Population Stratification, **Statistical Power** and Experimental Design

Christian Werner

(Computational and quantitative geneticist) **EiB CIMMYT**, Texcoco (Mexico)

Filippo Biscarini

HerrFalloppio

(Biostatistician, bioinformatician and quantitative geneticist) **CNR-IBBA**, Milan (Italy)

Oscar González-Recio

OscarGenomics

(Computational biologist and quantitative geneticist) **INIA-UPM**, Madrid (Spain)

Physalia
Courses

Statistical Power (**A needle in a haystack**)

- Find causal mutation

GENETICS

Can SNPs Deliver on Susceptibility Genes?

Minor differences in people's DNA ought to predict their risk of certain diseases. Is research on so-called SNPs living up to its promise?

- Altshuler et al. (2000) discussed retested 13 published associations of SNPs with type II diabetes in an independent population.
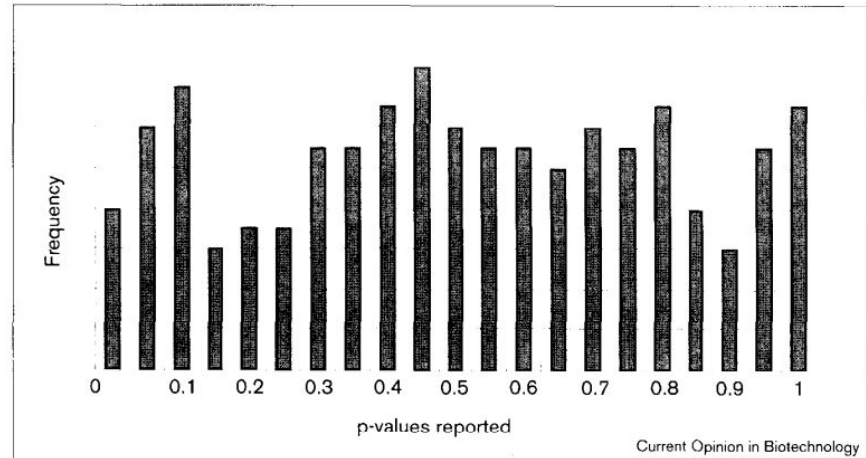
Only one was significant.

Statistical Power (A needle in a haystack)

- Terwilliger and Weiss (1998)

Figure 4

The distribution of all reported p-values from association studies in either *American Journal of Medical Genetics* (*Neuropsychiatric Genetics*) or *Psychiatric Genetics* in 1997 is shown. A total of 222 reported p-values are graphed in the figure, and an additional 39 tests were listed as 'nonsignificant' at the 0.05 level with no statistical details in the manuscript. If all of the results were obtained under the null hypothesis, the expected distribution would be uniform. As can be seen in this figure, there is very good fit to the uniform expectation ($\chi^2_{(20)} = 12.98$; $p > 0.87$), indicating that the published p-values are consistent with the absence of gene effects in all the published analyses.

Current Opinion in Biotechnology

Legend:
- ■ Real effect, significant
- ░ Real effect, not significant
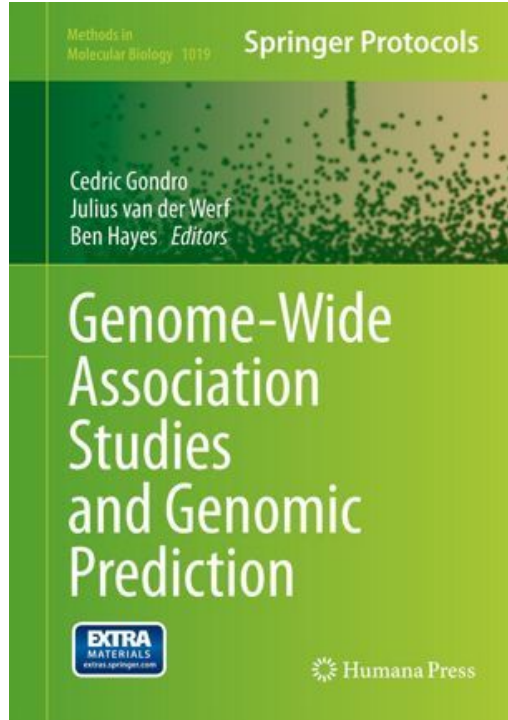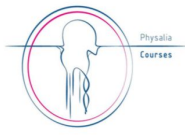- ▨ No effect, significant
- ■ No effect, not significant

$$P(\text{Real effect}|\text{Signif.}) = \frac{P(\text{Real effect} \cap \text{Signif.})}{P(\text{Signif.})} = \frac{0.24}{0.275} = 0.873$$

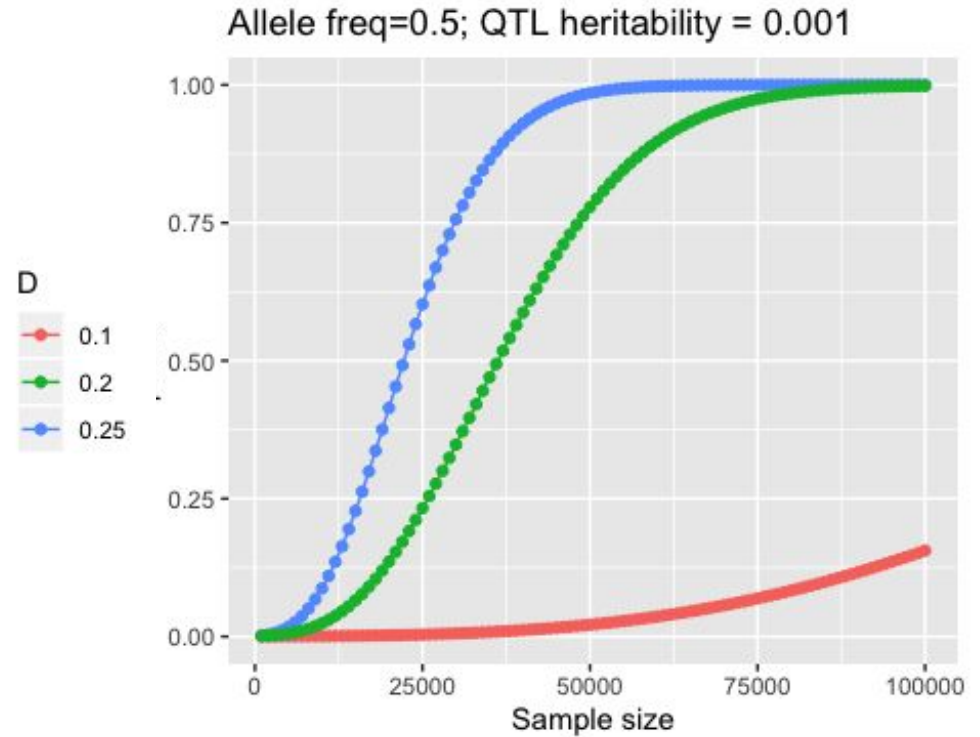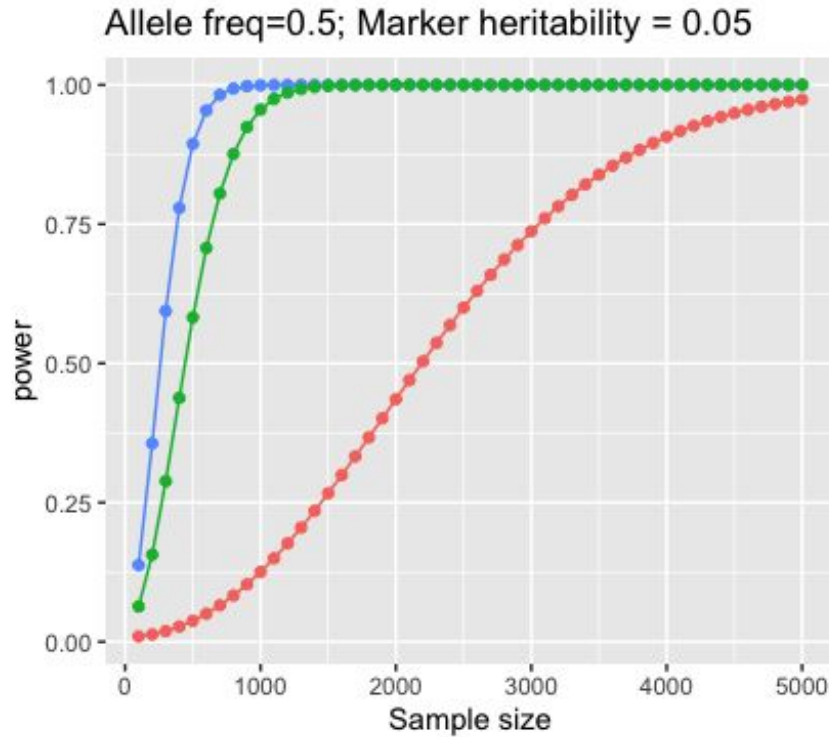http://shiny-eio.upc.edu/bne/efectos2/
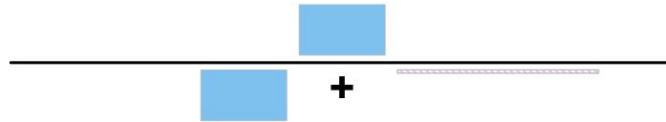
Chapter 3

power of GWAS experiments (common variants)

Allele freq=0.5; Marker heritability = 0.05

Allele freq=0.5; QTL heritability = 0.001

D
- 0.1
- 0.2
- 0.25

R. D. Ball (2013) Genomewide Association studies and Genome-Wide prediction.
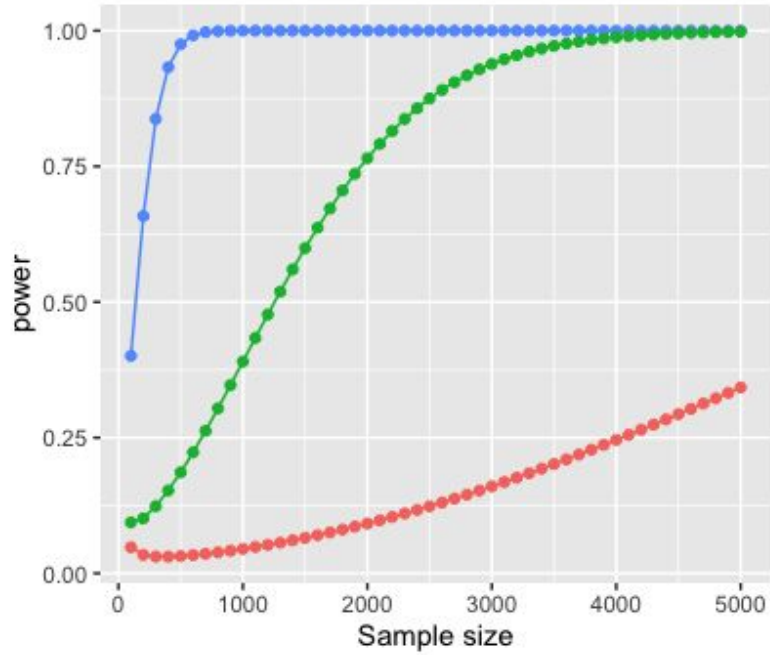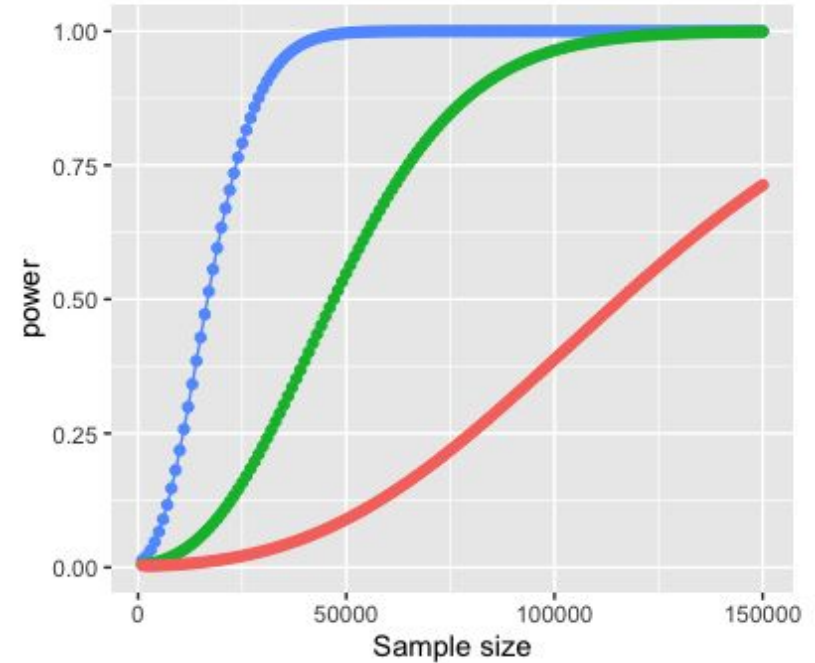
power of GWAS experiments (uncommon variants)

R. D. Ball (2013) Genomewide Association studies and Genome-Wide prediction.

power of GWAS experiments (rare variants)
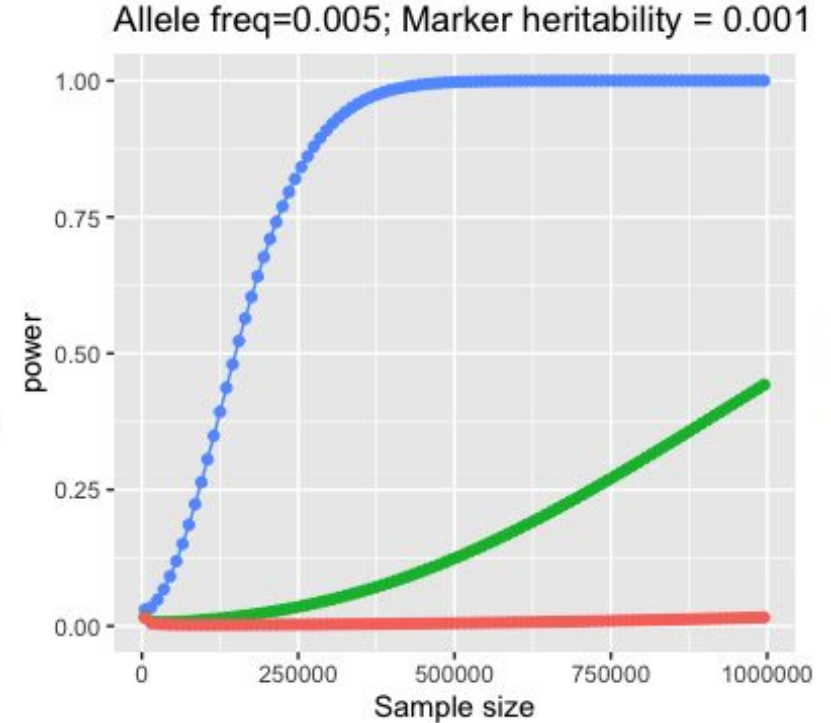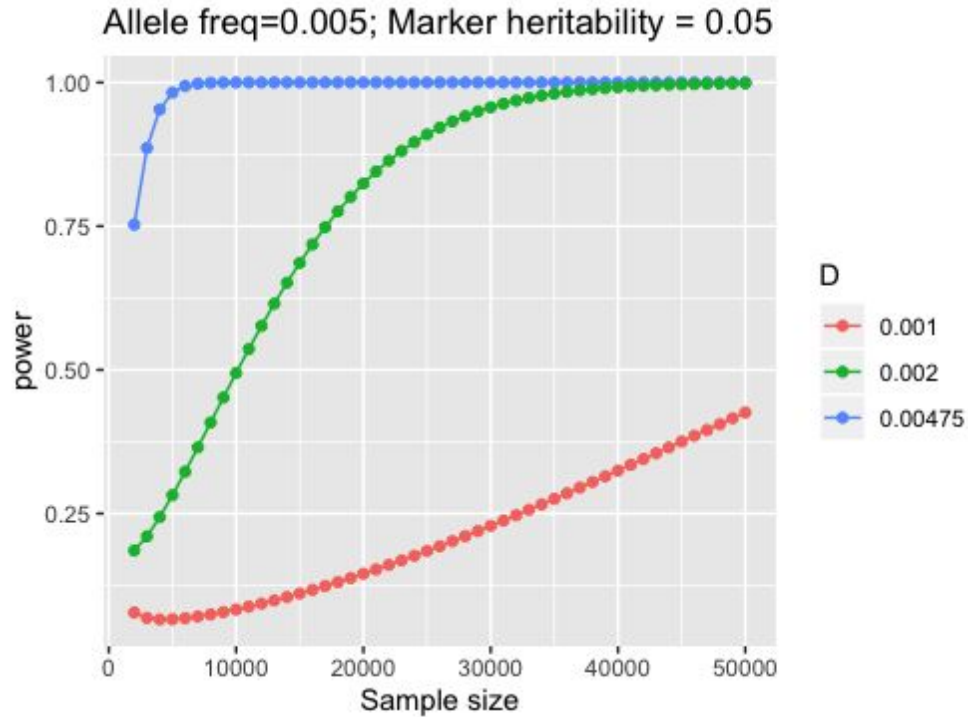
R. D. Ball (2013) Genomewide Association studies and Genome-Wide prediction.

power of GWAS experiments

- Sample size
- Magnitude of effect
- MAF marker
- MAF qtl
- Range of LD
- Likelihood of the model
- Experimental design

power of GWAS experiments

- Sample size
- Magnitude of effect
- MAF marker
- MAF qtl
- Range of LD
- Likelihood of the model
- Experimental design

Legend:
■ Highest power
■ Lowest power

Zondervan and Cardon (2004)
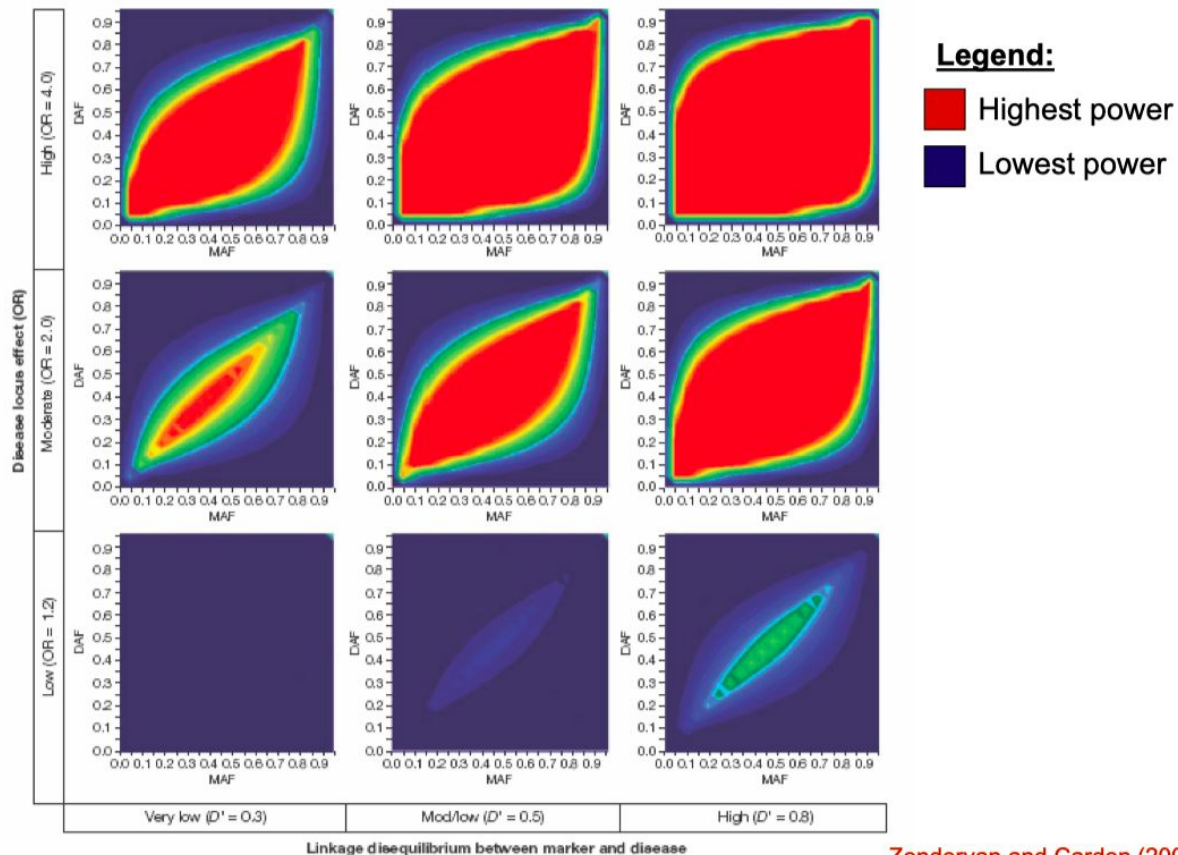
power of GWAS experiments

- MAIN DIFFICULTIES
- Low **effect size**
- Low LD

- When the allele-frequencies are mismatched, power is dramatically reduced
- We never know true disease allele frequency, so having a range of allele frequencies across markers is helpful

CAUTION

power of GWAS experiments

introduction_to_gwas/**5.power_and_significance**/StatisticalPower_exercise.R

POPULATION STRATIFICATION

Population **stratification**
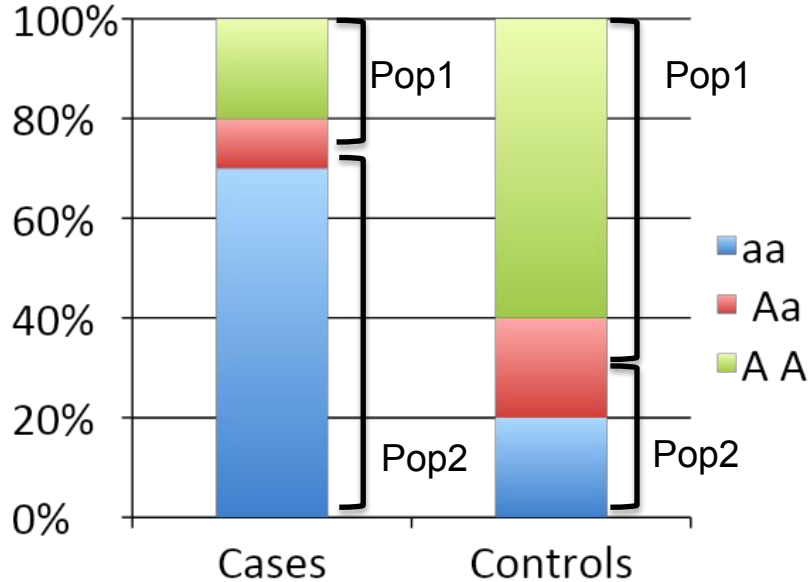
- Distribution of genotypes differs between cases and controls

- Might conclude that allele A (or genotype AA) related to disease

Separated populations

Population 1   Cases   Population 2

Genotype   aa   Aa   AA
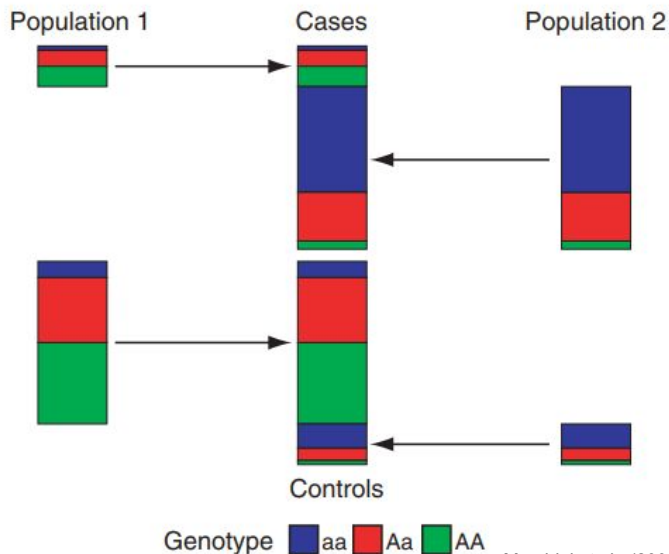
Marchini et al., (2004)

- If cases and controls not well-matched ethnically:
  - Disease is more common in one population than another AND
  - allele frequencies differ between populations at markers (chromosomal positions) unrelated to outcome

- Any allele more common in population with increased risk of disease may appear to be associated with disease

Population **stratification**

- Causes.

Unequal distribution of alleles may result from:

- – Subpopulations: sample made up of more than one distinct population, and cases and controls made up of different proportion of one or more of the subpopulations

- – Admixture: sample made up of individuals each with differing fractions of ancestry from distinct populations (e.g. African American individuals with differing levels of Caucasian ancestry) and the average level of admixture differs between cases and controls

Population **stratification**

Population **stratification**

- What can you do about this?
  - Carefully match cases and controls in the data collection stage
    - Easy at gross level, difficult when structure is subtle
    - Recommend recording grandparental birthplace

  - Use a family-based study design

  - Detect and/or control for stratification in analysis stage

Population **stratification**

- Methods to detect and control for stratification
    - Test for stratification
    - Genomic control (GC)
    - Structured analysis (SA)
    - Principal components
    - Genetic covariance structure (Kinship matrix)

Population **stratification**

- Methods to detect and control for stratification
  - Test for stratification
    - Rationale: if stratification exists, should see association with disease status across many loci, not just the candidate locus of interest
    1. Genotype a set of L unlinked (independent) marker loci in all samples of study (e.g. 15 to 20 polymorphic markers).
    2. Assume markers are chosen randomly so that the probability that any one marker is tightly linked to a disease locus is very low.
    3. Compare differences between genotype or allele frequencies between cases and controls using the χ^2 test for each marker.
    4. To test for stratification, sum individual marker test statistics to get final χ^2 statistic with L degrees of freedom.

Population **stratification**

- Methods to detect and control for stratification
  - Test for stratification
    - H_0: the allele frequencies at each of the marker loci are the same in the case and control groups.
    - H_A: the allele frequency distribution across the L loci differs between the case and control groups.

    - Strategy <u>attractive</u> for its simplicity and ability to formally test, but it <u>doesn't offer a solution</u>.

(Pritchard, JK and Rosenberg NA (1999) Use of unlinked genetic markers to detect population stratification in association studies. Am J Hum Genet 65: 220-228)

Population **stratification**

- Methods to detect and control for stratification
    - Genomic Control
        - Rationale: if population stratification exists in the study population then it should be present at many markers across the genome
        - Strategy:
        1. Genotype a set of L unlinked (independent) marker loci in all samples of study (e.g. 20 to 50 SNPs)
        2. Assume markers are chosen randomly so that the probability that any one marker is tightly linked to a disease locus is very low
        3. Compare differences between genotype or allele frequencies between cases and controls using the χ^2 test for each marker.
        4. Adjust test statistic for association based on the inflation (λ) seen across a collection of unlinked (independent) markers

Population **stratification**

- Methods to detect and control for stratification
    - Genomic Control
        - Assume <u>constant inflation factor (λ) across genome</u> (it is thought to be true regardless [almost] of allele frequency, but cannot be generalized)

        - If λ = 1 then no population stratification
        - If λ < 1 then set to 1 (bounded λ)

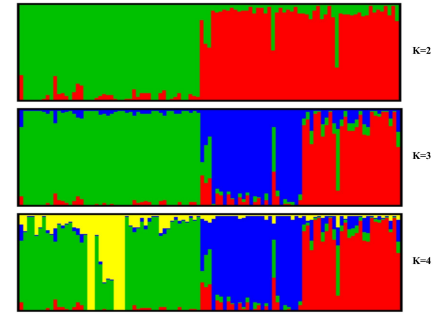   Adjust χ 2 statistic for each markers of interest: $\chi^2 = \dfrac{\chi^2}{\hat{\lambda}}$

        (Devlin B and Roeder K (1999) Genomic control for association studies. Biometrics 55:369-387)

Population **stratification**

S1 Figure. Admixture plots of K=2-4 showing population structure of different Angora sub-populations

- Methods to detect and control for stratification
  - Structured analysis
    - Rationale: genotype data on a sample of individuals for many markers can give us information about sub populations in the sample

    - Strategy: assign and control for population membership, performing analysis <u>in each</u> population (*reduce statistical power*)

    - Performed in STRUCTURE or STRAT type analyses

(Pritchard JK, Stephens M and Donnelly P (1999) Inference of population structure using multilocus genotype data. Genetics 155: 945-959)

Population **stratification**

- Methods to detect and control for stratification
  - Principal Component Analysis
    - Rationale: Similar to SA approach in that goal is to use many markers to capture variation that is due to ancestry (*GWAS lot of markers*)
    - Strategy:
    1. Get a covariate (or set of covariates) for each individual that represents their genetic ancestry from PC.
    2. Adjust phenotype and genotypes for ancestry based on the covariate that represents ancestry (*Test association of PC to detect PS, or visualization*)
    3. Adjust phenotype (outcome) and genotype (predictor) for ancestry (PC), in the association test as usual

Price et al. (2006) Principal components analysis corrects for stratification in genome-wide association studies. Nature Genetics 38:904-909

Population **stratification**

- Methods to detect and control for stratification
  - Adjust Genetic Covariance (Kinship matrix).
    - Rationale:Similar to PCA approach, but uses the whole genomic variability in the data. Additionally, it adjust the model by an overall genomic background. (need *lot of markers to be efficient*)
    - Strategy:
    1. Calculate a genomic relationship matrix (GRM)

    $$A_{jk} = \frac{1}{N}\sum_i A_{ijk} = \begin{cases} \frac{1}{N}\sum_i \frac{(x_{ij} - 2p_i)(x_{ik} - 2p_i)}{2p_i(1 - p_i)}, & j \neq k \\ 1 + \frac{1}{N}\sum_i \frac{x_{ij}^2 - (1 + 2p_i)x_{ij} + 2p_i^2}{2p_i(1 - p_i)}, & j = k \end{cases} \quad (6)$$

    2. Include a polygenic effect in a mixed model taking into account GRM

    $$\mathbf{y} = \mu\mathbf{1} + \mathbf{X}\beta + \mathbf{Z}\mathbf{u} + \mathbf{e} \qquad \mathbf{u} \sim N(0, \mathbf{A}\sigma_u^2)$$

    3. Alternative, PC from the GRM and implement PCA strategy

Population **stratification**

- Comparison of methods
  - stratification level

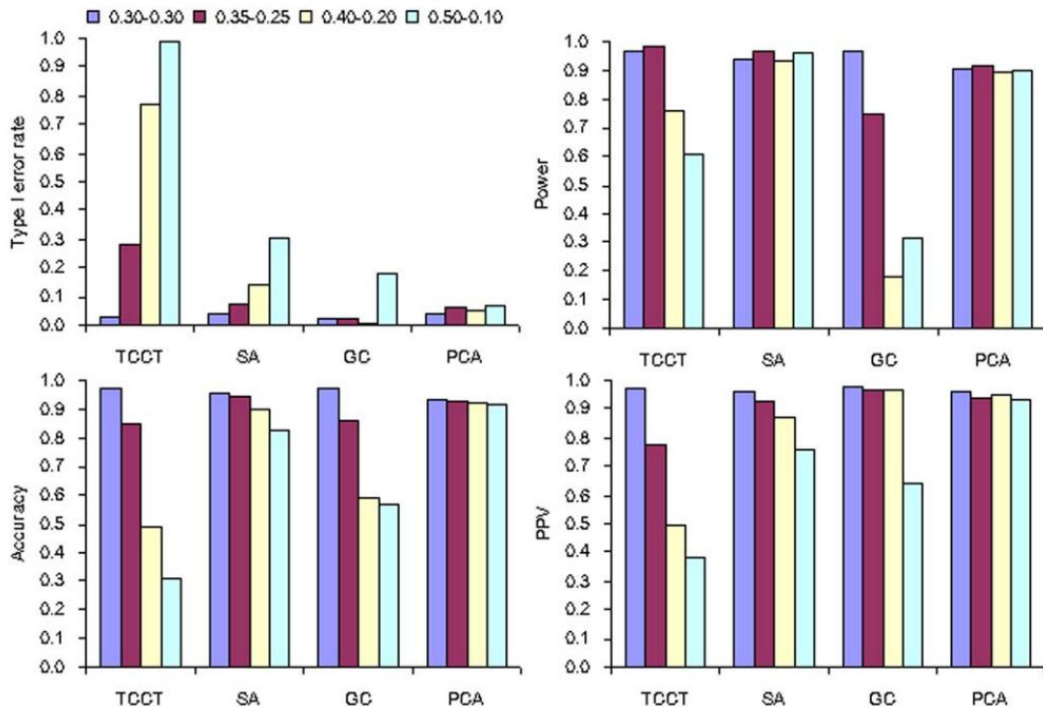Legend: ☐ 0.30-0.30 ■ 0.35-0.25 ☐ 0.40-0.20 ☐ 0.50-0.10

Figure 1. Performance of the four analytical methods in stratified populations with stratification levels varying from 0.3−0.3 to 0.5−0.1 (sample size = 1200, frequency of disease susceptible allele = 0.20±0.02 and number of AIMs = 40).
doi:10.1371/journal.pone.0003392.g001

Population **stratification**
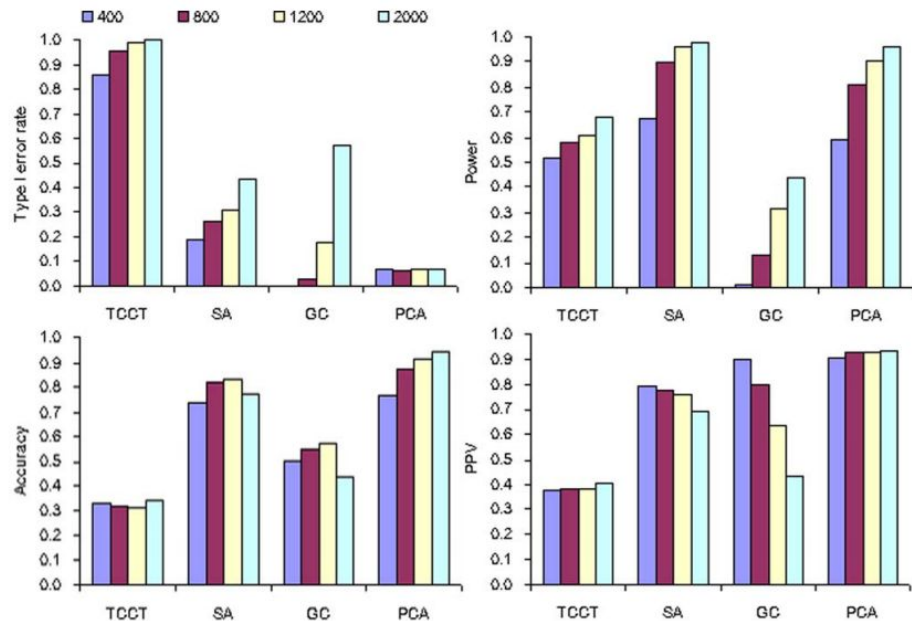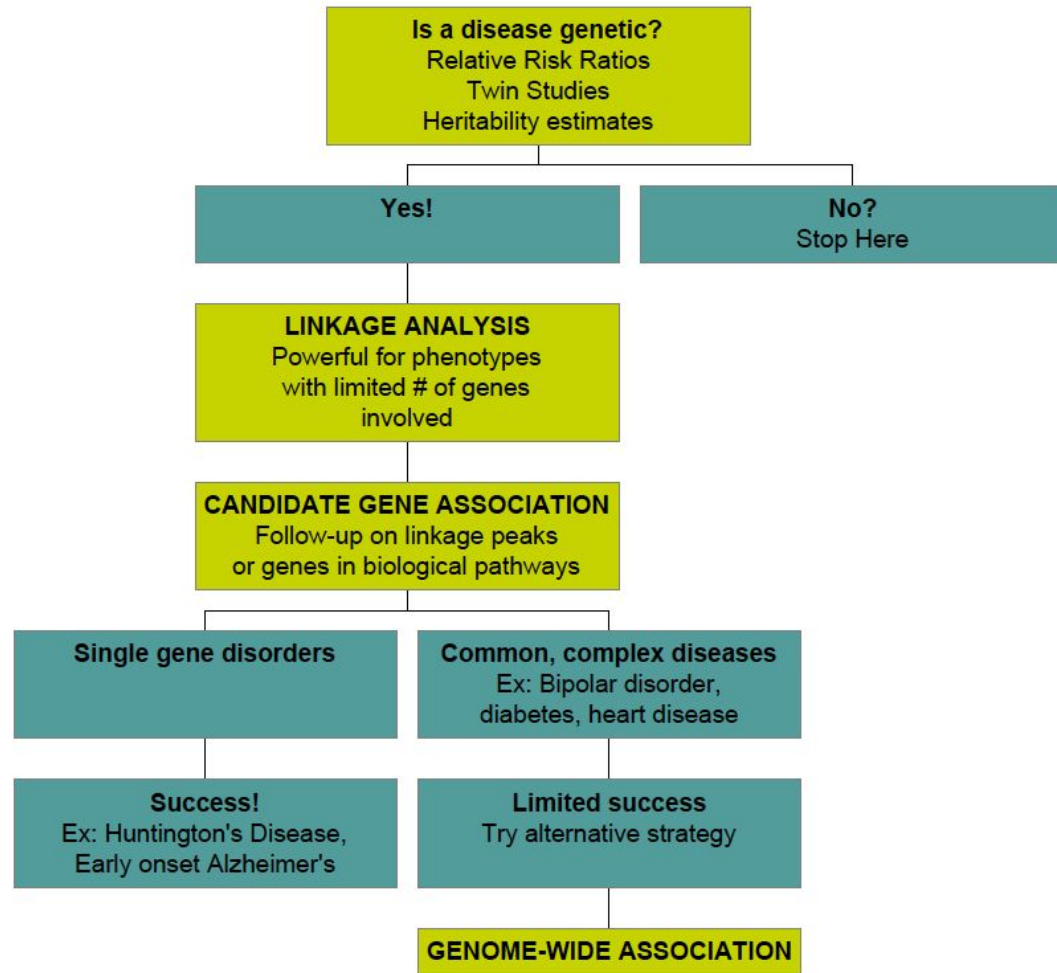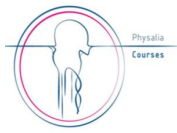
- Comparison of methods
  - sample size

Figure 2. Performance of the four analytical methods in stratified populations with sample sizes varying from 400 to 2000 (stratification level = 0.5−0.1, frequency of disease susceptible allele = 0.20±0.02 and number of AIMs = 40).
doi:10.1371/journal.pone.0003392.g002

Zhang F, Wang Y, Deng H-W (2008) Comparison of Population-Based Association Study Methods Correcting for Population Stratification. PLoS ONE 3(10): e3392.
doi:10.1371/journal.pone.0003392

Experimental **design**

Experimental **design**

- Collaboration, collaboration, collaboration

- Collaboration with many disciplines epidemiologists, biostatisticians, bioinformatics, clinicians, geneticists, etc.

- Collaboration across funded research centers to pool and share results

- Analysis methods and techniques are constantly changing

- High dimensional data (p >>>>> n)
  - Large p, small n problem

Experimental **design**

1. SNP QUALITY CONTROL

Filter process to end up with the highest quality set of SNPs
- SNP genotyping rates: usually >95%
- Sample call rates: usually >95%
- Minor allele frequency: ranges >0.002 - 0.05
- Hardy Weinberg Equilibrium: p-value threshold ranges >10^{-3} - 10^{-6}
- Mendelian inconsistencies - need "trios"
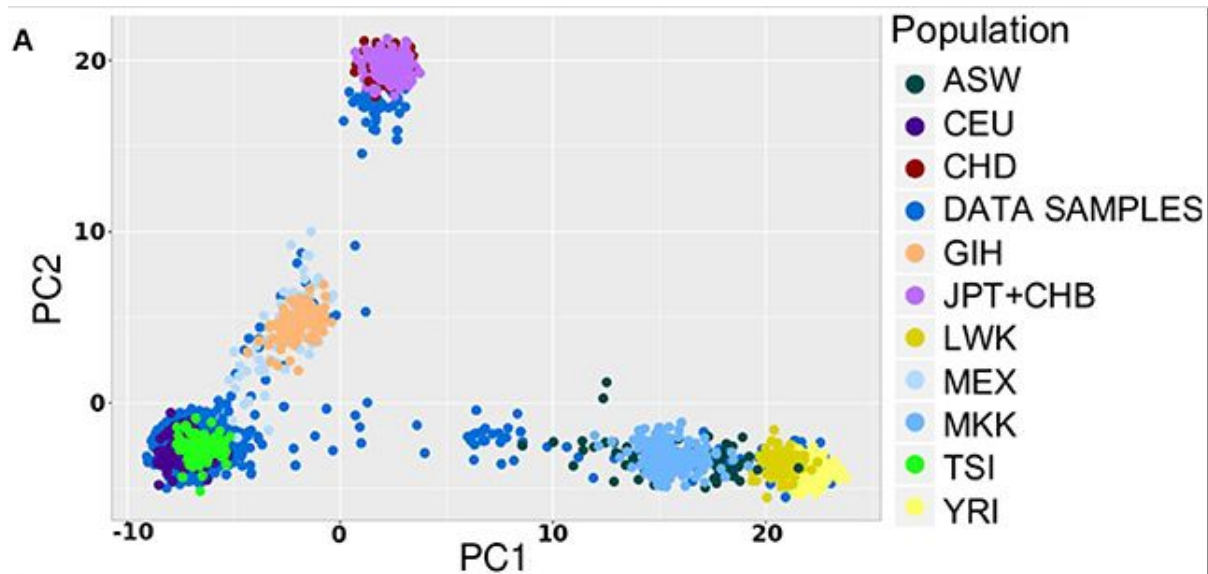
Retain approximately 75-80% (or more) of the SNPs

2. POPULATION STRATIFICATION

Classic issue of confounding

- Test for PS
- Correct for PS

3. ADJUSTMENT OF COVARIATES
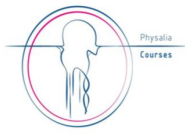
Rationale for adjustment in GWAS

1. Confounding

2. Increase ability to predict outcome or explain more variation in the trait -- "what can genetics provide beyond established risk factors?"

What did everyone else do?

- Replication or comparing results across studies requires a similar analysis strategy and adjustment model.

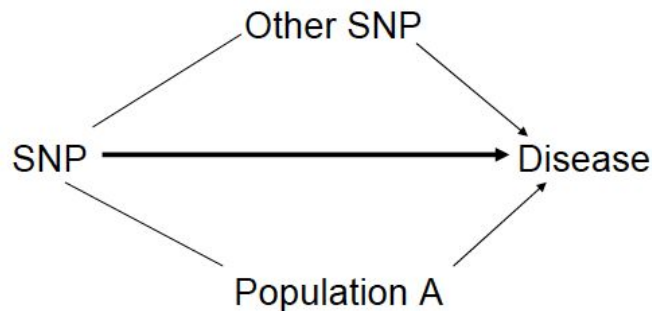4. MULTIPLE TESTING

5. INTERPRETING THE RESULTS

So you think you have a significant SNP?

- Direct causal relationship
- Indirect association - linkage disequilibrium
- Spurious association - population stratification or false positive

6. REPLICATION