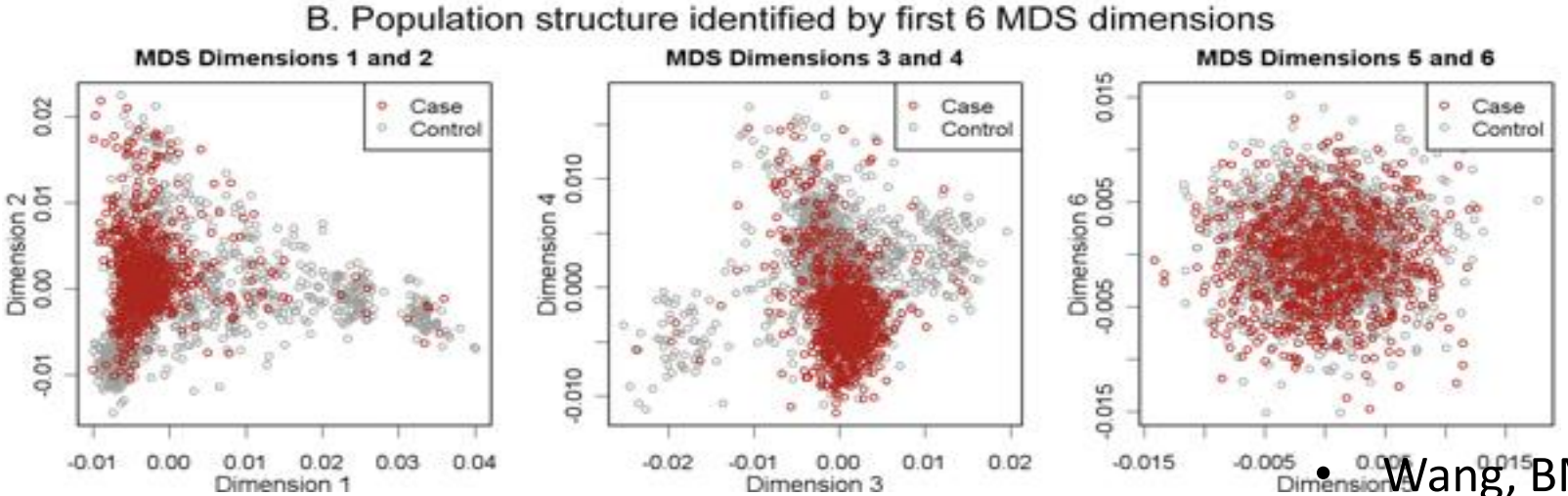



- Razib, Current Biology 2008

# Adjusting for PC's

Do not want separate cluster for cases as controls! – Why want individuals from similar population for controls (and randomization). [These look reasonable, but if see a separation…]



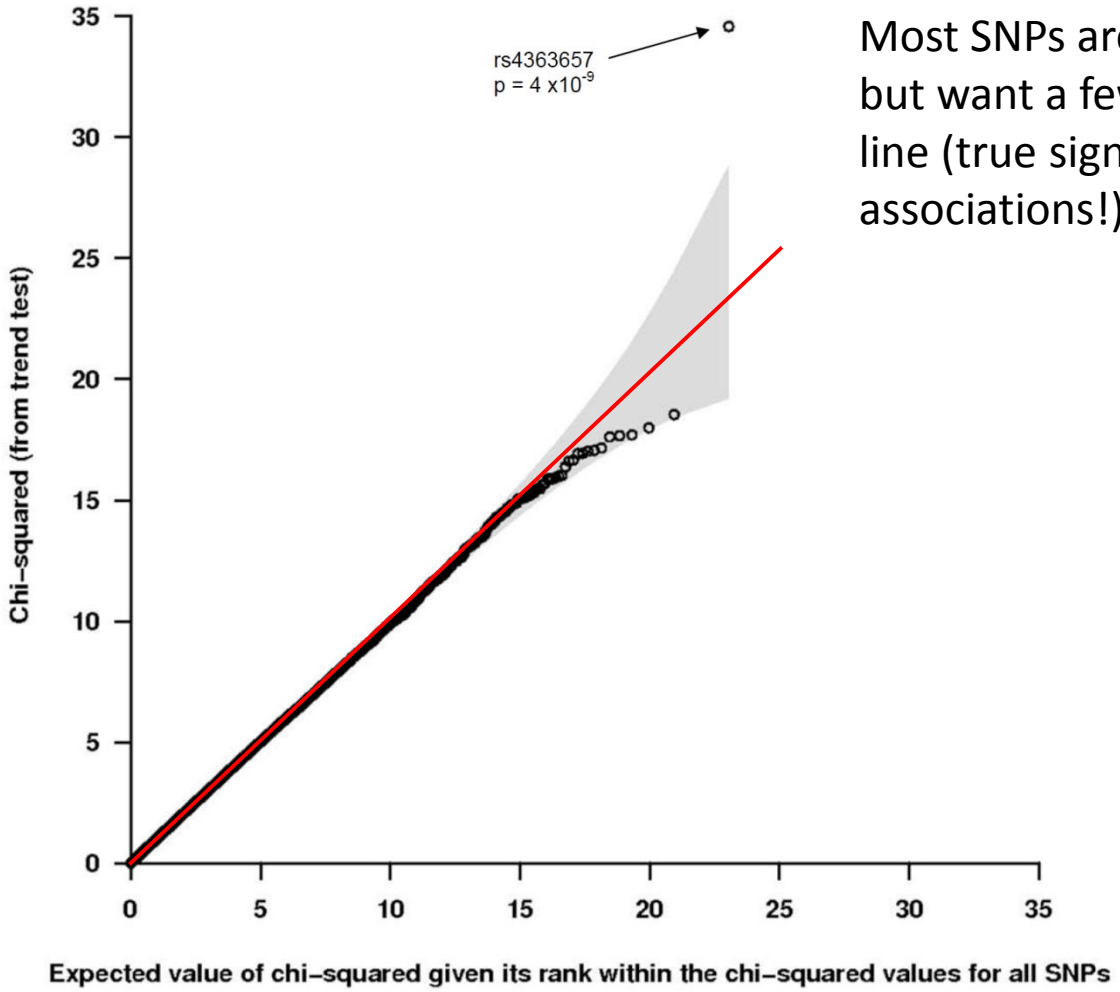




- Wang, BMC Proc 2009

# After run analysis

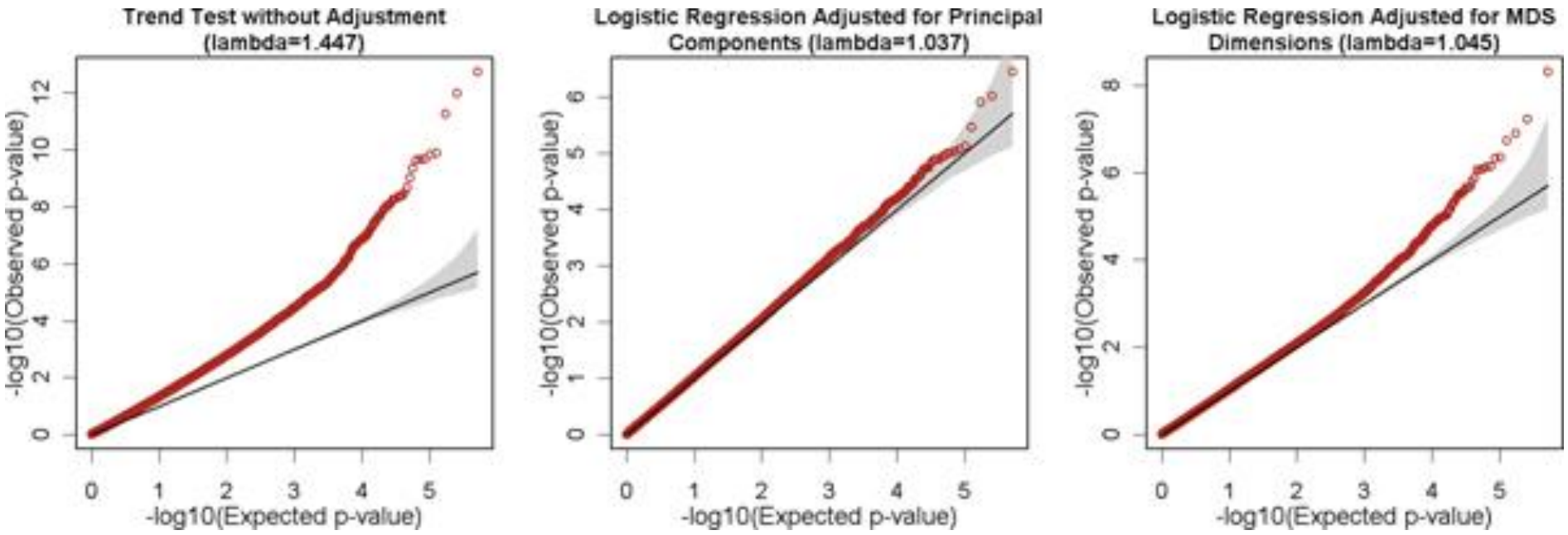
- Assess the fit with QQ-plot (saw last lecture)
- Look at results in Manhattan plots
- **Replication** of hits in a separate cohort

# Quantile-quantile (QQ) plot review




Most SNPs are on the line, but want a few hits off the line (true significant associations!)

# QQ-plots and PC adjustment recap




- Exactly on line if no signal at all.
- If don't adjust for PC's, then p-values are all inflated artificially.
- Adjusting for PC's fixes the problem.

Wang, BMC Proc 2009

# Manhattan plot example: Kaiser GWAS of Prostate Cancer

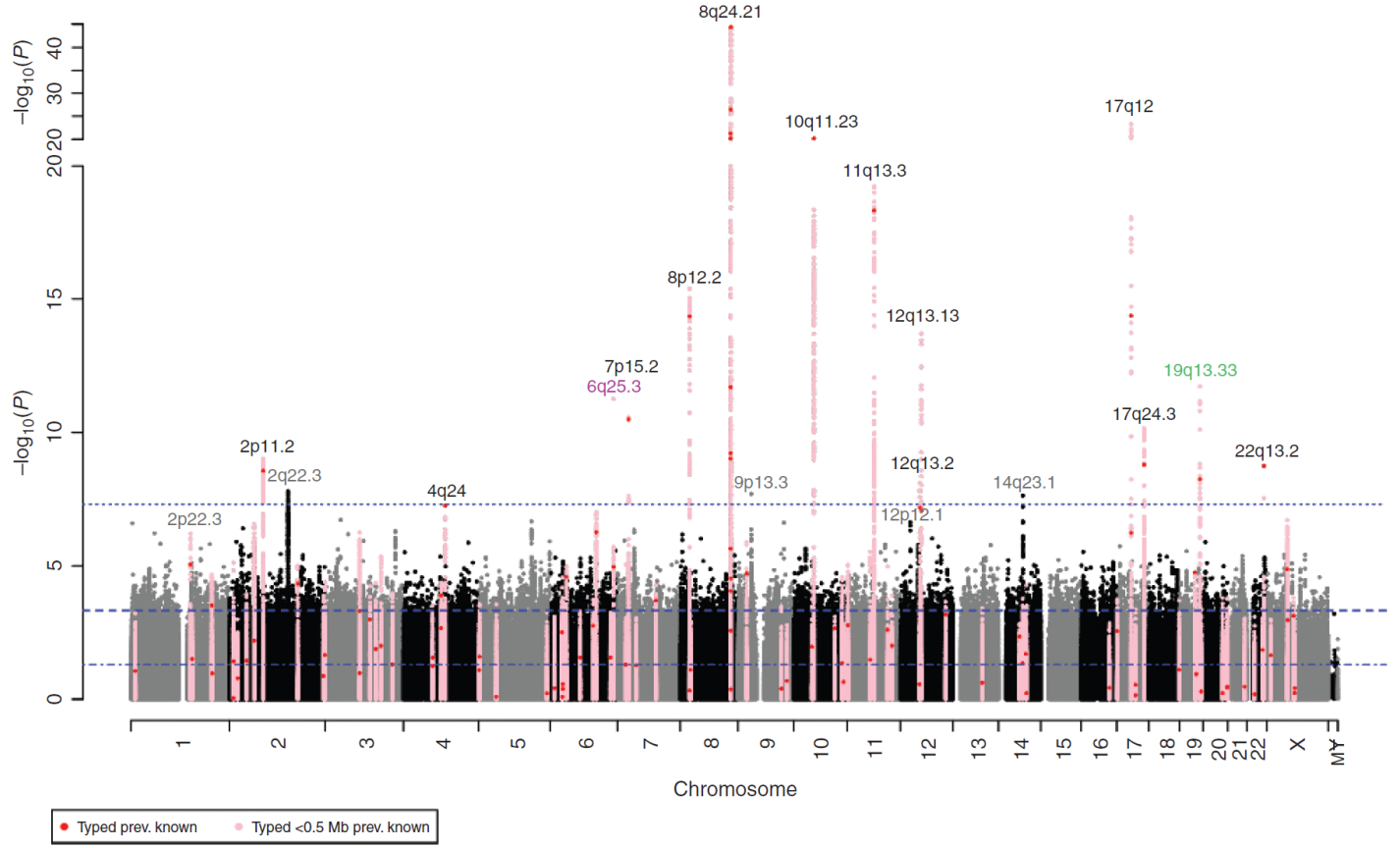
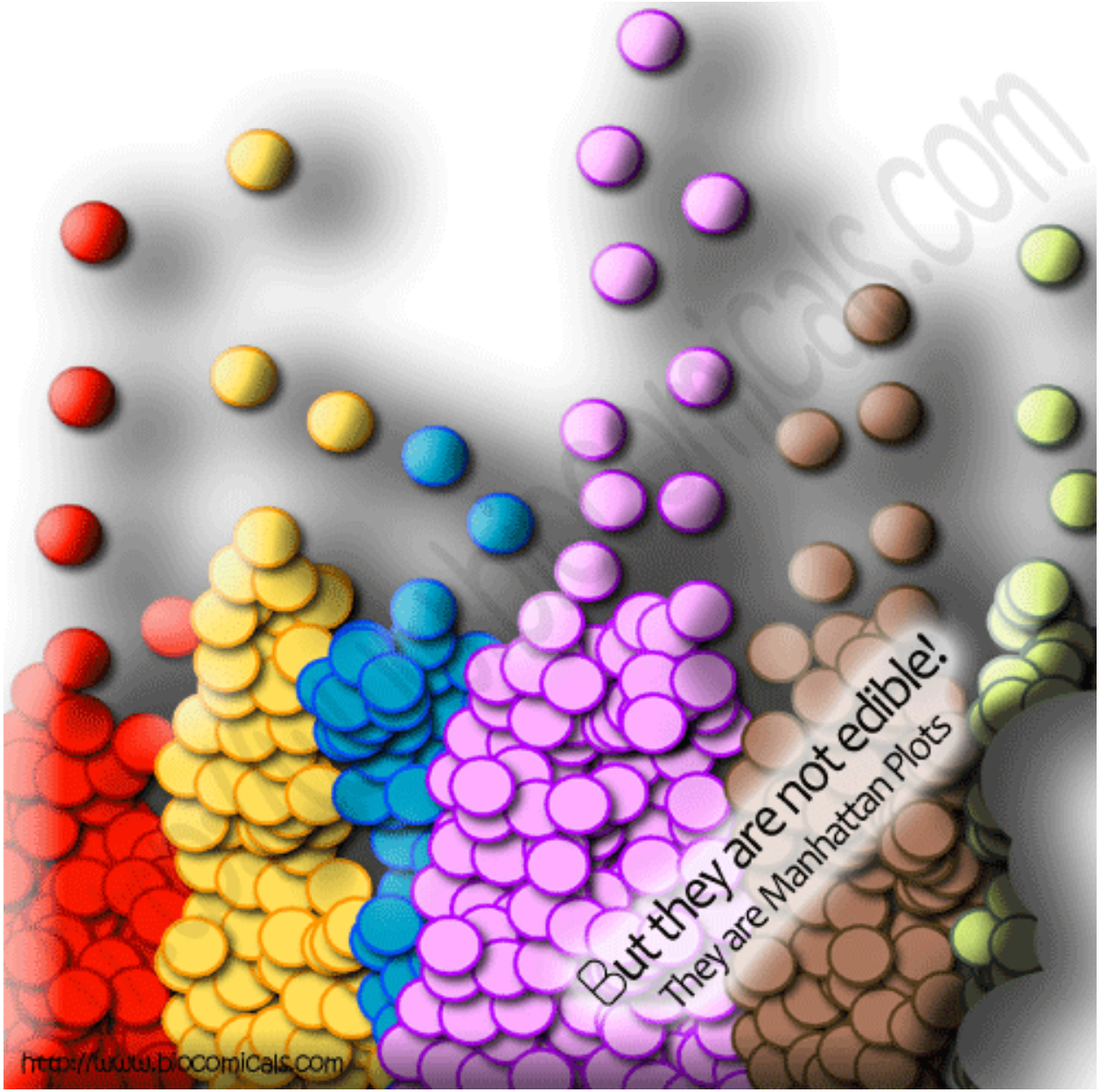



**Figure 1.** Results from a GWAS of prostate cancer in the KP population (8,399 cases and 38,745 controls), highlighting key chromosomal regions. *P* values are for variant associations with prostate cancer from transethnic fixed-effects meta-analysis of four race/ethnicity GWAS (non-Hispanic white, Latino, African-American, East Asian), each adjusted for age and ancestry principal components. Horizontal dashed lines indicate genome-wide statistical significance ($P < 5 \times 10^{-8}$), Bonferroni-corrected significance for 105 known prostate cancer risk variants ($P < 0.05/105$), and nominal significance ($P < 0.05$). Red points denote our findings for the 105 known risk SNPs, and pink indicates results for SNPs within a 0.5 Mb window around these SNPs. The new 6q25.3 indel rs4646284 is colored magenta, and the 19q13.33 prostate cancer or PSA SNP rs2659124 is noted in green. Those loci containing previously reported variants that we replicated at genome-wide significance are noted in black text. Loci with variants that were novel in the KP GWAS but failed to replicate are noted in gray text.

Hoffmann et. Al, Cancer Discovery 2015


But they are not edible!
They are Manhattan Plots
http://www.biocomicals.com

# Replication, Replication, Replication

- To replicate:
  - Association test for replication sample significant at 0.05/{Number of SNPs replicating} alpha level
  - Same genetic model (e.g. additive, dominant)
  - Same direction
  - Sufficient sample size for replication!
    - [e.g., do a power calculation]
- Non-replications not necessarily a false positive
  - LD structures, different populations (e.g., flip-flop)
  - covariates, phenotype definition, underpowered
  - Winner's curse

# Meta-analysis

- Combine multiple studies to increase power
- Either combine p-values (Fisher's test),
- or coefficient estimates + standard error (better)

# Replication & Meta-analysis

**Table 2. Prostate cancer genome-wide association study results for new risk indel rs4646284 at 6q25.3 and for risk SNP rs2659124 at 19q13.33**

A

| Variant | Risk allele | Ref. allele | Group | Risk AF | No conditioning | | Conditioning on other 6q25.3 SNPs | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | OR (95% CI) | *P* | OR (95% CI) | *P* | $r^2_{info}$ |
| Indel: rs4646284 | TG (indel) | T | KP NH white | 0.298 | 1.17 (1.12–1.23) | $6.7 \times 10^{-11}$ | 1.16 (1.10–1.23) | $3.3 \times 10^{-7}$ | 0.91 |
| | | | KP Latino | 0.250 | 1.13 (0.94–1.36) | 0.20 | 1.06 (0.85–1.31) | 0.61 | 0.89 |
| | | | KP Asian | 0.324 | 1.03 (0.84–1.27) | 0.76 | 0.91 (0.68–1.23) | 0.56 | 0.89 |
| | | | KP Af-Amer | 0.365 | 1.18 (1.02–1.37) | 0.029 | 1.21 (1.03–1.42) | 0.020 | 0.85 |
| | | | KP Meta FE | — | 1.17 (1.12–1.22) | $5.5 \times 10^{-12}$ | 1.15 (1.09–1.21) | $8.5 \times 10^{-8}$ | — |
| | | | KP Meta RE | — | 1.17 (1.12–1.22) | $5.5 \times 10^{-12}$ | 1.15 (1.07–1.22) | $7.7 \times 10^{-5}$ | — |
| | | | MEC | 0.361 | 1.13 (1.03–1.25) | 0.0094 | 1.13 (1.03–1.25) | 0.014 | 0.81 |
| | | | PEGASUS | 0.278 | 1.27 (1.17–1.37) | $1.4 \times 10^{-8}$ | 1.20 (1.09–1.32) | 0.00024 | 0.80 |
| | | | Overall Meta FE | — | 1.18 (1.14–1.22) | $1.0 \times 10^{-19}$ | 1.16 (1.11–1.21) | $5.4 \times 10^{-12}$ | — |
| | | | Overall Meta RE | — | 1.18 (1.13–1.23) | $9.8 \times 10^{-17}$ | 1.16 (1.11–1.21) | $5.4 \times 10^{-12}$ | — |

Hoffmann et. Al, Cancer Discovery 2015`

# Beyond Genotyped SNPs: Imputation of SNP Genotypes

- Combine data from different platforms (e.g., Affy & Illumina) (for replication / meta-analysis).
- Estimate unmeasured or missing genotypes.
- Based on measured SNPs and external info (e.g., haplotype structure of HapMap).
- Increase GWAS power (impute and analyze all), e.g. Sick sinus syndrome, most significant was 1000 Genomes imputed SNP (Holm et al., Nature Genetics, 2011)
- HapMap as reference, now 1000 Genomes Project; UK10K, Haplotype reference consortium?
- Hybrid sequencing/genotyping approaches

# Generalization of Tag SNPs: Imputed SNPs using a Reference Panel

- Generalization of pairwise $r^2$ of tagSNPs to multi-marker correlation

**Study sample**

....A.....A....A...
....G.....C....A...

**Reference haplotypes**

CGAGATCTCCTTCTTCTGTGC
CGAGATCTCCCGACCTCATGG
CCAAGCTCTTTTCTTCTGTGC
CGAAGCTCTTTTCTTCTGTGC
CGAGACTCTCCGACCTTATGC
TGGGATCTCCCGACCTCATGG
CGAGATCTCCCGACCTTGTGC
CGAGACTCTTTTCTTTTGTAC
CGAGACTCTCCGACCTCGTGC
CGAAGCTCTTTTCTTCTGTGC

***Class Exercise!***

How would you fill in the remaining genotypes of this study sample individual, given the reference haplotypes?

- http://www.shapeit.fr/, http://mathgen.stats.ox.ac.uk/impute/impute_v2.html
- http://faculty.washington.edu/browning/beagle/beagle.html
- http://www.sph.umich.edu/csg/abecasis/MACH/download/

Li et al., Ann Rev Human Genet, 2009

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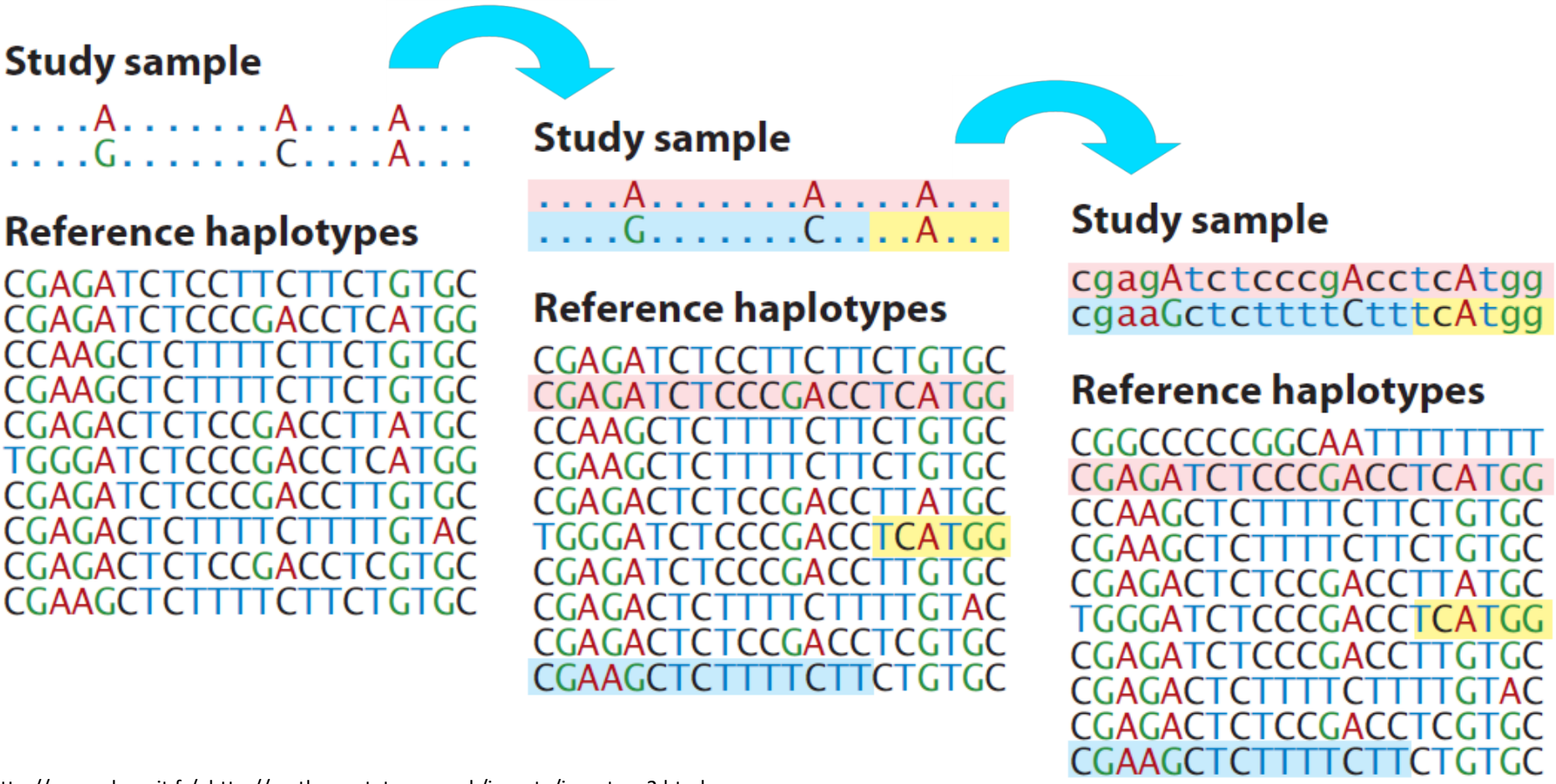
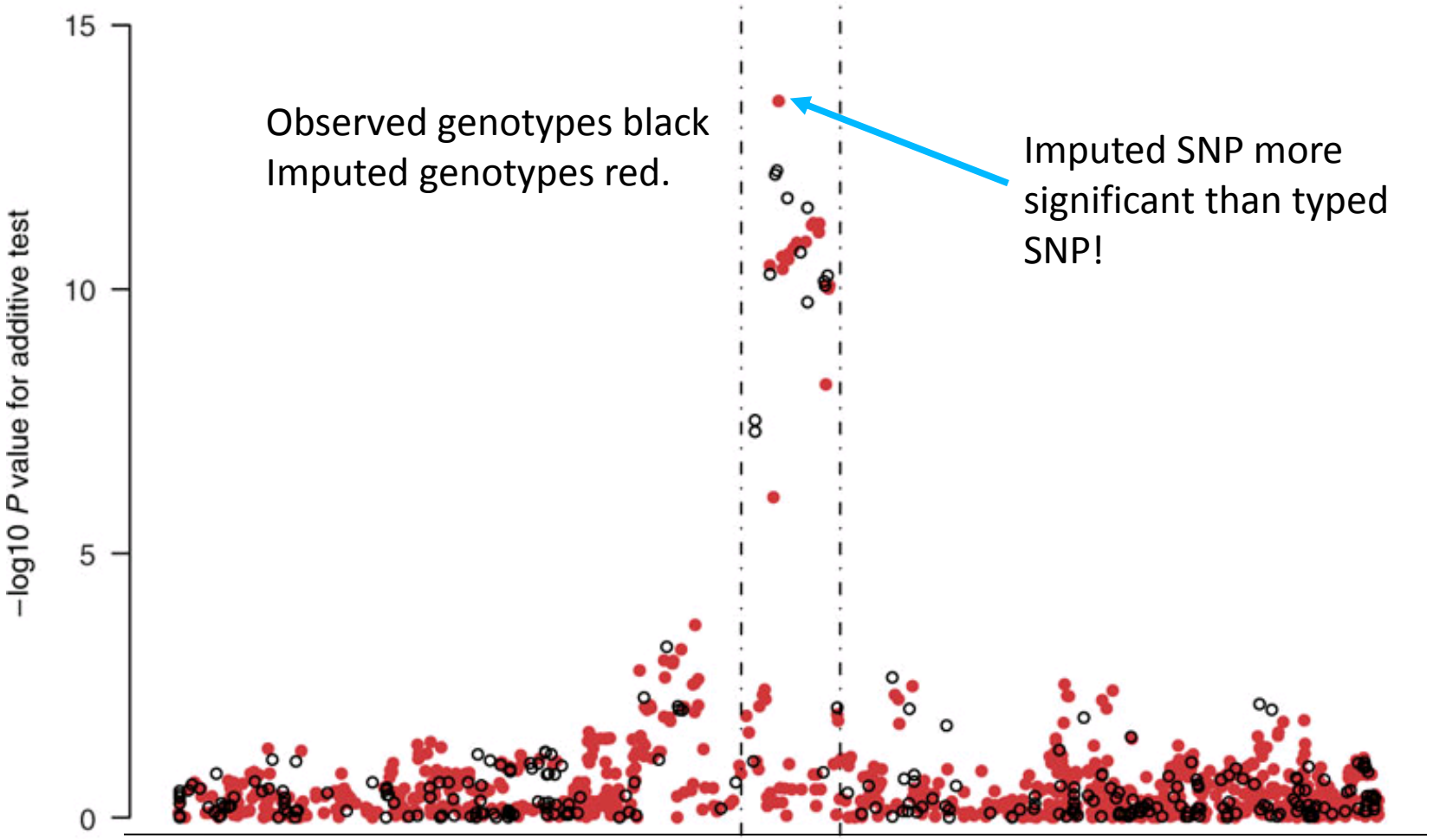



- http://www.shapeit.fr/, http://mathgen.stats.ox.ac.uk/impute/impute_v2.html
- http://faculty.washington.edu/browning/beagle/beagle.html
- http://www.sph.umich.edu/csg/abecasis/MACH/download/

Li et al., Ann Rev Human Genet, 2009

# Imputation Application

## *TCF7L2* gene region & T2D from the WTCCC data



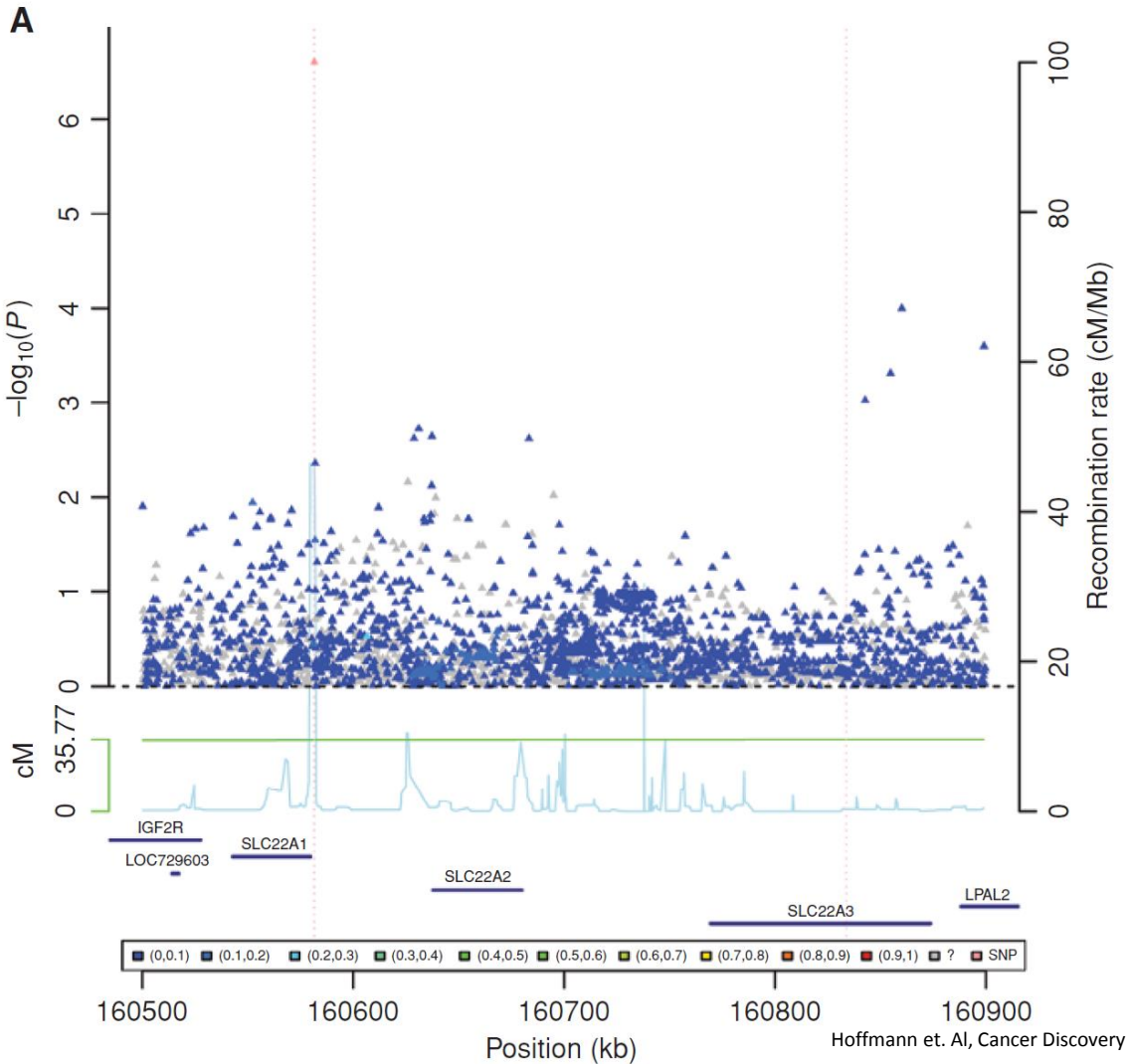

Marchini Nature Genetics2007

http://www.stats.ox.ac.uk/~marchini/#software

# Imputation Application #2 – Do not always see a jet of points, even for common SNP…

**Figure 2.** Regional fixed-effects meta-analysis plots from GWAS in KP population of the two risk variants that replicated: (**A**) 6q25.3 (rs4646284), (**B**) 19q13.33 (rs2659124). The color code for the points represents the $r^2$ of each SNP with the risk variant (ranges defined in the legend). The dotted vertical lines pass through the risk variants and the other significant SNPs in the region, on which analyses were conditioned.

(Same SNP as 4 slides ago)




Hoffmann et. Al, Cancer Discovery 2015`

# Imputation example: How rare can you go?

- G84E example talked about in Austin pg. 62
- MAF 0.0034 in European ancestry. $r^2$=0.57.
- In general, a moving target, still unknown how rare is possible

PLOS | GENETICS

RESEARCH ARTICLE

## Imputation of the Rare *HOXB13* G84E Mutation and Cancer Risk in a Large Population-Based Cohort

**Thomas J. Hoffmann[1,2], Lori C. Sakoda[3], Ling Shen[3], Eric Jorgenson[3], Laurel A. Habel[3], Jinghua Liu[2], Mark N. Kvale[2], Maryam M. Asgari[3], Yambazi Banda[2], Douglas Corley[3], Lawrence H. Kushi[3], Charles P. Quesenberry Jr.[3], Catherine Schaefer[3], Stephen K. Van Den Eeden[3,4], Neil Risch[1,2,3], John S. Witte[1,2,4]***

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# HOXB13 G84E (continued)

**Table 1.** Pleiotropic effect of G84E mutation on risk of cancer.

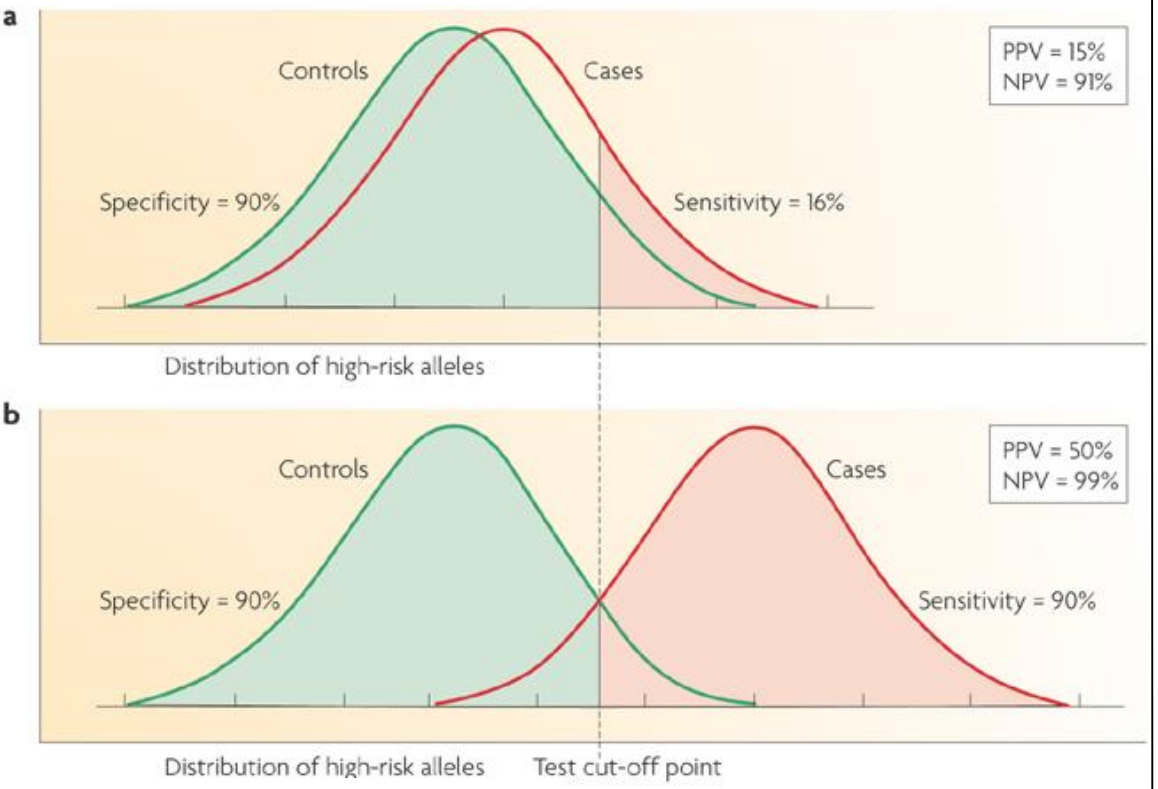
| Cancer[a] | # Case carriers[b] | # Cases[b] | Frequency[c] | Odds Ratio[d] (95% CI) | Corrected Odds Ratio[e] (95% CI) | P-value[d] |
|---|---|---|---|---|---|---|
| Kidney | 5 | 303 | 0.94% | 2.32 (0.94, 5.76) | 3.32 (0.89, 9.99) | 0.034 |
| Bladder | 5 | 335 | 0.85% | 2.05 (0.81, 5.22) | 2.84 (0.70, 8.94) | 0.065 |
| Non-Hodgkin's Lymphoma | 10 | 846 | 0.67% | 1.81 (0.98, 3.36) | 2.42 (0.96, 5.52) | 0.029 |
| Melanoma | 15 | 1301 | 0.66% | 1.50 (0.89, 2.55) | 1.88 (0.81, 3.95) | 0.066 |
| Pancreas | 2 | 149 | 0.77% | 1.49 (0.28, 7.83) | 1.86 (0.18, 13.70) | 0.32 |
| Breast | 38 | 3183 | 0.68% | 1.48 (1.04, 2.10) | 1.84 (1.07, 3.16) | 0.014 |
| Endometrium | 6 | 578 | 0.59% | 1.44 (0.64, 3.24) | 1.77 (0.49, 5.22) | 0.19 |
| Colon | 11 | 1119 | 0.56% | 1.42 (0.77, 2.60) | 1.74 (0.65, 4.06) | 0.13 |
| Thyroid | 3 | 217 | 0.79% | 1.35 (0.35, 5.22) | 1.61 (0.23, 8.81) | 0.33 |
| Ovary | 2 | 198 | 0.58% | 1.32 (0.32, 5.49) | 1.56 (0.20, 9.26) | 0.35 |
| Multiple Myeloma | 1 | 135 | 0.42% | 1.26 (0.20, 7.92) | 1.46 (0.12, 13.75) | 0.40 |
| Lung | 5 | 667 | 0.43% | 1.10 (0.44, 2.72) | 1.18 (0.30, 4.22) | 0.42 |
| Oral | 2 | 283 | 0.40% | 0.98 (0.23, 4.10) | 0.97 (0.14, 6.78) | NA |
| Lymphocytic Leukemia | 1 | 218 | 0.26% | 0.53 (0.05, 5.24) | 0.39 (0.03, 9.06) | NA |
| | | | | | | |
| Any[f] | 123 | 10493 | 0.67% | 1.36 (1.13, 1.63) | 1.63 (1.22, 2.29) | $5.8\times10^{-4}$ |
| Any single cancer[g] | 106 | 9532 | 0.63% | 1.41 (1.14, 1.75) | 1.72 (1.23, 2.51) | $8.9\times10^{-4}$ |
| Two or more cancers[h] | 17 | 961 | 1.01% | 2.21 (1.35, 3.61) | 3.12 (1.60, 6.14) | 0.0011 |

# Outline for GWAS

- Review / Overview
- Design
- Analysis
  - QC
  - Prostate cancer example
  - Imputation
  - Replication & Meta-analysis
- Advanced analysis
  - Limitations & “missing heritability”
  - Gene/pathway tests
  - Polygenic models

# Limitations of GWAS

- Not very predictive




Witte, Nat Rev Genet 2009

Example:
AUC for Breast Cancer Risk

58%: Gail model (# first degree relatives w bc, age menarche, age first live birth, number of previous biopsies) + age, study, entry year
58.9%: SNPs
61.8%: Combined

Wacholder et al., NEJM 2010

# Limitations of GWAS

- Not very predictive
- Explain little heritability
- Focus on common variation
- Many associated variants are not causal

# Where's the heritability?

**Table 1. Population Variation Explained by GWAS for a Selected Number of Complex Traits**

| Trait or Disease | $h^2$ Pedigree Studies | $h^2$ GWAS Hits[a] | $h^2$ All GWAS SNPs[b] |
|---|---|---|---|
| Type 1 diabetes | 0.9[98] | 0.6[99,c] | 0.3[12] |
| Type 2 diabetes | 0.3–0.6[100] | 0.05-0.10[34] | |
| Obesity (BMI) | 0.4–0.6[101,102] | 0.01-0.02[36] | 0.2[14] |
| Crohn's disease | 0.6–0.8[103] | 0.1[11] | 0.4[12] |
| Ulcerative colitis | 0.5[103] | 0.05[12] | |
| Multiple sclerosis | 0.3–0.8[104] | 0.1[45] | |
| Ankylosing spondylitis | >0.90[105] | 0.2[106] | |
| Rheumatoid arthritis | 0.6[107] | | |
| Schizophrenia | 0.7–0.8[108] | 0.01[79] | 0.3[109] |
| Bipolar disorder | 0.6–0.7[108] | 0.02[79] | 0.4[12] |
| Breast cancer | 0.3[110] | 0.08[111] | |
| Von Willebrand factor | 0.66–0.75[112,113] | 0.13[114] | 0.25[14] |
| Height | 0.8[115,116] | 0.1[13] | 0.5[13,14] |
| Bone mineral density | 0.6-0.8[117] | 0.05[118] | |
| QT interval | 0.37–0.60[119,120] | 0.07[121] | 0.2[14] |
| HDL cholesterol | 0.5[122] | 0.1[57] | |
| Platelet count | 0.8[123] | 0.05–0.1[58] | |

[a] Proportion of phenotypic variance or variance in liability explained by genome-wide-significant and validated SNPs. For a number of diseases, other parameters were reported, and these were converted and approximated to the scale of total variation explained. Blank cells indicate that these parameters have not been reported in the literature.

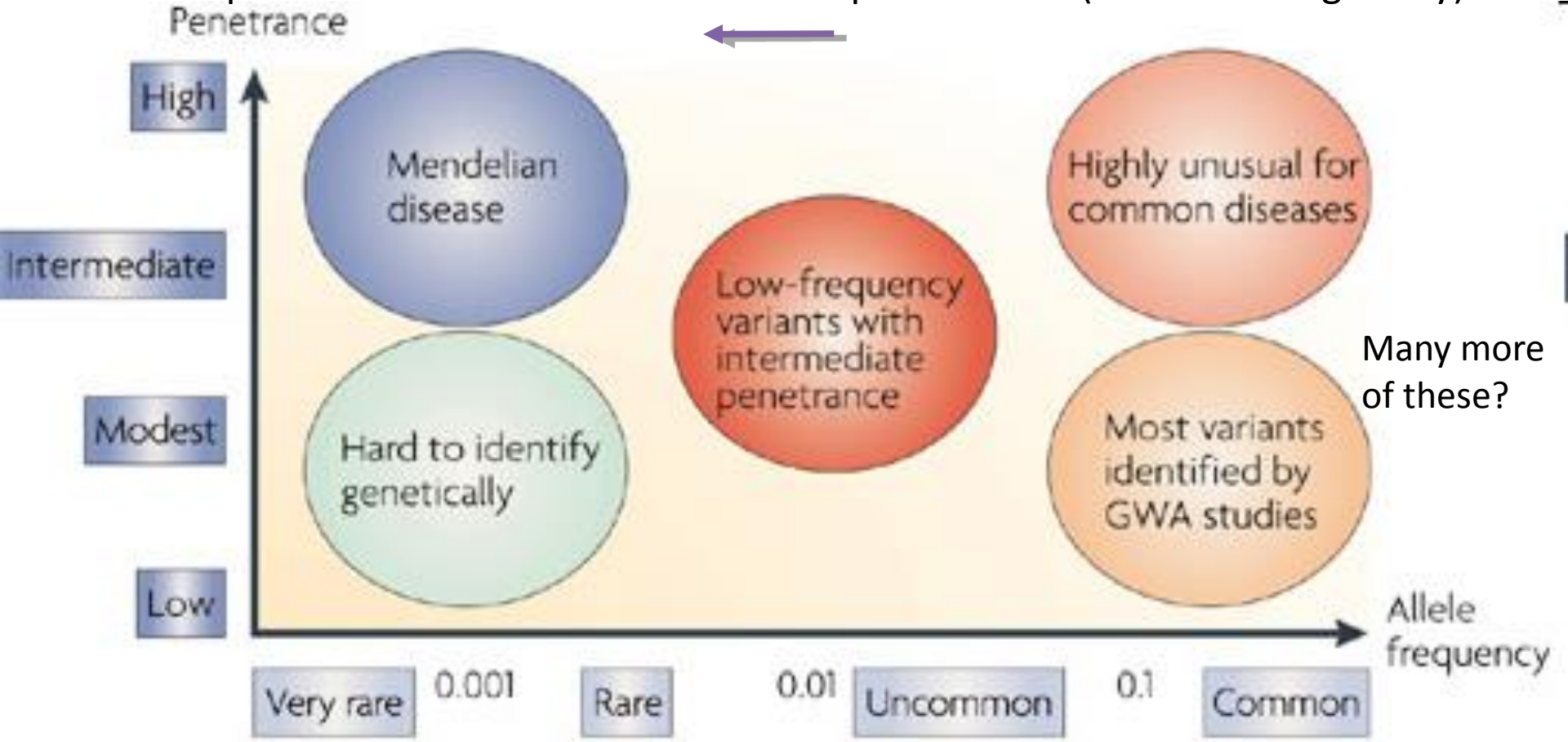
[b] Proportion of phenotypic variance or variance in liability explained when all GWAS SNPs are considered simultaneously. Blank cell indicate that these parameters have not been reported in the literature.

[c] Includes pre-GWAS loci with large effects.

Visscher, AJHG 2011

# Where's the heritability?

Common disease rare variant (CDRV) hypothesis: diseases due to multiple rare variants with intermediate penetrances (allelic heterogeneity)




See: NEJM, April 30, 2009

Nature Reviews | Genetics
McCarthy et al., 2008

# Will GWAS results explain more heritability?

Possibly, if…

1. Causal SNPs not yet detected due to power issues – weak effects
   - Previous GWAS designed/powered for MAF>0.05/0.10; lower MAFs for newer arrays and larger reference panels
2. More complicated modeling necessary, e.g. gene-gene interaction, gene-environment interaction, etc.

# “Array” heritability

- Compute heritability using **all** SNPs
  - Be careful with QC, esp. case-control
- If you adjust for the known hits, how much remains yet to be found?
- Restricted to narrow sense heritability
- E.g.,

http://www.complextraitgenomics.com/software/gcta/

ANALYSIS

nature genetics

# Common SNPs explain a large proportion of the heritability for human height

Jian Yang[1], Beben Benyamin[1], Brian P McEvoy[1], Scott Gordon[1], Anjali K Henders[1], Dale R Nyholt[1], Pamela A Madden[2], Andrew C Heath[2], Nicholas G Martin[1], Grant W Montgomery[1], Michael E Goddard[3] & Peter M Visscher[1]



**SNPs discovered by genome-wide association studies (GWASs) account for only a small fraction of the genetic variation of complex traits in human populations. Where is the remaining heritability? We estimated the proportion of variance for human height explained by 294,831 SNPs genotyped on 3,925 unrelated individuals using a linear model analysis, and validated the estimation method with simulations based on the observed genotype data. We show that 45% of variance can be explained by considering all SNPs simultaneously. Thus, most of the heritability is not missing but has not previously been detected because the individual effects are too small to pass stringent significance tests. We provide evidence that the remaining heritability is due to incomplete linkage disequilibrium between causal variants and genotyped SNPs, exacerbated by causal variants having lower minor allele frequency than the SNPs explored to date.**

GWASs in human populations have discovered hundreds of SNPs significantly associated with complex traits[1,2], yet for any one of variation that their effects do not reach stringent significance thresholds and/or the causal variants are not in complete linkage disequilibrium (LD) with the SNPs that have been genotyped. Lack of complete LD might, for instance, occur if causal variants have lower minor allele frequency (MAF) than genotyped SNPs. Here we test these two hypotheses and estimate the contribution of each to the heritability of height in humans as a model complex trait.

Height in humans is a classical quantitative trait, easy to measure and studied for well over a century as a model for investigating the genetic basis of complex traits[9,10]. The heritability of height has been estimated to be ~0.8 (refs. 9,11–13). Rare mutations that cause extreme short or tall stature have been found[14,15], but these do not explain much of the variation in the general population. Recent GWASs on tens of thousands of individuals have detected ~50 variants that are associated with height in the population, but these in total account for only ~5% of phenotypic variance[16–19].

Data from a GWAS that are collected to detect statistical associations between SNPs and complex traits are usually analyzed by testing each SNP individually for an association with the trait. To account for the

# Gene/pathway-based tests

- Various ways of collapsing the genotype information in multiple genes
  - Less multiple comparison adjustment
- logit (Prob(y=1| **x, c**)) = $\alpha + \beta\mathbf{x} + \gamma\mathbf{c}$

  e.g.=$\alpha+\beta_1 x_1+\ldots+\beta_{12} x_{12}$+{PC's, other covariates}
  - y: disease status)
  - **x** is a vector of genotypes (e.g., a gene, or a pathway)
  - **c** is a vector of covariates
- $H_0$: $\beta$=**0**
- Rare variant "burden" tests are along these lines

# Pathways - how to define?

- Many websites / companies provide ‘dynamic’ graphic models of molecular and biochemical pathways.
- Examples: GO (Gene Ontology), KEGG, BioCarta, Reactome
- May be interested in potential joint and/or interaction effects of multiple genes in one pathway.

# Other Extreme: Polygenic Models

- Many weak associations combine to risk?
- Score model (use all GWAS SNPs):

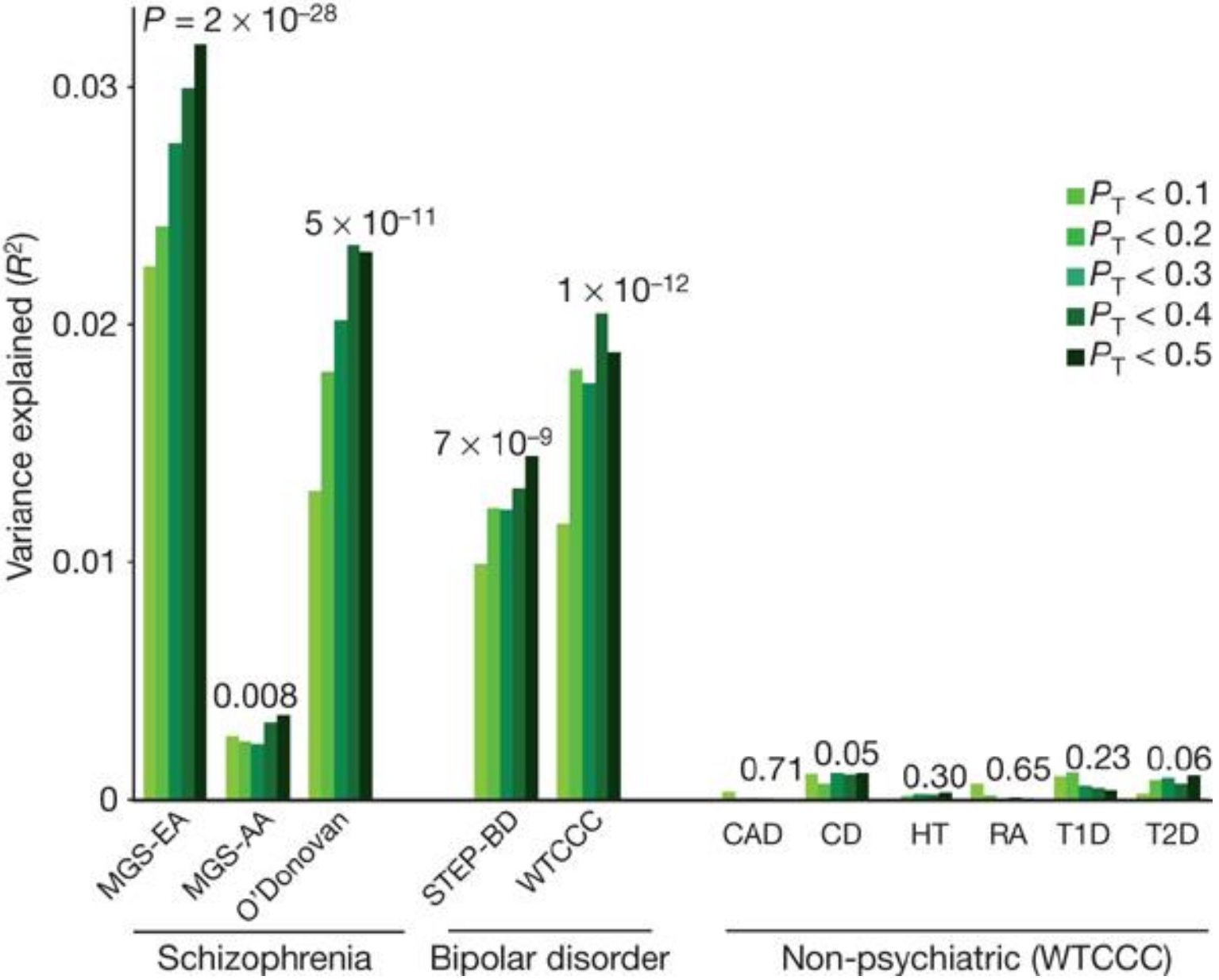
$$x_j = \frac{\sum_{i=1}^{m} \ln\left(OR_i\right) \times SNP_{ij}}{m}$$

where

  - $\ln(OR_i)$ = 'score' for $SNP_i$ from 'discovery' sample
  - $SNP_{ij}$ = # of alleles (0,1,2) for $SNP_i$, person j in 'validation' sample.
  - Large number of SNPs ($m$)
- $x_j$ associated with disease?
- ASIDE: Genetic risk score this idea, but only on ***known hits***

ISC / Purcell et al. *Nature* 2009

# Application of Model




Purcell / ISC et al. *Nature* 2009

# Other things than SNPs

- Copy number variants (CNVs)
- Epigenetics, e.g., methylation, histone modification
- Gene expression (RNA levels)
- Proteomics (measures of proteins in cells)
- …