Genome-wide association studies (GWAS) - Part 1

Heather J. Cordell

Population Health Sciences Institute
Faculty of Medical Sciences
Newcastle University, UK

heather.cordell@ncl.ac.uk

Genome-wide association studies (GWAS)

- Popular (and highly successful) approach over past 12 years
- Enabled by advances in high-throughput (microarray-based) genotyping technologies
- Idea is to measure the genotype at a set of single nucleotide polymorphisms (SNPs) across the genome, in a large set of unrelated cases and controls
  - Or related individuals (family data) – but need to analyse differently

Two individuals

Person 1	ACCTGTGTGCCCAATGGCGTCCCATACTATCGG
	ACCTGTGCGCCCAATGGCGTCCCATACTATCGG
Person 2	ACCTGTGCGCCCAGTGGCGTCCCATACTATCGG
	ACCTGTGCGCCCAGTGGCGTCCCATAGTATCGG

- Test each SNP for association/correlation with disease phenotype

Association testing: case/control studies

- Collect sample of affected individuals (cases) and unaffected individuals (controls)
  - Or a else a sample of random "population" controls
    - Most of whom will not have the disease of interest
- Examine the association (correlation) between alleles present at a genetic locus and presence/absence of disease
  - By comparing the distribution of genotypes in affected individuals with that seen in controls

Case/control studies

- Each person can have one of 3 possible genotypes at a SNP (with alleles coded 1 and 2)

Genotype	Cases	Controls
2|2	500 $(= a)$	200 $(= b)$
1|2	1100 $(= c)$	820 $(= d)$
1|1	400 $(= e)$	980 $(= f)$
Total	2000	2000

- Test for association (correlation) between genotype and presence/absence of disease using standard χ^2 test for independence on 2 df
- Two odds ratios can be estimated
  - $\mathrm{OR}\,(2|2 : 1|1) = \frac{af}{be}$
  - $\mathrm{OR}\,(1|2 : 1|1) = \frac{cf}{de}$

Odds ratios

- Odds of disease are defined as P(diseased)/P(not diseased)
  - Odds ratio OR$(2|2:1|1)$ repesents the factor by which your odds of disease must be multiplied, if you have genotype $2|2$ as opposed to $1|1$
    - i.e. the 'effect' of genotype $2|2$
- Similarly, we can define the OR for $1|2$ vs $1|1$
  - As the factor by which your odds of disease must be multiplied, if you have genotype $1|2$ as opposed to $1|1$
    - i.e. the 'effect' of genotype $1|2$
- ORs are closely related (often $\approx$) genotype relative risks
  - The factor by which your probability of disease must be multiplied, if you have genotype $1|2$ as opposed to $1|1$ (say)
- If your genotype has no effect on your probability (and therefore on your odds) of disease, then the ORs=1.
  - So the association test can be thought of as a test of the null hypothess that the ORs=1

Genotype relative risks

- If a disease is reasonably rare, the odds ratio approximates the genotype relative risk (GRR, RR)

Genotype	Penetrance	GRR	Odds	OR
$1/1$	0.01	1.0	$0.01/0.99 = 0.0101$	1.00
$1/2$	0.02	2.0	$0.02/0.98 = 0.0204$	2.02
$2/2$	0.05	5.0	$0.05/0.95 = 0.0526$	5.21

- If your genotype has no effect on your probability (and therefore your RR) of disease, then both the ORs and the GRRs=1.

Dominant/recessive effects

Dominant:

Genotype	Cases	Controls	Total
$2|2$ and $1|2$	500+1100	200+820	700+1920
$1|1$	400	980	1380
Total	2000	2000	4000

Recessive:

Genotype	Cases	Controls	Total
$2|2$	500	200	700
$1|2$ and $1|1$	1100+400	820+980	1920+1380
Total	2000	2000	4000

- Can also rearrange table to examine effects of alleles (1 df tests):

Counting alleles

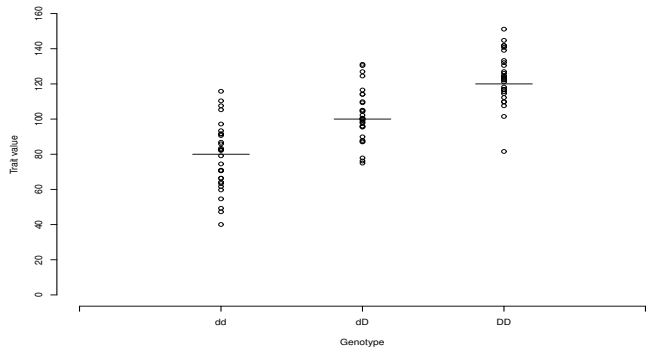
	Counts in	
Allele	Cases	Controls
2	2100 ($=a$)	1220 ($=b$)
1	1900 ($=c$)	2780 ($=d$)
Total	400	400

Allelic OR $= ad/bc$

- χ^2 test statistic on 1 df $= \sum_i (O_i - E_i)^2/E_i$ where O_i and E_i are the observed and expected values in cell i.
  - Assumes HWE under null and multiplicative allelic effects under alternative: considers chromosomes as independent units
  - Better approach: use counts in previous genotype table to perform a Cochran-Armitage trend test
  - Even better approach: use linear or logistic regression

Testing for association: quantitative traits

- Linear regression provides a natural test for quantitative traits
  - Testing the null hypothesis that the slope = 0



Logistic regression

- Used in case/control studies
  - Outcome is affected or unaffected
  - Model probability (and thus odds) of disease p as function of variable x coding for genotype:

$$\ln \frac{p}{1-p} = \beta_0 + \beta_1 x \quad \equiv c + mx$$

  - Use observed genotypes in cases and controls to estimate the values of regression coefficients β_0 and β_1
    - And to test whether $\beta_1 = 0$

Logistic regression

- Standard method used in standard epidemiological studies e.g. of risk factors such as smoking in lung cancer
- Main advantage is you can include more than one predictor in the regression equation e.g.

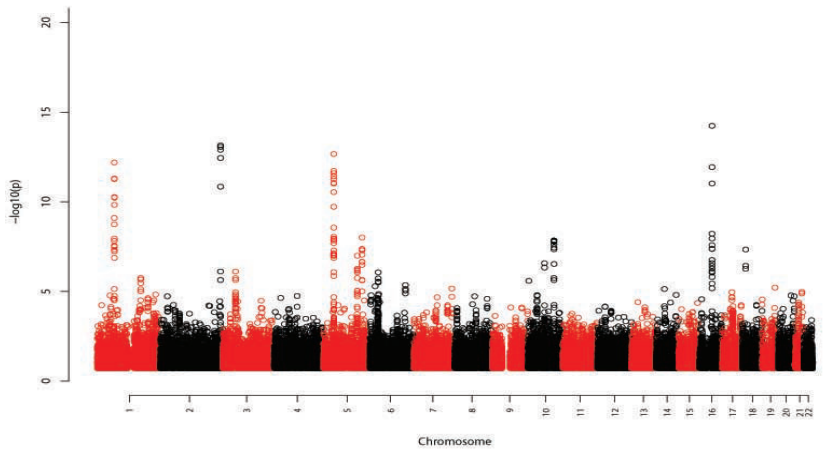
$$\ln \frac{p}{1-p} = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3$$

where x_1, x_2, x_3 code for
  - genotypes at 3 loci
  - measured environmental covariates (e.g. age, sex, smoking etc),
  - genetic principal component scores (to adjust for population substructure),
  - interactions between loci etc. etc.

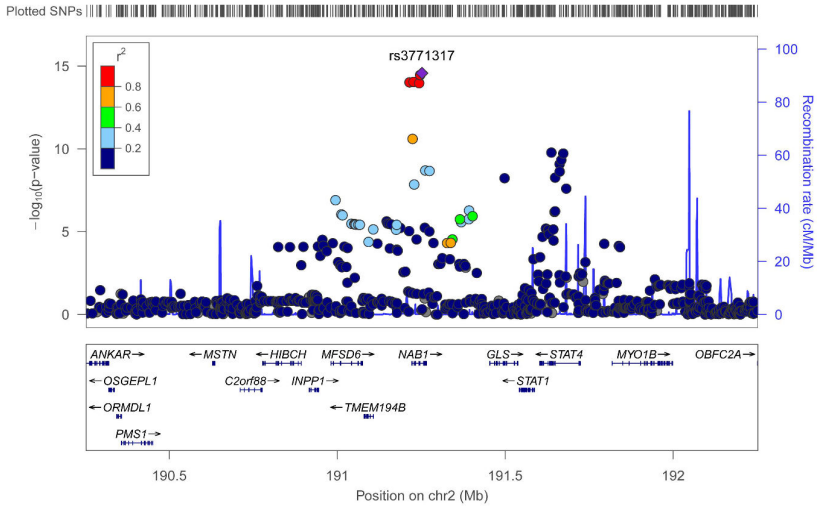
Testing for association

- All methods produce a test statistic and a p value at each SNP, indicating how significant the association/correlation observed appears to be
  - i.e. how likely it was to have occurred by chance
- At any location showing 'significant' association, we expect to see several SNPs in the same region showing association/correlation with phenotype
  - Due to the correlation or linkage disequilibrium (LD) between neighbouring SNPs

Manhattan Plots



Close-up of hit region



Historical Perspective: Complement Factor H in AMD

- First (?) GWAS was by Klein et al. (2005) Science 308:385-389
- Typed 116,204 SNPs in 96 cases (with age-related macular degeneration, AMD) and 50 controls
  - Very small sample size – they were very lucky to find anything!
  - Luck was due to the fact the polymorphism has a very large effect (recessive OR=7.4)
- Klein et al. followed up on two SNPs passing threshold ($p < 4.8 \times 10^{-7}$)
  - Plus a third SNP that just failed to pass significance threshold, but lay in same region as first SNP

Complement Factor H in AMD

- Of the 3 SNPs followed up:
  - One appeared to be due to genotyping errors: significance disappeared on filling in some missing genotypes
  - First and third SNP lie in intron of Complement Factor H (*CFH*) gene
    - Lies in region previously implicated by family-based linkage studies
- Resequencing of the region identified a polymorphism of plausible functional effect
- Immunofluorescence experiments in the eyes of AMD patients supported the involvement of *CFH* in disease pathogenesis.

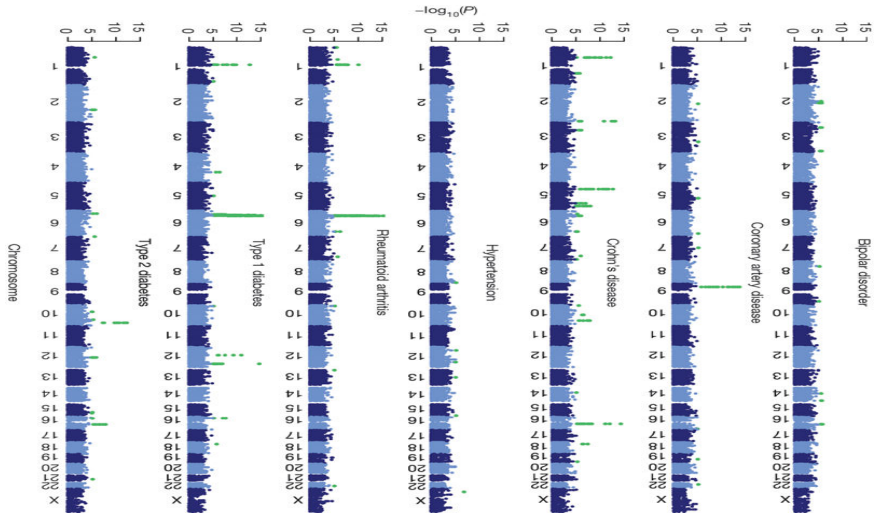
GWAS

- GWAS really got going about 12 or 13 years ago
  - See Visscher et al. (2012) AJHG 90:7-24 "Five Years of GWAS Discovery"
  - And Visscher et al. (2017) AJHG 101:5-22 "10 Years of GWAS Discovery: Biology, Function and Translation"
- 2007/2008 saw a slew of high-profile GWAS publications
  - Breast cancer (Easton et al. 2007)
  - Rheumatoid Arthritis (Plenge et al. 2007)
  - Type 1 and Type 2 diabetes (Todd et al. 2007; Zeggini et al. 2008)
- Arguably the most influential was the Wellcome Trust Case Control Consortium (WTCCC) study of 7 different diseases
  - http://www.wtccc.org.uk/

WTCCC

- Nature 447: 661-678 (2007)
- Considered 2000 cases for each of the following diseases:
  - Bipolar disorder, coronary artery disease, Crohn's disease, hypertension, rheumatoid arthritis, type 1 diabetes, type 2 diabetes
- Compared each disease cohort to common control panel
  - 3000 population-based controls
  - From 1958 birth cohort and National Blood Service
- Highly successful
  - WTCCC found 24 separate association signals
  - Including highly convincing signals in 5 out of the 7 diseases studied
  - All were replicated in subsequent independent follow-up studies

Manhattan plots for 7 diseases



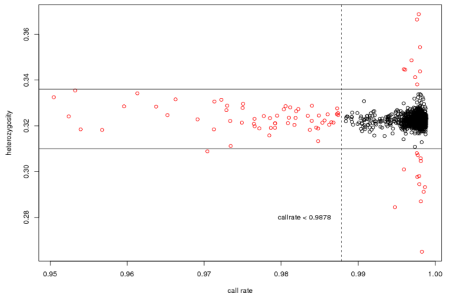
Lessons from WTCCC (and others)

- Typically used rather standard statistical/epidemiological methods (χ^2 tests, t tests, logistic regression etc.)
- Success largely due to:
  - An appreciation of the importance of large sample size ($>$ 2000 cases, similar or greater number of controls)
  - Stringent quality control procedures for discarding low-quality SNPs and/or samples
  - Stringent significance thresholds ($p = 5 \times 10^{-8}$) to account for multiple testing and/or low prior prob of true effect
  - Importance of replication in an independent data set

Quality Control

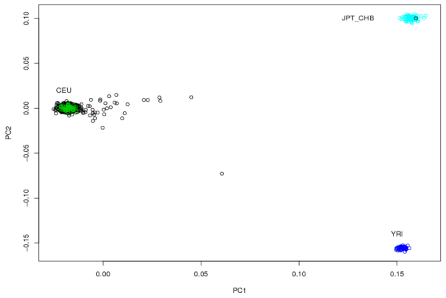
- Stringent QC checks are required for GWAS data
- Discard samples (people) deemed unreliable
  - Low genotype call rates, excess heterozygosity etc.
  - X chromosomal markers useful for checking gender
    - Males should 'appear' homozygous at all X markers
  - Genome-wide SNP data useful for checking relationships and ethnicity
- Discard data from SNPs deemed unreliable
  - On basis of genotype call rates, Mendelian misinheritances, Hardy-Weinberg disequilibrium
  - Exclude SNPs with low minor allele frequency (MAF)

QC: call rates and heterozygosity



- 61 sample exclusions (low call-rate); 23 exclusions (heterozygosity)
- SNP exclusions also made based on call-rates, MAF and Hardy-Weinburg equilibrium (HWE)

QC: ethnicity tests



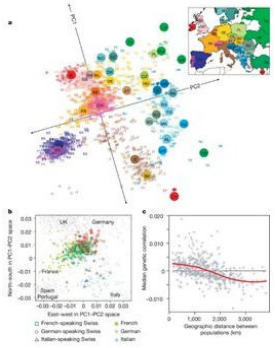
- Multidimensional scaling (with 210 HapMap individuals) identifies 33 samples with non-Caucasian ancestry
- Similar methods can be used to model more subtle population differences between samples

Multivariate Analysis

- Several related multivariate analysis techniques have been proposed for detecting population structure in genome-wide association studies
  - Principal components analysis (PCA)
  - Principal coordinates analysis (PCoA)
  - Multidimensional scaling (MDS)
- If population differences can be detected (and adjusted for) in association analysis, this offers a way to deal with the problem of population stratification
  - Population sampled actually consists of several 'sub-populations' that do not really intermix
  - Can lead to spurious false positives (type 1 errors) in case/control studies
- These techniques can also be used in quality control (QC) procedures, to check for (and discard) population outliers

Principal components analysis (PCA)

Genes mirror geography within Europe



J Novembre *et al.* (2008) *Nature* **456(7218):98-101**, doi:10.1038/nature07331

Principal Components Analysis

- Price et al. (2006) Nature Genetics 38:904-909; Patterson et al. (2006) PLoS Genetics 2(12):e190
  - Based on popn genetics ideas from Cavalli-Sforza (1978)
- Idea is to form a large matrix M of SNP counts (0,1,2) corresponding to the genotype at a L loci (=rows) for n individuals (=columns)

$$\mathrm{M} = \begin{pmatrix} g_{11} & g_{12} & . & g_{1n} \\ g_{21} & g_{22} & . & g_{2n} \\ g_{31} & g_{32} & . & g_{3n} \\ . & . & . & . \\ . & . & . & . \\ g_{L1} & g_{L2} & . & g_{Ln} \end{pmatrix}$$

Principal Components Analysis

- Subtract row means and normalise by function of row allele frequency $\sqrt{f_l(1-f_l)}$ to give matrix X

$$\mathrm{X} = \begin{pmatrix} x_{11} & x_{12} & . & x_{1n} \\ x_{21} & x_{22} & . & x_{2n} \\ x_{31} & x_{32} & . & x_{3n} \\ . & . & . & . \\ . & . & . & . \\ x_{L1} & x_{L2} & . & x_{Ln} \end{pmatrix}$$

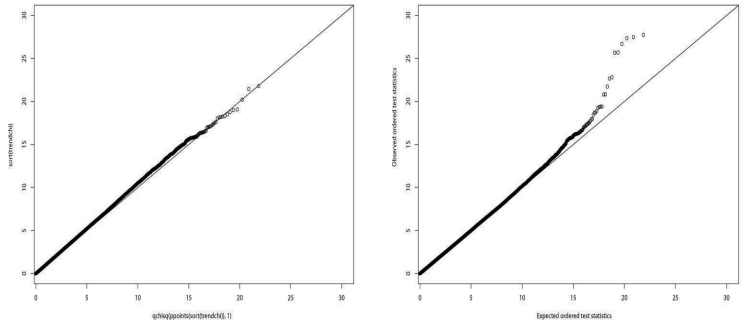
- This matrix will be used as starting point for PCA
  - In principal we could start with a different matrix – in particular not all PCA approaches would normalise by $\sqrt{f_l(1-f_l)}$

Multivariate Analysis

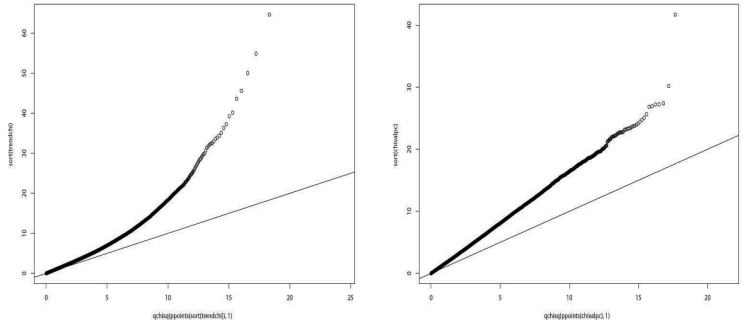
- Estimate covariance matrix $\Psi = \mathrm{X}^T\mathrm{X}$ between all pairs of individuals, with entries ψ_{ij} defined as the covariance (summing over SNPs) between column i and j of X
  - Represents average genome-wide IBD (estimated from IBS)
  - Compute the eigenvectors $\vec{v}_j$ and eigenvalues λ_j of matrix Ψ
    - Co-ordinate j of the kth eigenvector represents the ancestry of individual j along 'axis' k
- For technical details, see McVean (2009) PLoS Genetics 5;10:e1000686
- Many genetics packages e.g. (PLINK) will allow you to calculate the top 10 (or more) PCs
  - Different geographic populations can often be well separated by just the first two or three PCs
    - Useful for outlier detection
  - For more subtle differences, you may need to calculate more PCs
    - And include them as covariates in the regression equation
    - Post-GWAS QC can determine whether you have included 'enough'

Post GWAS QC: Q-Q Plots (good)

- Plot ordered test statistics (y axis) against their expected values (x axis)



Q-Q Plots (bad)



Population stratification

- A QQ plot showing constant inflation (straight line with slope > 1) can indicate population stratification/population substructure
- Simple solution: Genomic Control (Devlin and Roeder 1999)
  - Use your observed test statistics to estimate the slope (=inflation factor λ)
  - Divide each test statistic by λ to get an adjusted (deflated) test statistic
- More complicated solution: use PCA/MDS or similar
- Even more complicated solution: use linear mixed models

Relatedness

- With genome-wide data, can also infer relationships based on average identity by descent (IBD) $\Psi = X^T X$ or identity by state (IBS)
  - Using 'thinned' subset of markers with high minor allele frequency (MAF) and in approximate linkage equilibrium
  - Simple relationships (PO, FS, MZ/duplicates) can identified with only a few hundred markers
  - More complicated relationships require 10,000-50,000 SNPs
- Various software packages, including PLINK, KING and TRUFFLE

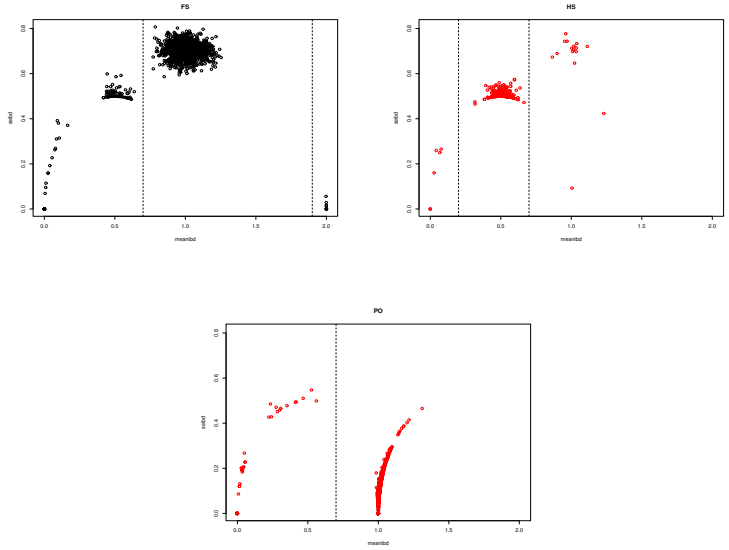
Expected IBD sharing

- Assuming no inbreeding, the IBD state probabilities are:

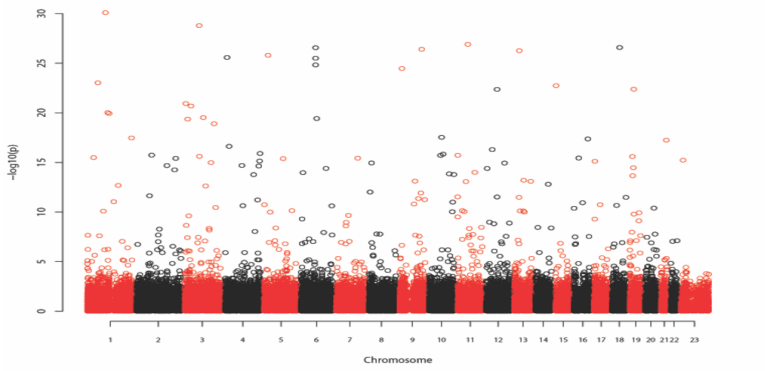
	Number of alleles shared IBD		
Relationship	2	1	0
MZ twins	1	0	0
Parent–Offspring	0	1	0
Full siblings	1/4	1/2	1/4
Half siblings	*0*	*1/2*	*1/2*
Grandchild–grandparent	*0*	*1/2*	*1/2*
Uncle/aunt–nephew/niece	*0*	*1/2*	*1/2*
First cousins	0	1/4	3/4
Second cousins	0	1/16	15/16
Double 1st cousins	1/16	6/16	9/16

- A useful visualisation tool is to plot SE(IBD) vs mean(IBD)

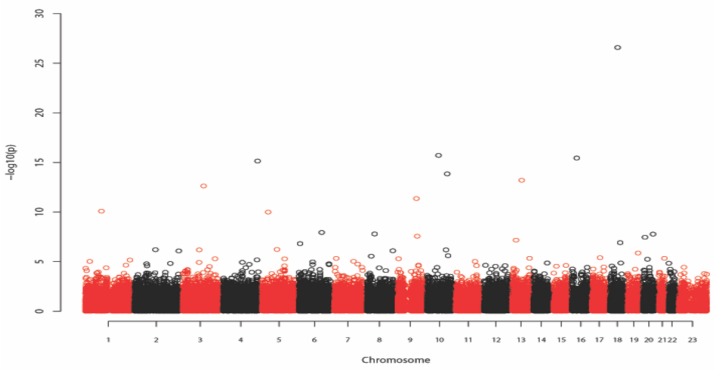
Full/half sibs and parent-offspring



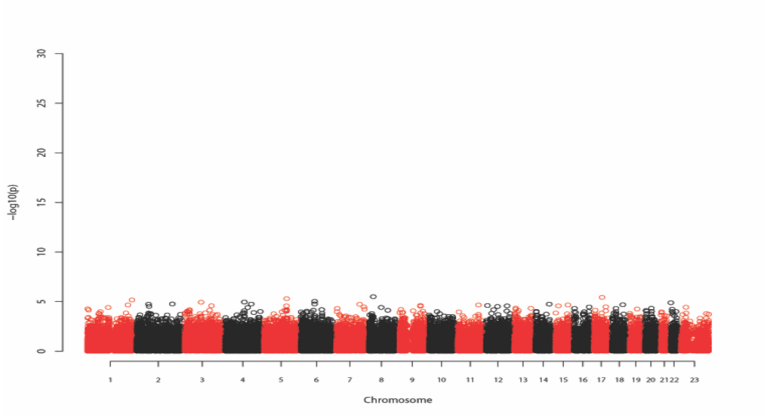
CHD GWAS results (low QC)



CHD GWAS results (better QC)



CHD GWAS results (final QC)



Genome-wide meta-analysis

- Puts together data (or results) from a number of different studies
  - Could analyse as one big study
  - But preferable to analyse using meta-analytic techniques
    - At each SNP construct an overall test based on the results (log ORs and standard errors) from the individual studies
- Meta-analysis is often made easier by using *imputation*
  - Inferring (probabilistically) the genotypes at SNPs which have not actually been genotyped
    - On the basis of their known correlations with nearby SNPs that have been genotyped
    - Using a reference panel of people (e.g. 1000 Genomes) who have been genotyped at all SNPs
- Enables meta-analysis of studies that used different genotyping platforms
  - By imputing to generate data at a common set of SNPs
    - Ideally while accounting for the imputation uncertainty in the downstream statistical analysis
    - In practice often don't bother - use post-imputation QC to remove poorly-imputed SNPS