# Tutorial on Genome-Wide Association Studies

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- Dana Crawford
- Holli Dilks-Hutchinson
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# Key References

OPEN ACCESS Freely available online

PLOS | COMPUTATIONAL BIOLOGY

Education

## Chapter 11: Genome-Wide Association Studies

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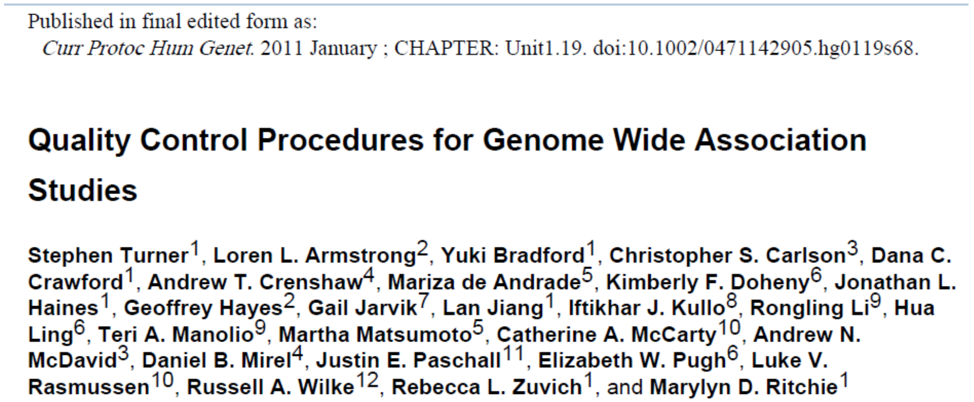
- www.ploscollections.org/translationalbioinformatics



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## Quality Control Procedures for Genome Wide Association Studies

Stephen Turner[1], Loren L. Armstrong[2], Yuki Bradford[1], Christopher S. Carlson[3], Dana C. Crawford[1], Andrew T. Crenshaw[4], Mariza de Andrade[5], Kimberly F. Doheny[6], Jonathan L. Haines[1], Geoffrey Hayes[2], Gail Jarvik[7], Lan Jiang[1], Iftikhar J. Kullo[8], Rongling Li[9], Hua Ling[6], Teri A. Manolio[9], Martha Matsumoto[5], Catherine A. McCarty[10], Andrew N. McDavid[3], Daniel B. Mirel[4], Justin E. Paschall[11], Elizabeth W. Pugh[6], Luke V. Rasmussen[10], Russell A. Wilke[12], Rebecca L. Zuvich[1], and Marylyn D. Ritchie[1]

# Overview

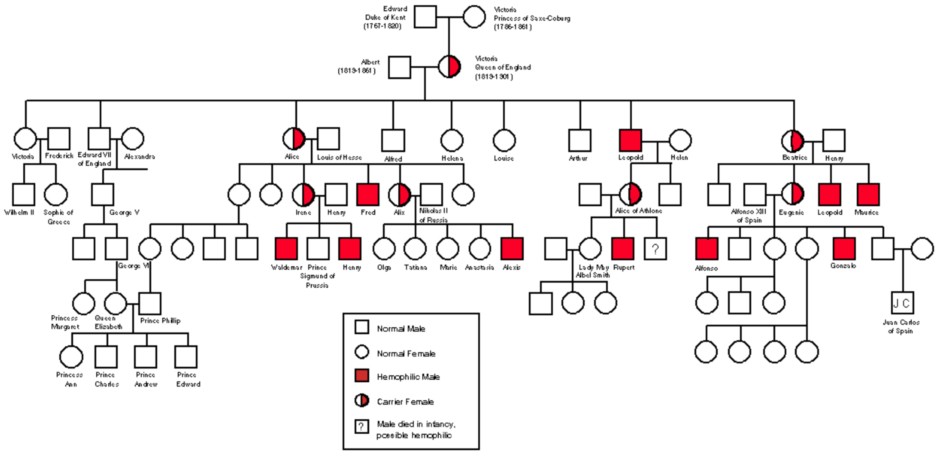
- Common Study Designs for GWAS
- Quality Control Procedures for GWAS Data
- Statistical Analysis
- Replication

# Goals of a Genetic Study

- Determine if there is a genetic component (heritability)
- Describe mode of inheritance (segregation analysis)
- Determine the effect size of the genetic component
- Identify the gene causing the disease

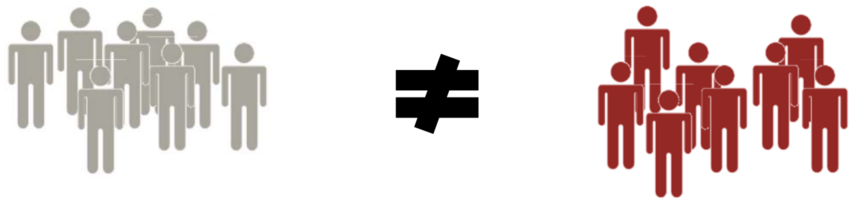
# Family Studies

- Allows estimation of genetic component
- Allows examination of mode of inheritance
- Difficult to collect
- Power derived from the number of families



# Population Studies

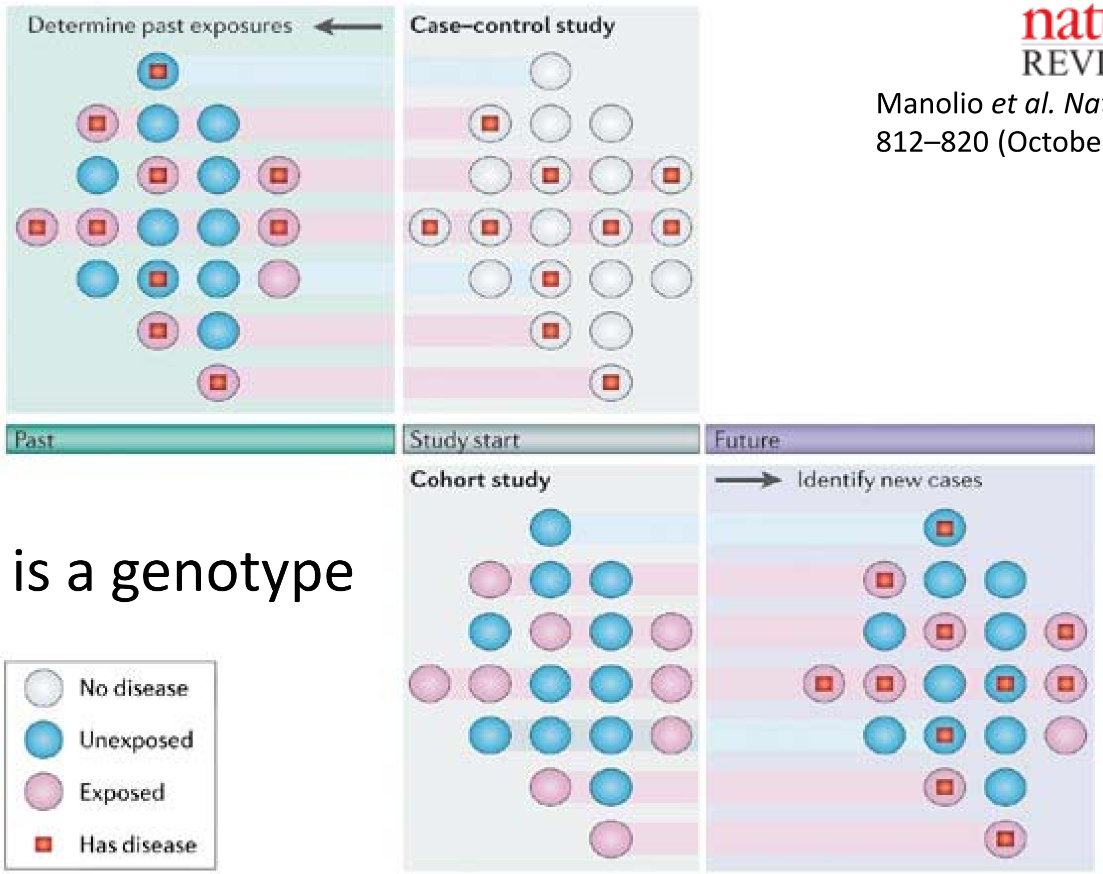
- Easier to collect
- Larger sample sizes
- Assumes there is a genetic component
- Power derived from ratio of controls to cases



# Retrospective vs. Prospective

nature REVIEWS GENETICS

Manolio *et al. Nature Reviews Genetics* 7, 812–820 (October 2006)



Exposure is a genotype

Nature Reviews | Genetics

# Choosing a Study Design

- What samples are available?
- Is a genetic component known?
- Details of the trait being studied (age at onset, disease frequency, penetrance, etc.)
- Interest in other factors of disease (environmental exposures, survival, effect size)

# Choosing a Study Design

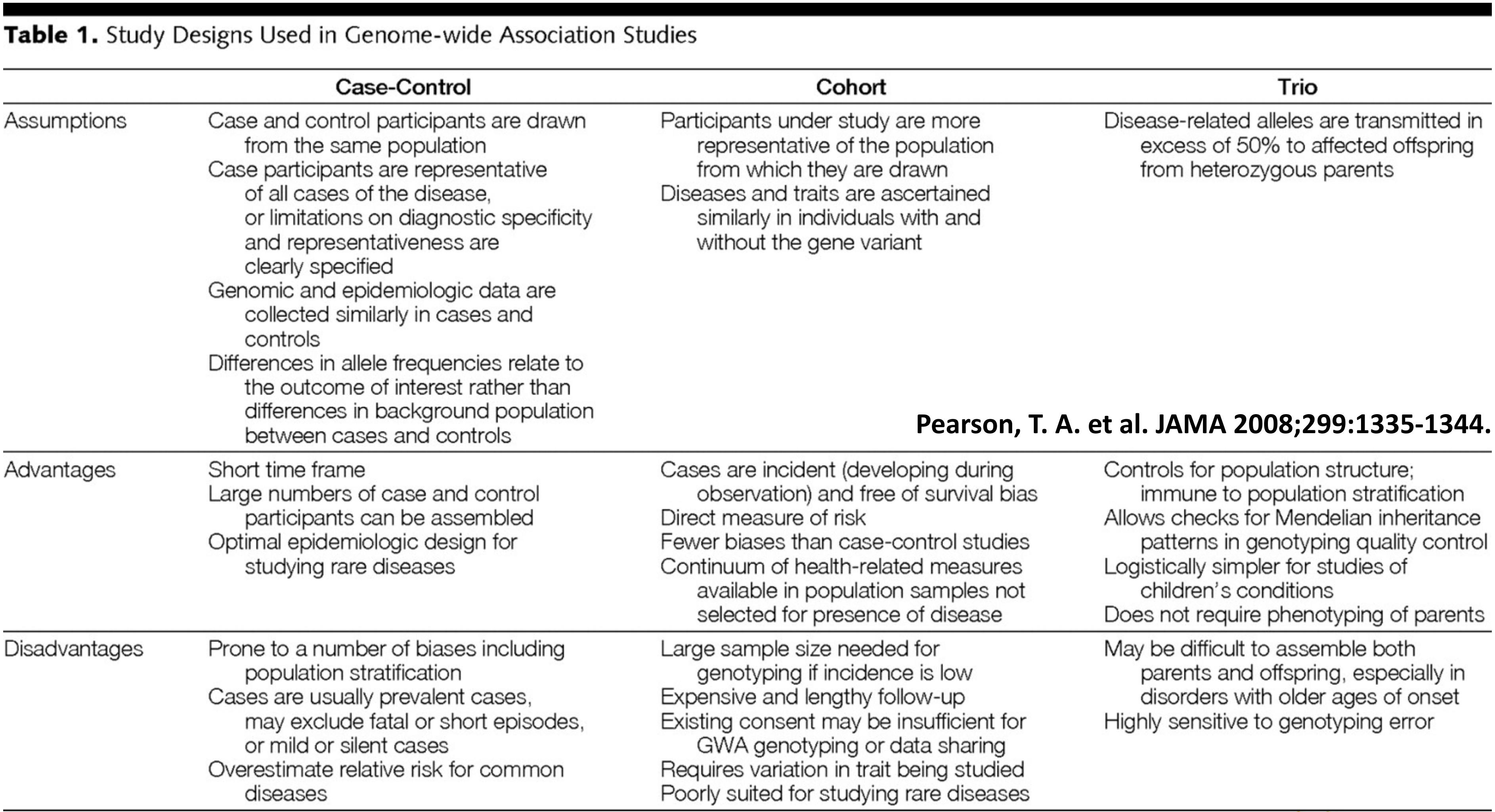


**Table 1.** Study Designs Used in Genome-wide Association Studies

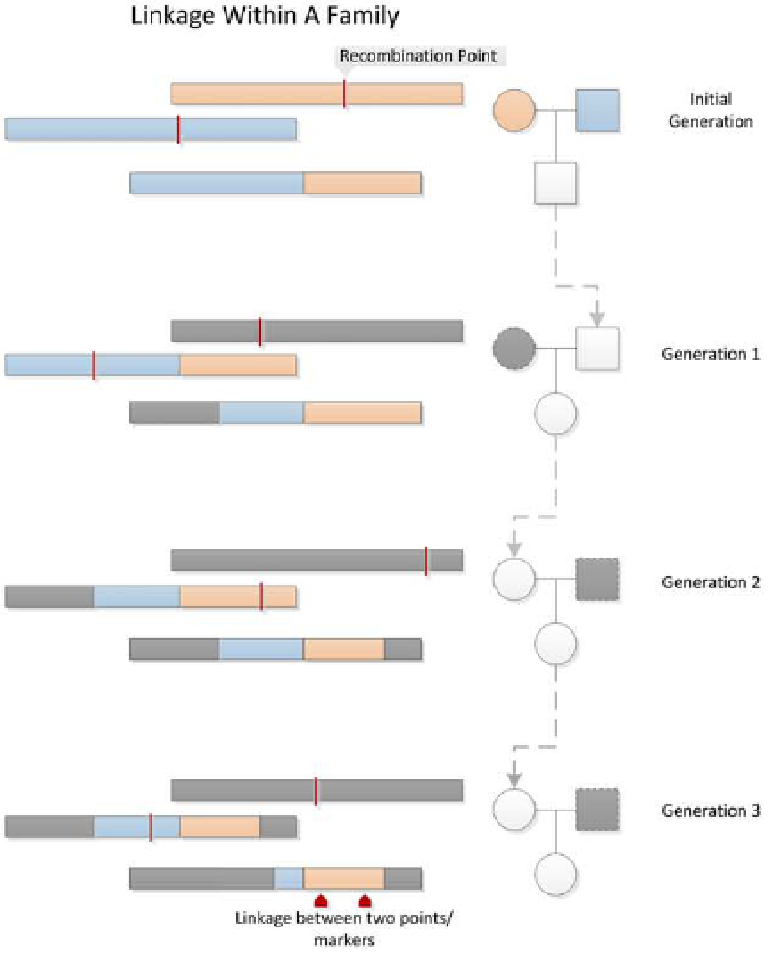
| | Case-Control | Cohort | Trio |
|---|---|---|---|
| Assumptions | Case and control participants are drawn from the same population<br>Case participants are representative of all cases of the disease, or limitations on diagnostic specificity and representativeness are clearly specified<br>Genomic and epidemiologic data are collected similarly in cases and controls<br>Differences in allele frequencies relate to the outcome of interest rather than differences in background population between cases and controls | Participants under study are more representative of the population from which they are drawn<br>Diseases and traits are ascertained similarly in individuals with and without the gene variant | Disease-related alleles are transmitted in excess of 50% to affected offspring from heterozygous parents |
| Advantages | Short time frame<br>Large numbers of case and control participants can be assembled<br>Optimal epidemiologic design for studying rare diseases | Cases are incident (developing during observation) and free of survival bias<br>Direct measure of risk<br>Fewer biases than case-control studies<br>Continuum of health-related measures available in population samples not selected for presence of disease | Controls for population structure; immune to population stratification<br>Allows checks for Mendelian inheritance patterns in genotyping quality control<br>Logistically simpler for studies of children's conditions<br>Does not require phenotyping of parents |
| Disadvantages | Prone to a number of biases including population stratification<br>Cases are usually prevalent cases, may exclude fatal or short episodes, or mild or silent cases<br>Overestimate relative risk for common diseases | Large sample size needed for genotyping if incidence is low<br>Expensive and lengthy follow-up<br>Existing consent may be insufficient for GWA genotyping or data sharing<br>Requires variation in trait being studied<br>Poorly suited for studying rare diseases | May be difficult to assemble both parents and offspring, especially in disorders with older ages of onset<br>Highly sensitive to genotyping error |

**Pearson, T. A. et al. JAMA 2008;299:1335-1344.**

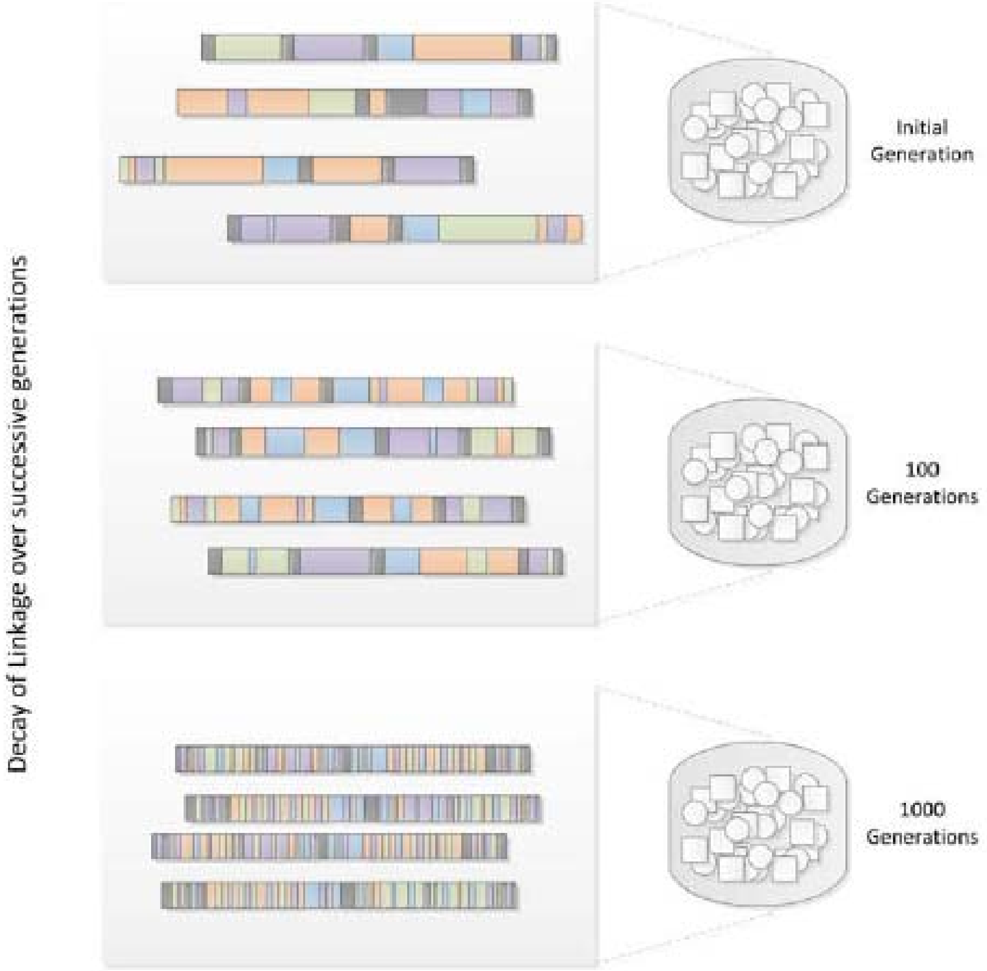
# Assumptions of GWAS

- Examines *only* the Common Disease – Common Variant hypothesis
- Relies on dense sets of genetic markers
- Exploits linkage disequilibrium to make "indirect associations"
- Goal: Identify markers with significant associations to disease

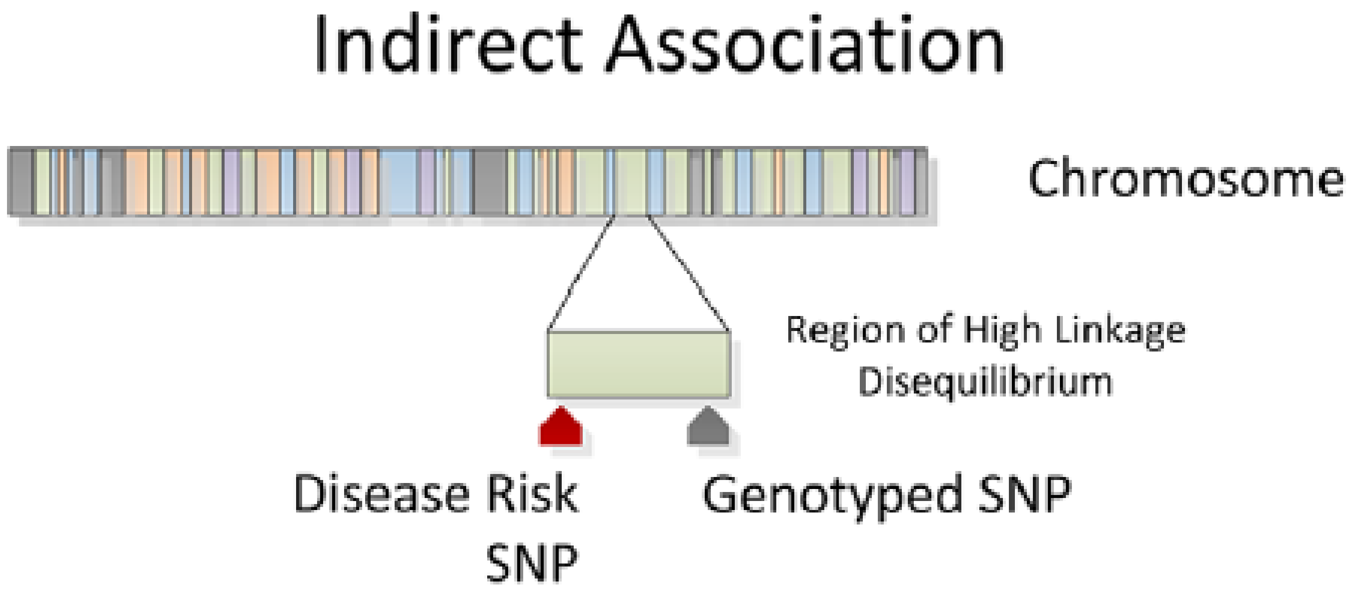
# Recombination



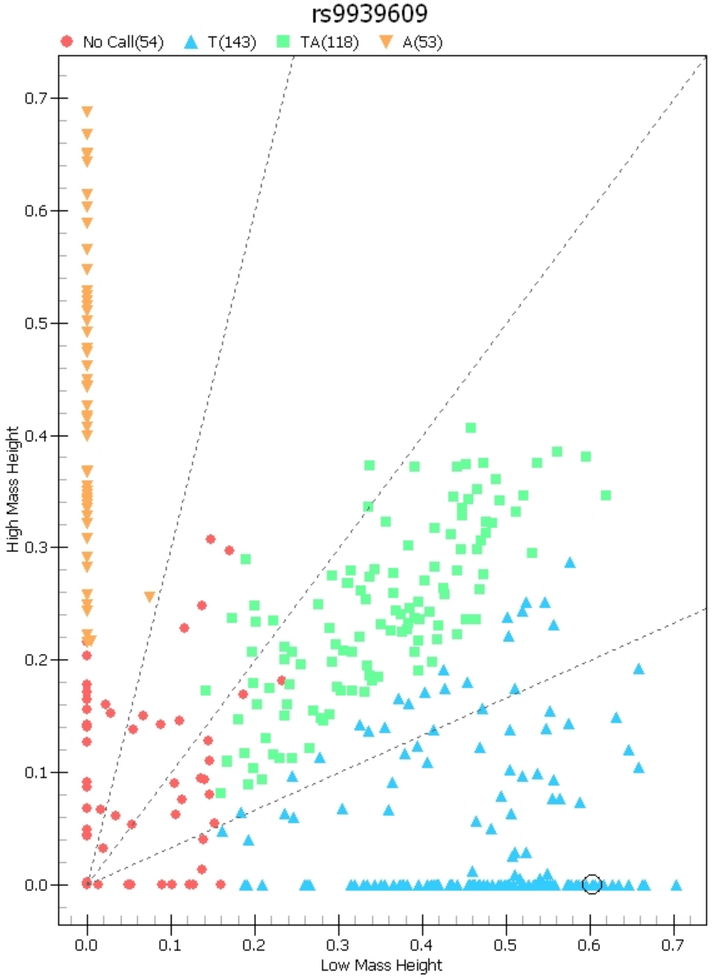
# Linkage Disequilibrium

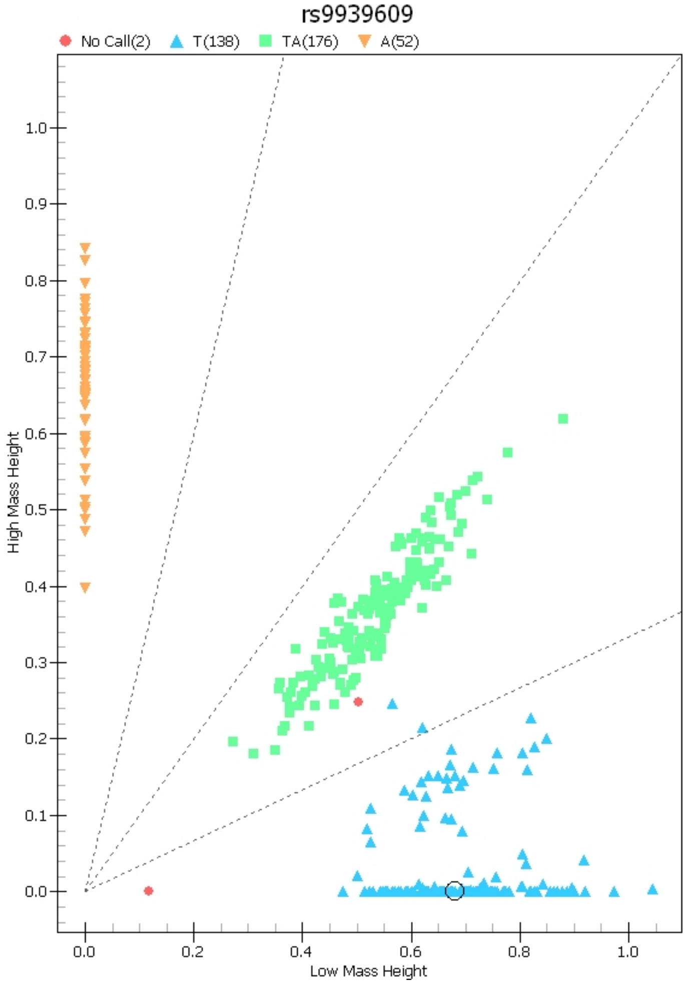


# Exploiting Linkage Disequilibrium



# How GWAS Data is Generated

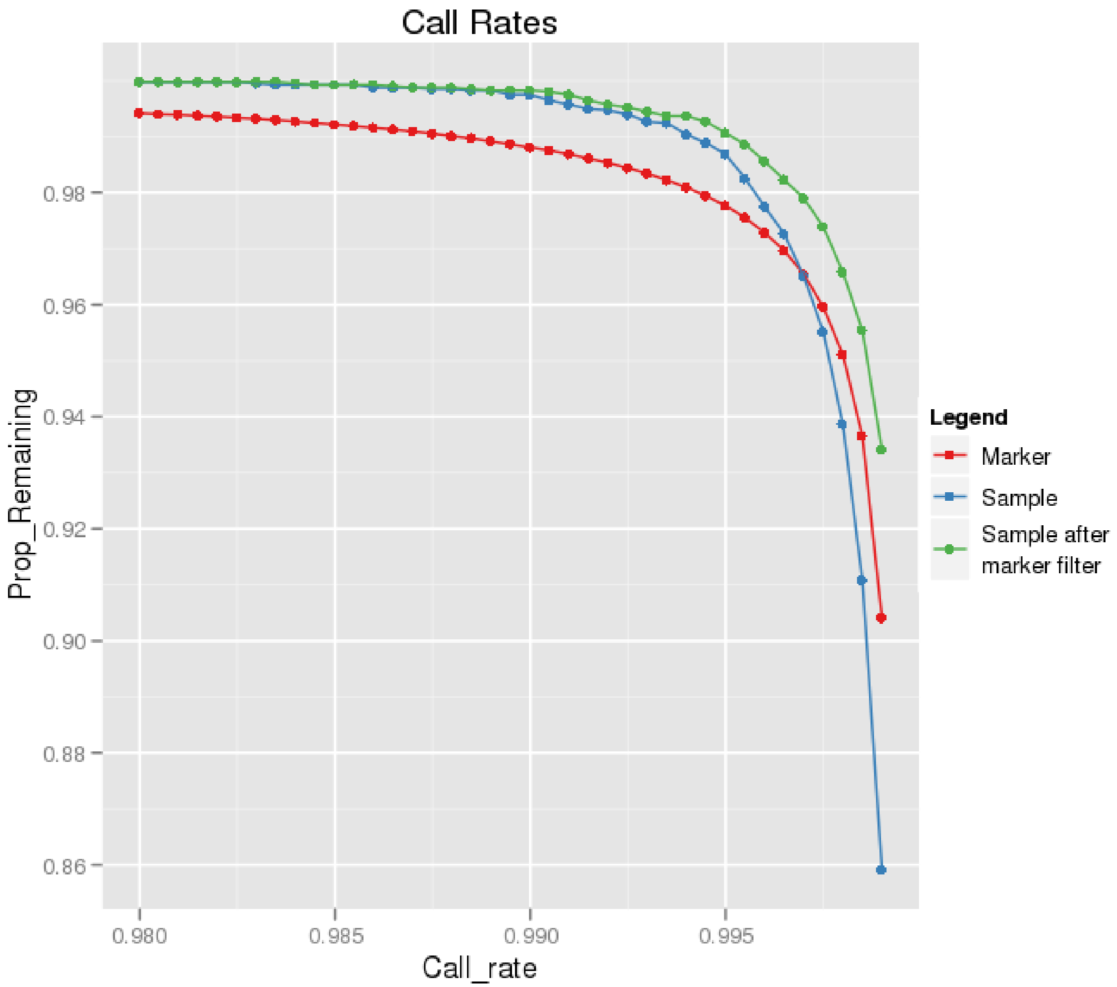




# Quality Control of GWAS Data

| Variable | Comments |
|---|---|
| Genotyping Call Rate | Low call rate often correlates with error. Some low call rate SNPs or samples may still be good. |
| Genotyping Quality | Worse quality score (GenCall) correlates strongly with error rate |
| Sex concordance | Check expectations for X marker heterozygosity and Y marker positive results. Can estimate error rate. |
| Sample Relatedness | Check for related samples (expected or unexpected) |
| Mendelian Inheritance Errors | For trio/family data, can identify problem samples and families. Can estimate error rate. |
| Replicate concordance | Check for consistent genotype calls in duplicate samples |
| Batch effects | Check for genotyping call differences due to plate |
| Hardy-Weinberg Equilibrium | Violation across all sample groups may indicate error, but can also be a good test of association |
| Population Stratification | Check for population substructure using the genome-wide data |

# Marker and Sample Call Rate



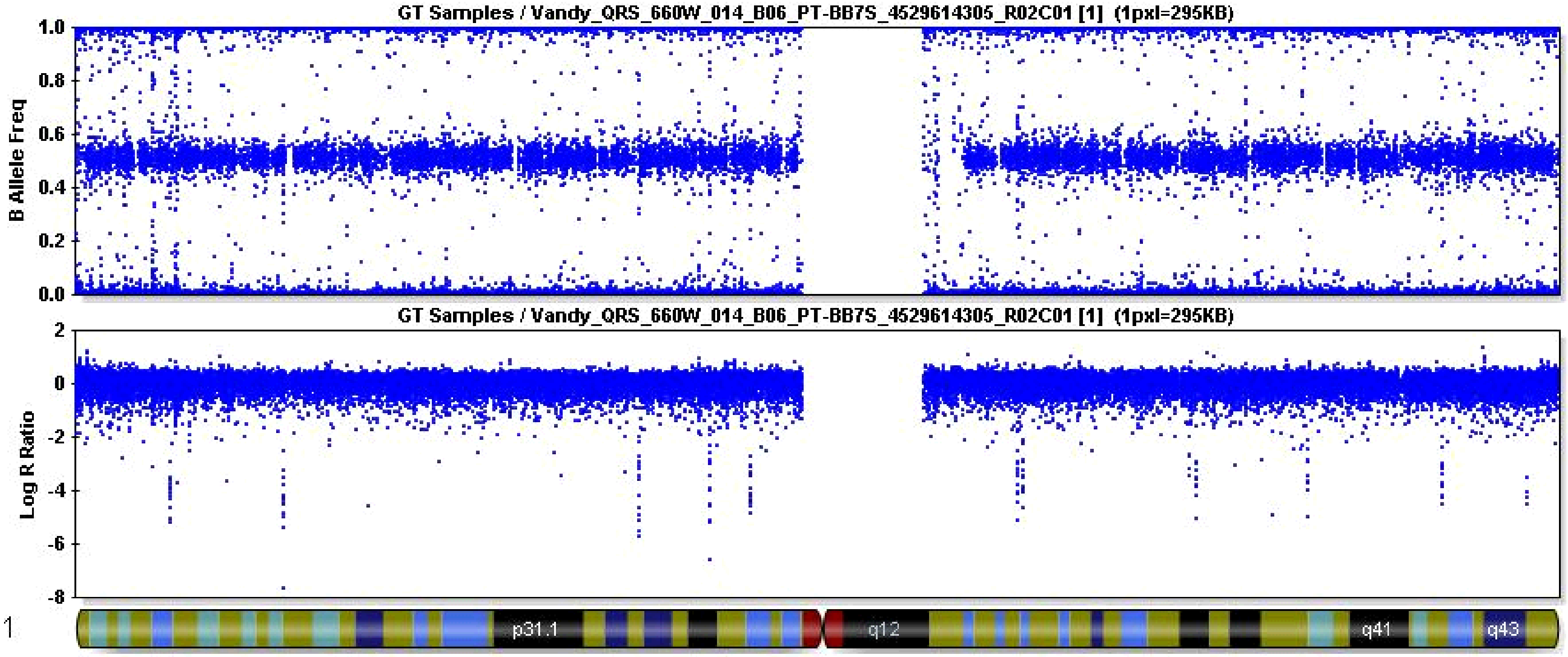
# Sex Concordance Check

| emerge_id | Pedsex | SNPsex | PLINK_F | Note |
|---|---|---|---|---|
| 16230834 | 2 | 0 | 0.4746 | CIDR comment after review of B allele freq and Log R ratio plots for all chromosomes: This sample has large loss-of-heterozygosity (LOH) blocks on X (and other autosomes). The sample is definitely female (2 X chromosomes by intensities). |
| 16228083 | 2 | 0 | 0.2654 | Same as above |
| 16231930 | 2 | 0 | 0.4376 | Same as above |
| 16233764 | 2 | 0 | 0.2603 | Same as above |
| 16221112 | 2 | 0 | 0.2048 | XX/XO mosaic not caught by initial check completed by CIDR |
| 16222319 | 2 | 0 | 0.7452 | Annotation by CIDR at data release: Appears to be XX/XO mosaic |
| 16228204 | 2 | 1 | 1 | Annotation by CIDR at data release: Appears to be XX/XO mosaic |
| 16233113 | 1 | 0 | 0.4752 | Annotation by CIDR at data release: Appears to be XXY |
| 16214881 | 1 | 2 | 0.136 | Annotation by CIDR at data release: Appears to be XXY/XY mosaic |

- Female: pedsex=2, SNPsex=2
- Male: pedsex= 1, SNPsex=1
- A male call is made if the F (actual X chromosome inbreeding estimate) is more than 0.8; a female call is made if the F is less than 0.2.

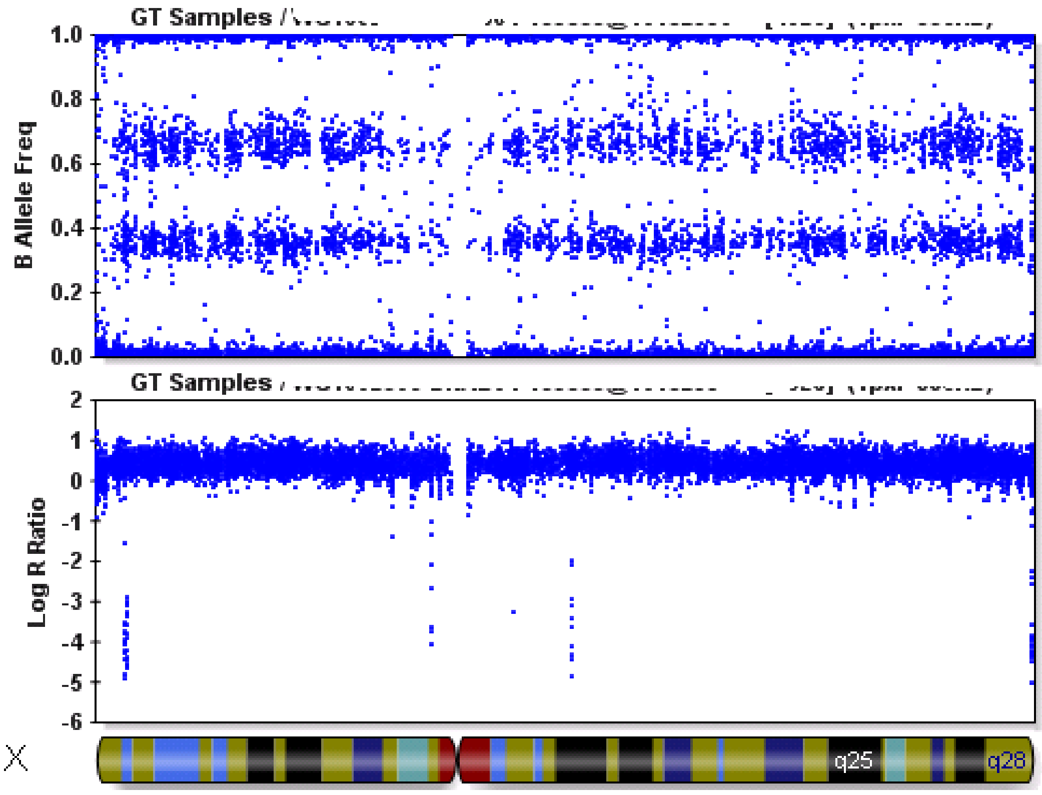
# Sex Concordance Check

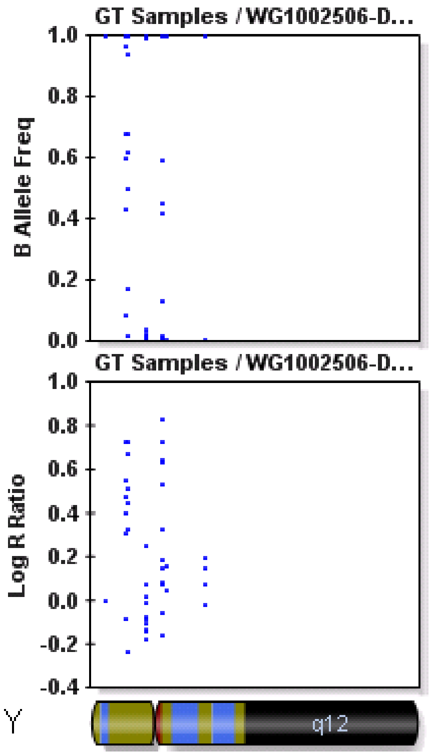
- Normal Chromosome 1



# Sex Concordance

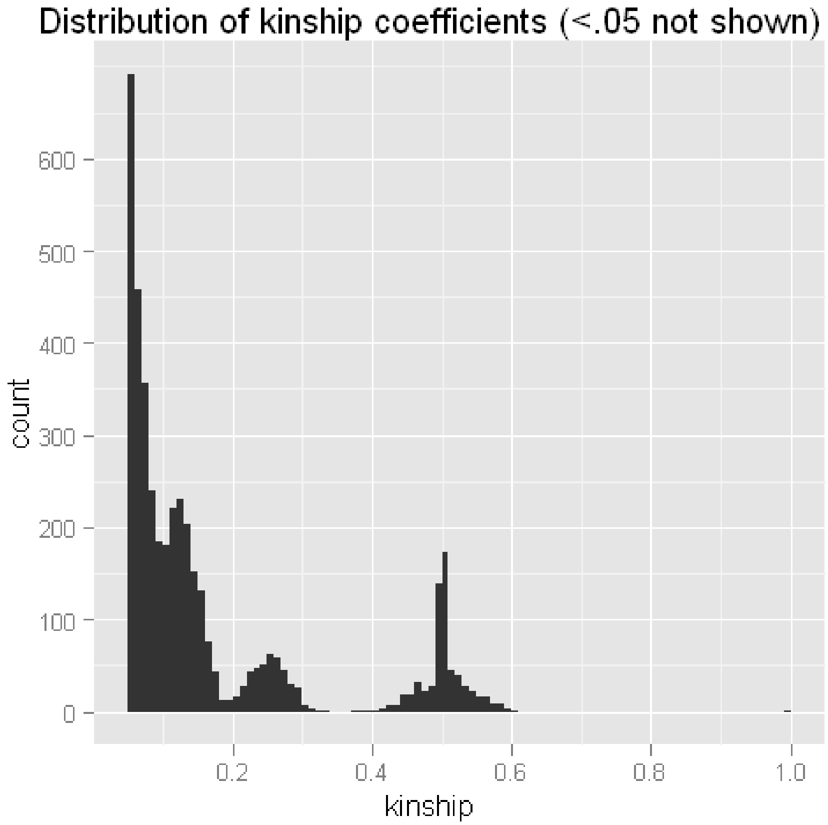
- Possible XXY/XY Mosaic



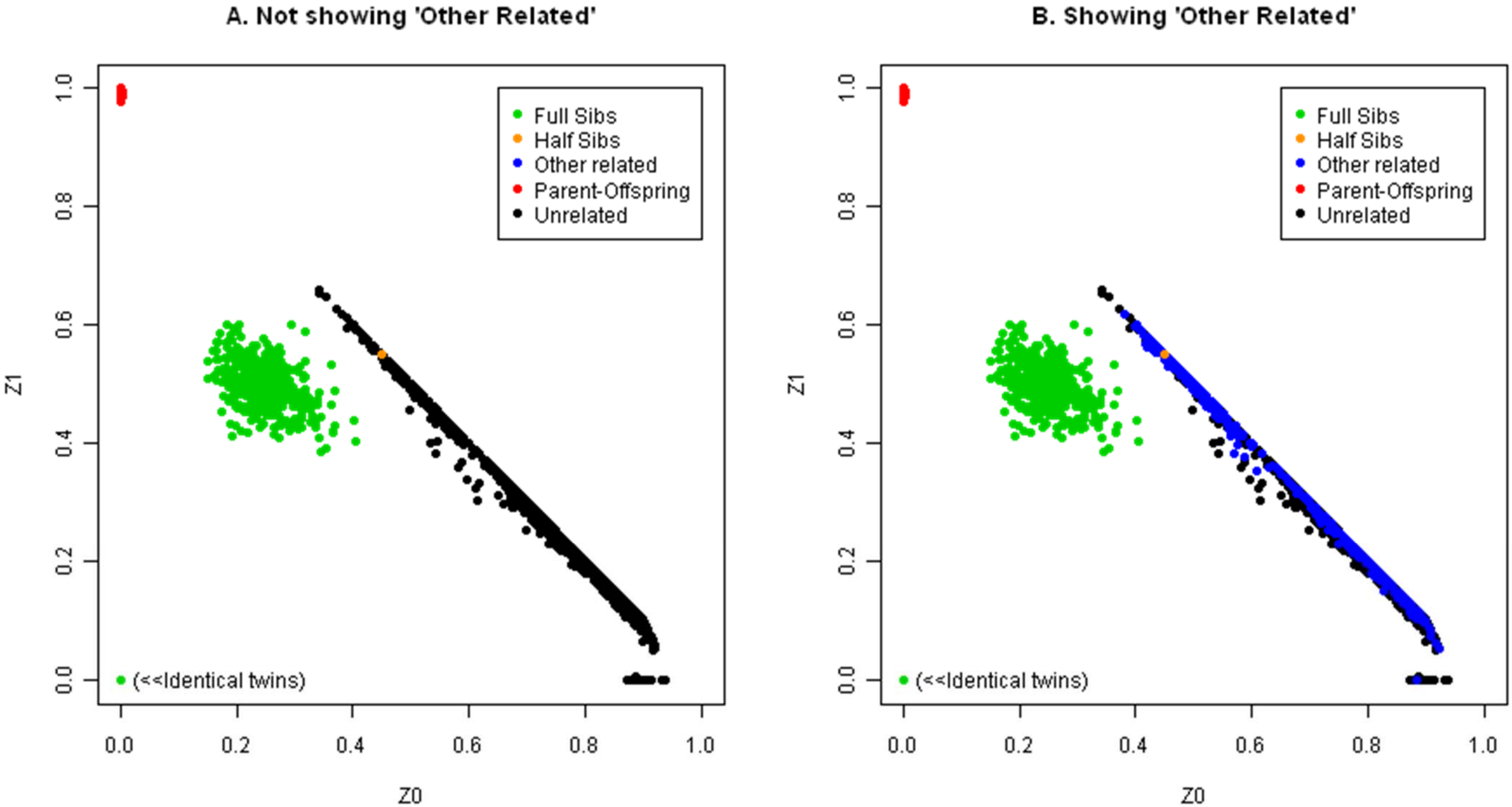


# Sample Relatedness

| Z0 | Z1 | Z2 | Kinship | Relationship |
|---|---|---|---|---|
| 0.0 | 0.0 | 1.0 | 1.0 | MZ twin or duplicate |
| 0.0 | 1.0 | 0.0 | 0.50 | Parent-offspring |
| 0.25 | 0.50 | 0.25 | 0.50 | Full siblings |
| 0.50 | 0.50 | 0.0 | 0.25 | Half siblings |
| 0.75 | 0.25 | 0.0 | 0.125 | Cousins |
| 1.0 | 0.0 | 0.0 | 0.0 | Unrelated |



# Sample Relatedness


A. Not showing 'Other Related'

B. Showing 'Other Related'

http://www.sph.umich.edu/csg/abecasis/publications/11524377.html

# Mendelian Inheritance Errors

- Even with Case/Control data, HapMap trios are typically plated with study samples for QC

| Number Mendelian Errors | Number SNPs pre QC | Number SNPs post marker QC |
|---|---|---|
| 0 | 558821 | 552346 |
| 1 | 1519 | 1353 |
| 2 | 97 | 64 |
| 3 | 5 | 1 |

# Sample Replicate Concordance

| emerge | Samp1 | samp2 | discordant | total | concordance_rate |
|---|---|---|---|---|---|
| 16231453 | A | B | 171 | 558882 | 0.99969 |
| 16223704 | A | B | 137 | 557783 | 0.99975 |
| 16216270 | A | B | 133 | 559711 | 0.99976 |
| 16230108 | A | B | 69 | 559341 | 0.99987 |
| 16224359 | A | B | 67 | 558868 | 0.99988 |
| 16234120 | A | B | 43 | 560202 | 0.99992 |
| 16232463 | A | B | 42 | 560355 | 0.99992 |
| 16234233 | A | B | 33 | 560384 | 0.99994 |
| 16216349 | A | B | 30 | 559345 | 0.99994 |
| 16215309 | A | B | 12 | 560041 | 0.99997 |
| 16224779 | A | B | 7 | 560412 | 0.99998 |
| 16231724 | A | B | 5 | 560427 | 0.99999 |
| 16233841 | A | B | 4 | 560519 | 0.99999 |
| 16221647 | A | B | 2 | 560457 | 0.99999 |
| 16230404 | A | B | 2 | 560309 | 0.99999 |
| 16226433 | A | B | 2 | 560500 | 0.99999 |
| 16234367 | A | B | 2 | 560373 | 0.99999 |
| 16224635 | A | B | 1 | 560560 | 0.99999 |
| 16219214 | A | B | 1 | 560535 | 0.99999 |
| 16231219 | A | B | 1 | 560547 | 0.99999 |
| 16220060 | A | B | 0 | 560580 | 1 |

# Hardy Weinberg Equilibrium

## All individuals

| threshhold | below | exp_below | excess_below |
|---|---|---|---|
| 0.05 | 37690 | 28022 | 9668 |
| 0.01 | 12774 | 5604 | 7170 |
| 0.001 | 4766 | 560 | 4206 |
| 1.00E-04 | 2949 | 56 | 2893 |
| 1.00E-05 | 2337 | 5 | 2332 |
| 1.00E-06 | 2004 | 0 | 2004 |
| 1.00E-07 | 1785 | 0 | 1785 |

## All cases

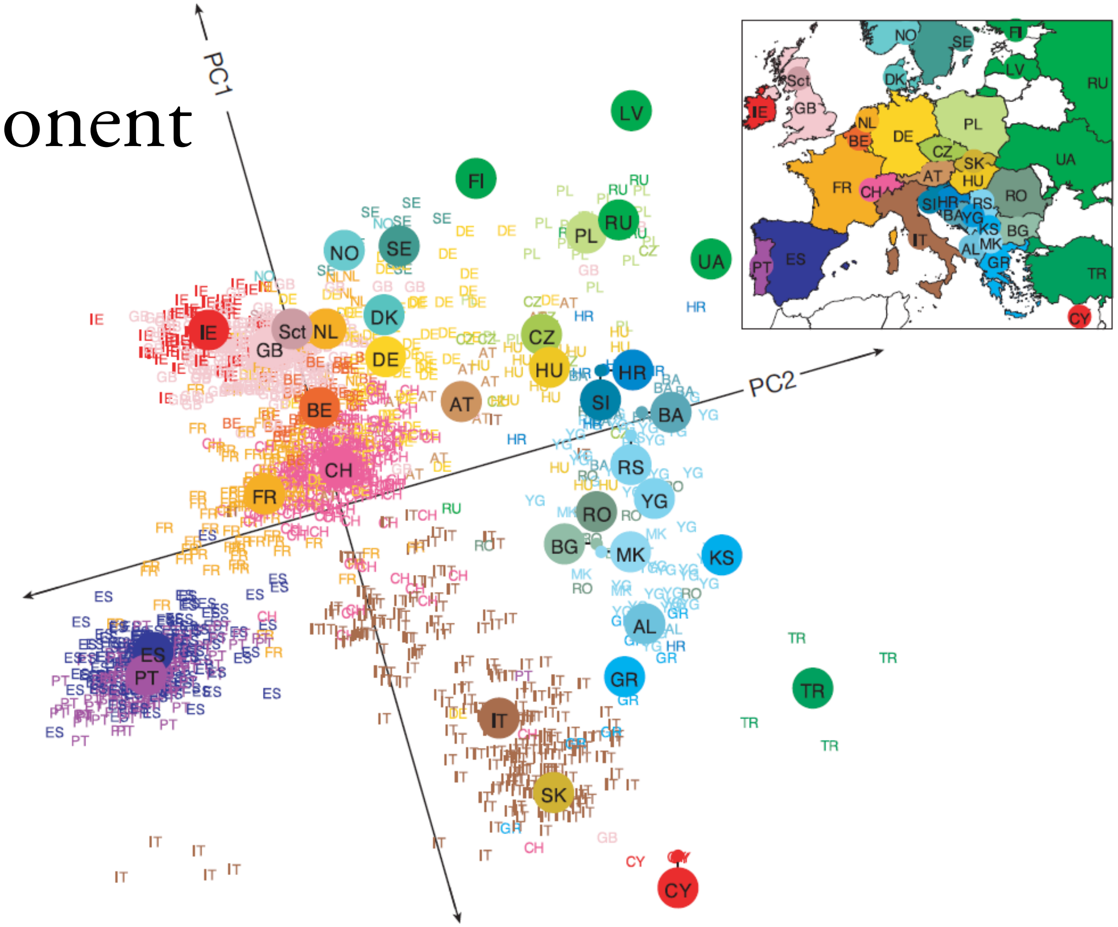
| threshold | below | exp_below | excess_below |
|---|---|---|---|
| 0.05 | 34646 | 28022 | 6624 |
| 0.01 | 10843 | 5604 | 5239 |
| 0.001 | 3642 | 560 | 3082 |
| 1.00E-04 | 2194 | 56 | 2138 |
| 1.00E-05 | 1792 | 5 | 1787 |
| 1.00E-06 | 1563 | 0 | 1563 |
| 1.00E-07 | 1394 | 0 | 1394 |

## All controls

| threshold | below | exp_below | excess_below |
|---|---|---|---|
| 0.05 | 30557 | 28022 | 2535 |
| 0.01 | 8859 | 5604 | 3255 |
| 0.001 | 2614 | 560 | 2054 |
| 1.00E-04 | 1517 | 56 | 1461 |
| 1.00E-05 | 1180 | 5 | 1175 |
| 1.00E-06 | 982 | 0 | 982 |
| 1.00E-07 | 860 | 0 | 860 |

# Population Stratification

- Principal Component Analysis (PCA)
- Can cause confounding



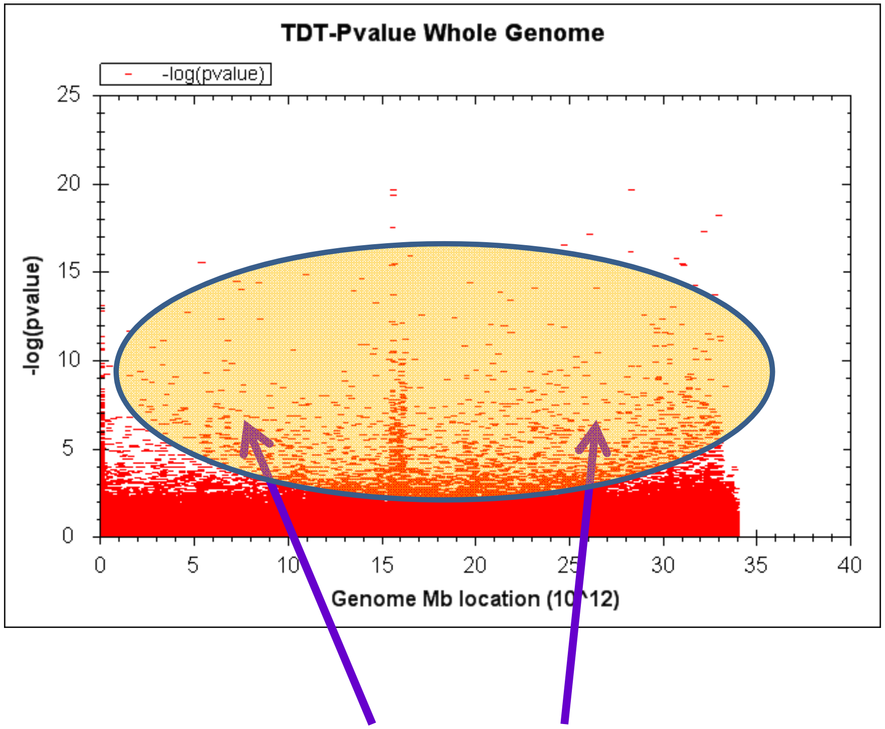
Genes Mirror Geography in Europe, Novembre, Nature Genetics, 2008

# Batch Effects

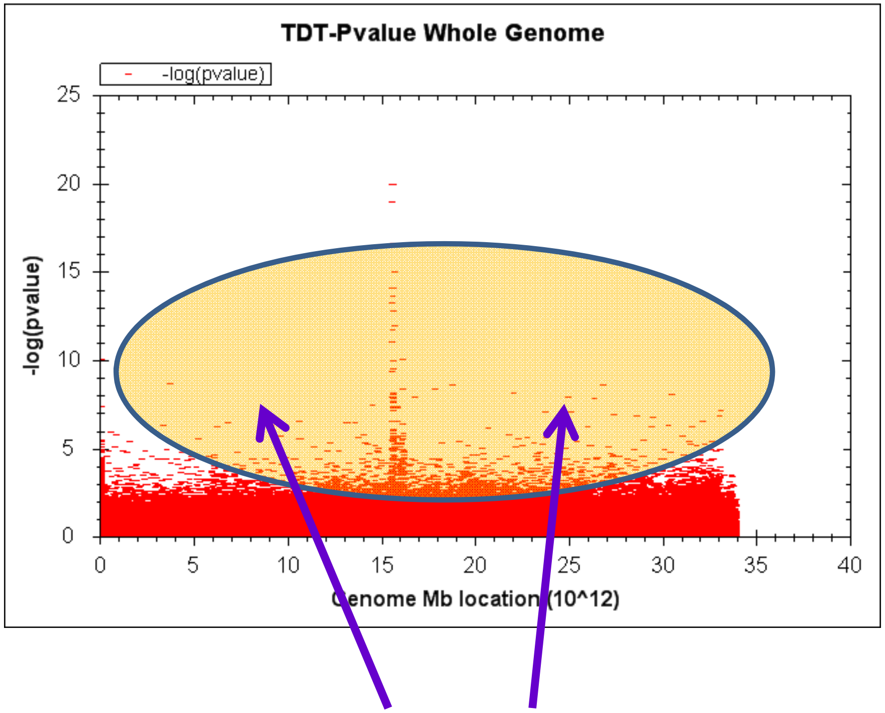
- Evidence that associations can result due to allele frequency difference due to plate effects
- Careful consideration when creating plate maps
  - Plate cases and controls together
  - Randomize by race, gender, age, BMI, others…
- After genotyping look for plate effects
  - MAF differences by plate
  - Call rate by plate
  - Association tests (one plate versus all others)

# Importance of QC

**Pre-QC Thresholds**



**Post-QC Thresholds**



Many false positives disappear after QC

# GWAS Analysis

- Consider 500,000 SNPs across the human genome
- Each SNP has its own statistical test
- Each SNP has a different statistical power (depending on allele frequency)

# Analysis of GWAS Data

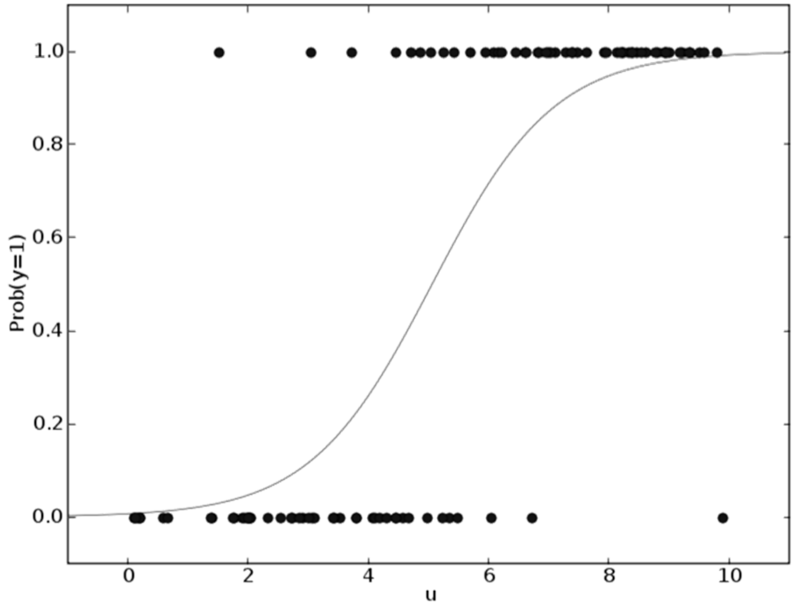
- Basic Statistical Methods are usually applied
- Linear Regression (continuous trait)
- Logistic Regression (dichotomous trait)
- Adjustments are critical to avoid confounding

# Software Tools

- PLINK - http://pngu.mgh.harvard.edu/~purcell/plink/
- PLATO – https://ritchielab.psu.edu/software/plato-download
- R – http://www.r-project.org/
  - Bioconductor - http://www.bioconductor.org/

# Logistic Regression

- Examines differences between two groups (cases and controls)
- Transforms the Y-Axis of a typical regression using a logit function
- Produces a probability of case or control status (Odds Ratio)



# QQ Plot

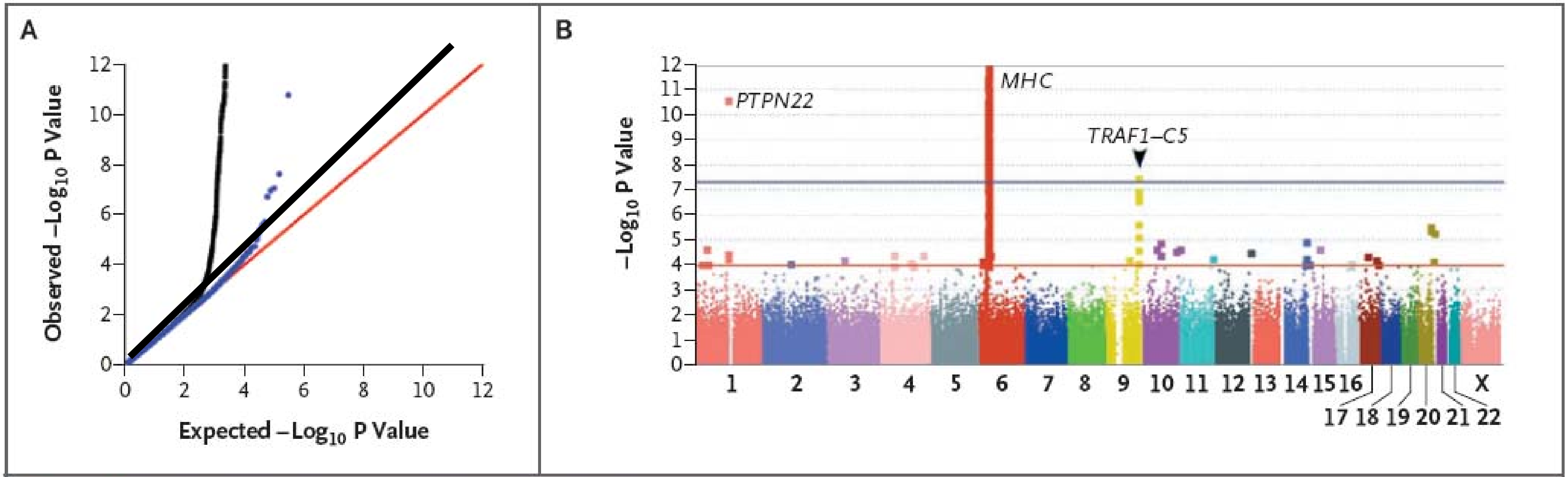


Figure 1. Results of the Genomewide Association Study.

- Systematic deviation from the line indicates population stratification / genomic inflation

# Multiple Testing

- Perform 500,000 analyses
  - Type I error set at 5%, we can expect 25,000 false positive results
    - Bonferroni correction
    - False Discovery Rate (FDR)
    - Gene-based correction (principal components)
- “Genome-wide significance” is $p<10^{-8}$
  - Can be problematic for non-European Populations

# Winner's Curse

- Consider an item with a fixed value (pashmina)
- If there are ten American tourists bidding on the same item, the bids will average around the item's true value
- By definition, the winner will ALWAYS overpay (Dana)

# Winner's Curse in GWAS

- Similarly when running a GWAS and discovering a SNP association, you will OVERESTIMATE the strength of the association
- Power calculations use an effect size to know how many samples you need to detect this effect
- If the effect size is actually SMALLER than you think, you'll need MORE samples to see you effect again

# Replication

- Now required for consideration in top journals
- Second sample, preferably with larger sample sizes to increase power
- Ideally should be interchangeable with the first sample in every way
  - Need all the covariates you used in the first dataset

# Great GWAS Examples

- Multiple Sclerosis GWAS
  - Trio design, extensive QC
  - http://www.ncbi.nlm.nih.gov/pubmed/17660530
- Type II Diabetes GWAS
  - http://www.ncbi.nlm.nih.gov/pubmed/17463246?dopt=Abstract&holding=npg