Session 2: Steps involved in Genome-wide association studies

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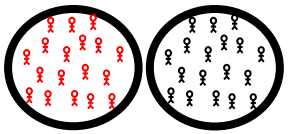
Learning objective

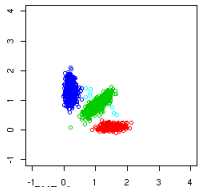
- Have a working knowledge of the different steps involved in the conduct of genome-wide association studies, including:
  - Study design
  - Quality control
  - Basic analyses
  - Replication/meta-analysis

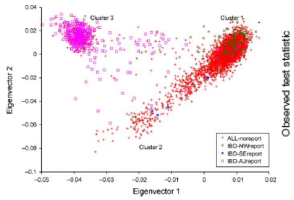
Genome-wide Association Study (GWAS) Recipe

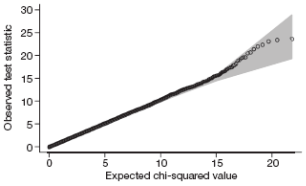
Genotype 100,000s **common** SNPs in 1000s of cases+controls



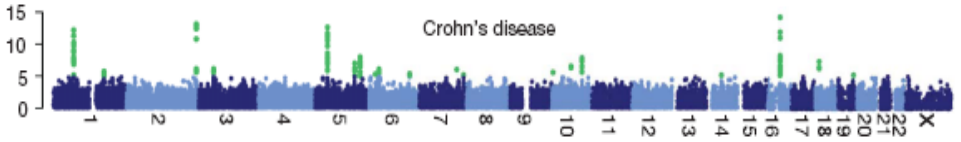
Quality-control analyses: e.g. genotype calling, population biases



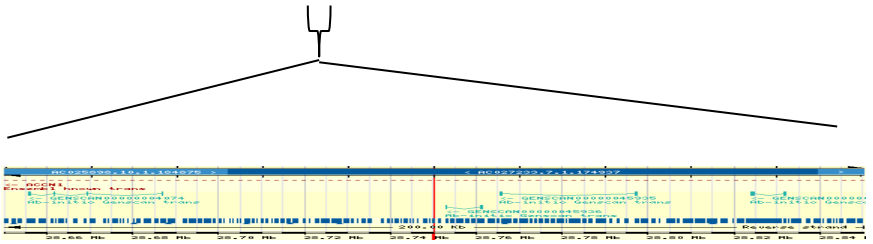




At each SNP test for allele frequency difference between cases& controls (chi^2, logistic regression)

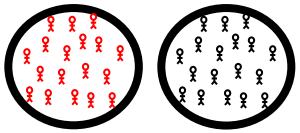


Identify significant associations, nominal p-value (5x 10^{-8})



Assess genomic info: genes, SNP density, regulatory regions, etc

Genotype selected SNPs in different case+control samples of same population: replication/meta-analysis



GWAS Study Design

Important steps

- Define the case phenotype in adequate detail
  - At least sufficient for replication studies.....
  - In practice.... paid variable attention to in GWAS

    'Lumpers' vs. 'Splitters'!

'Lumpers':
Very large case sets result in ↑ power that outweighs ↓ power due to (a degree of) misclassification and genetic heterogeneity of disease

'Splitters':
Lack of accurate phenotypic definitions result in need for much greater sample sizes, but also hide differential heterogeneity of 'subtypes'

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'Splitters':
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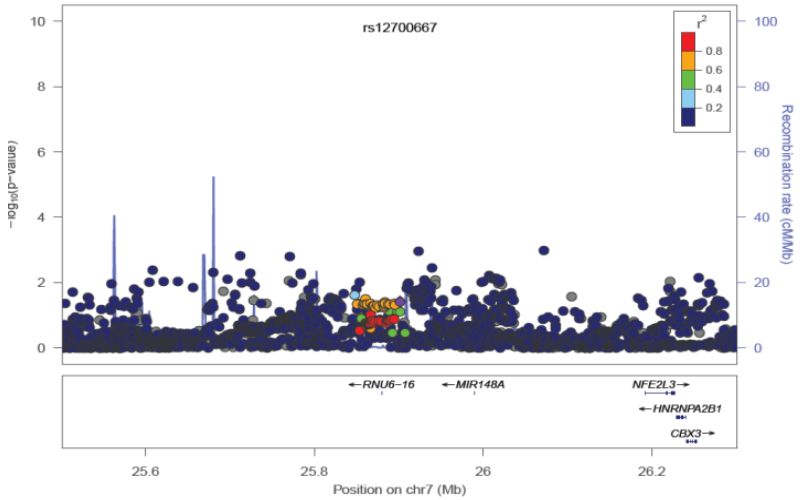
- Impact of definition accuracy likely disease/trait-specific
- Well-phenotyped datasets including sub-types disease/correlated traits are useful to dissect differential genetic origins (e.g. CAD and cholesterol levels, endometriosis and surgical stage of the disease)

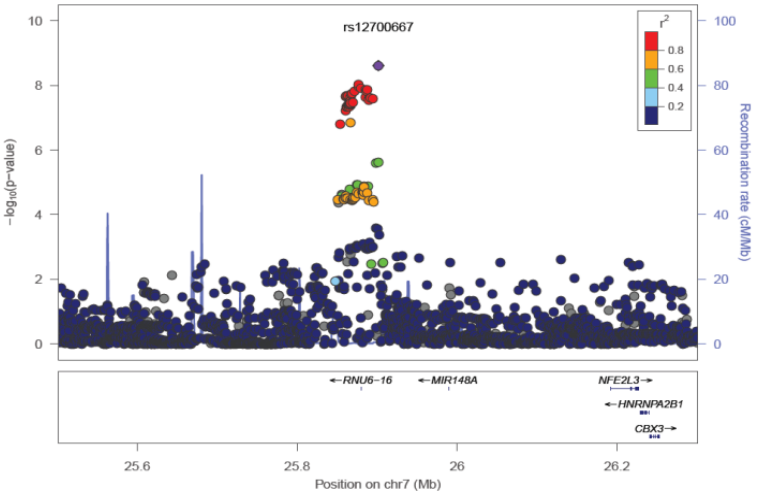
GWAS in Endometriosis (Sub-types)

Stage I/II endometriosis (1.6K cases)

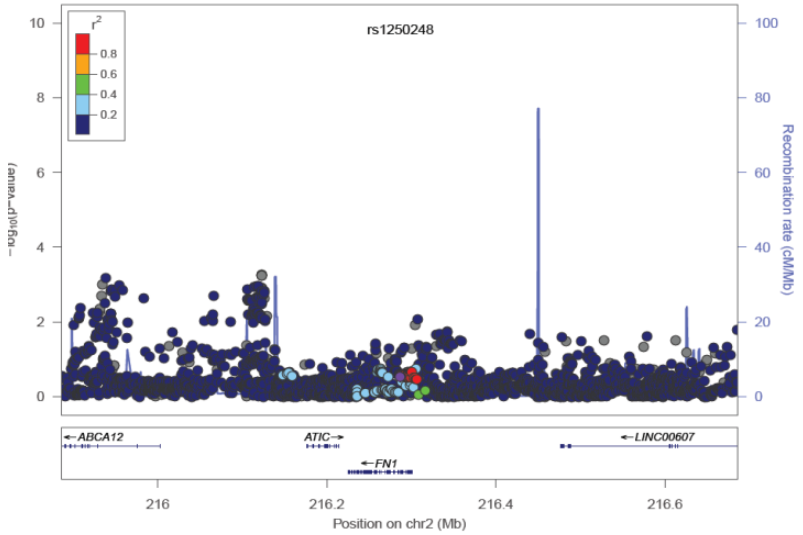
Stage III/IV endometriosis (1.3K cases)

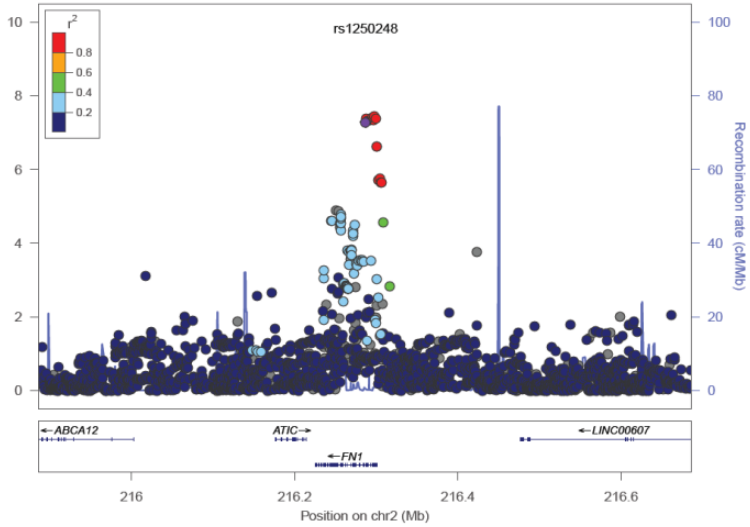
7p15.2 (rs12700667)





FN1, intronic (rs1250248)





GWAS Study Design

Important steps

- Define the case phenotype in adequate detail
- [Check the heritability of the disease in question]
  - Most diseases are ‘heritable’

GWAS Study Design

Important steps

- Define the case phenotype in adequate detail
- [Check the heritability of the disease in question]
- Consider whether a population-based study is the appropriate design for the research question
  - Are you interested in a disease sub-type that looks to be highly familial?

*

GWAS Study Design

Important steps

- Define the case phenotype in adequate detail
- [Check the heritability of the disease in question]
- Consider whether a population-based study is the appropriate design for the research question
- Select appropriate controls
  - Same ethnic population from which cases arose
  - 'Common controls' principle: publicly available genomic datasets on large numbers of individuals of a certain ancestry (e.g. WTCCC sets of blood donors and 1958BC members; many such datasets now available).
  - Female controls for female-specific conditions? Not necessary for analysis of autosomes (chromosomes 1-22)!

GWAS Study Design

Important steps

- Define the case phenotype in adequate detail
- [Check the heritability of the disease in question]
- Consider whether a population-based study is the appropriate design for the research question
- Select appropriate controls
- Calculate required sample size
  - Previous GWAS: allow for allelic ORs in the 1.1-1.5 range
  - Typically you need at least 2,000 cases (and 1:1 to 1:3 control ratio)

*

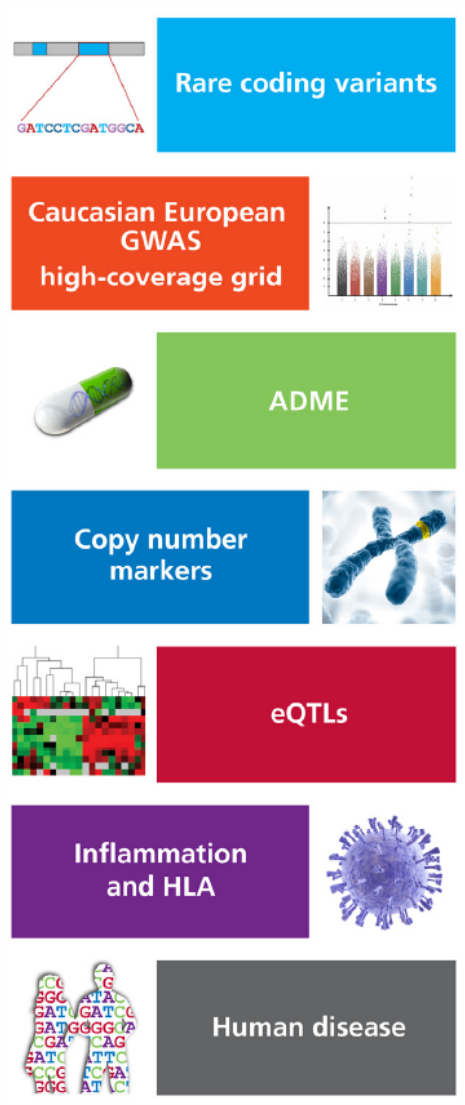
GWAS Marker (Chip) Selection

Considerations

- Many different genotyping arrays/chips on the market
- Evolved from increasingly large tagSNP panels (LD based), at ever decreasing cost
- To include supplements specific to certain analyses, as knowledge of the genomic aspects of these analyses increases
  - Low frequency and exome SNPs (following 1000G)
  - Disease-specific chips (e.g. Illumina Psych-chip/Metabo-chip)
  - Combination panels for Biobanking (e.g. Affymetrix UKBiobank Axiom 800K)

UK Biobank Axiom array

820,967 SNPs and indel markers, covering areas:



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  - Combination panels for Biobanking (e.g. Affymetrix UKBiobank Axiom 800K)
- Make sure cases and controls are genotyped on the same platform, and together (randomly distributed over chips, to avoid batch effects)

GWAS Quality Control (QC)

Important steps

- The most important part of GWAS analysis.

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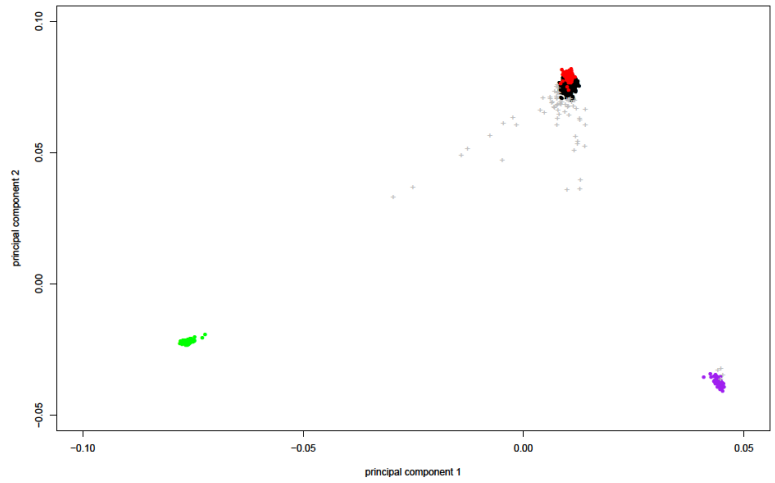
- Per-Individual QC:
  - Discordant sex information (comparing homozygosity rates for X chromosome)
  - Outlying missing genotypes (typically >5%) or heterozygosity rates
  - Duplicated or related individuals
  - Divergent ancestry:

    Using multivariate methods (e.g. principal component analysis). PCs each absorb decreasing amount of variance. Implemented in software to produce graphical representation of population stratification.

Remove population outliers



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- Per-SNP QC:
  - Excessive missing genotype rates (call rates < 95%)
  - Deviation from HWE (significance thresholds vary, most commonly 5×10^{-7} combined with post-analysis checks of genotype cluster plots)
  - Different missing genotype rates between cases and controls
  - MAF < 1%

GWAS Analysis

Important steps

- Select genotypic disease models to test. *Multiplicative model most powerful under most disease models!*

Genetic Data Analysis – Models

'per-allele' assumes an additive genetic model, i.e. an effect for each allele copy

looks for an incremental effect across the genotype groups

Allele Counts		
	C	T
Case	2a+b	b+2c
Control	2d+e	e+2f

Cochran Armitage Test for Trend			
	CC ➔	CT ➔	TT
Case	a	b	c
Control	d	e	❖

Full Data – Genotype Counts			
	CC	CT	TT
Case	a	b	c
Control	d	e	f

'per genotype' looks for any difference across the genotype groups without making any assumptions about the direction of the effect

Dominant Model (T risk)		
	CC	CT or TT
Case	a	b+c
Control	d	e+f

Recessive Model (T risk)		
	CC or CT	TT
Case	a+b	c
Control	d+e	f

Statistical Analysis

- Logistic regression (SNPTEST/PLINK): Binary Traits
- Linear regression (SNPTEST/PLINK): Continuous Traits
- Firth-test (EPACTS): Datasets with case/control imbalance.
- Linear Mixed Model (BOLT-LMM): Datasets with related individuals

GWAS Analysis

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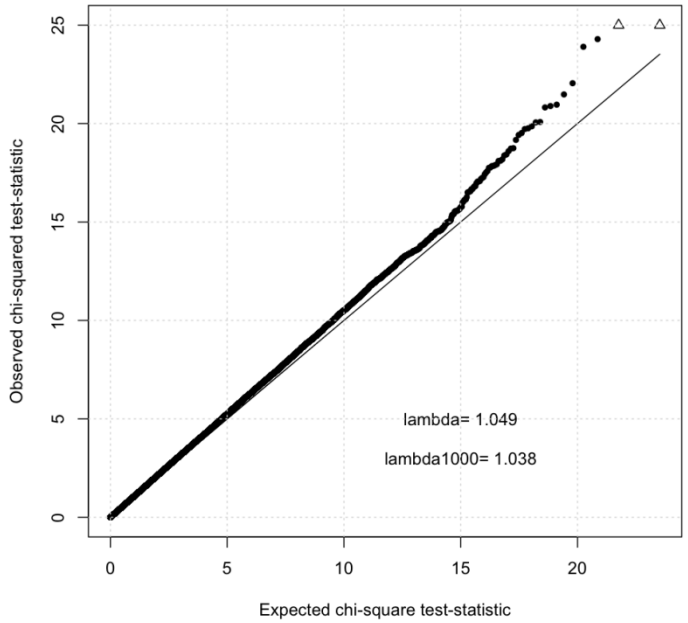
- Select genotypic disease models to test (e.g. multiplicative, recessive, dominant). *Multiplicative model most powerful under most disease models!*
- Post-analysis QC: QQ plots and lambda inflation score



Lambda >1 :
population structure

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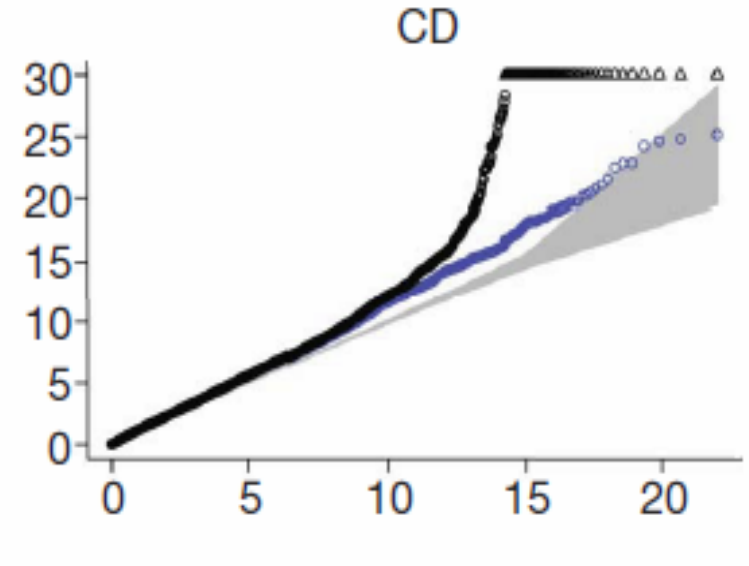
- Need to adjust for confounders*?*

  Principal components related to ancestry



Q-Q plot of GWAS of Crohn's Disease before (**black**) and after (**blue**) adjusting for principal components relating to ancestry (population stratification)

WTCCC, Nature 2007

GWAS Analysis

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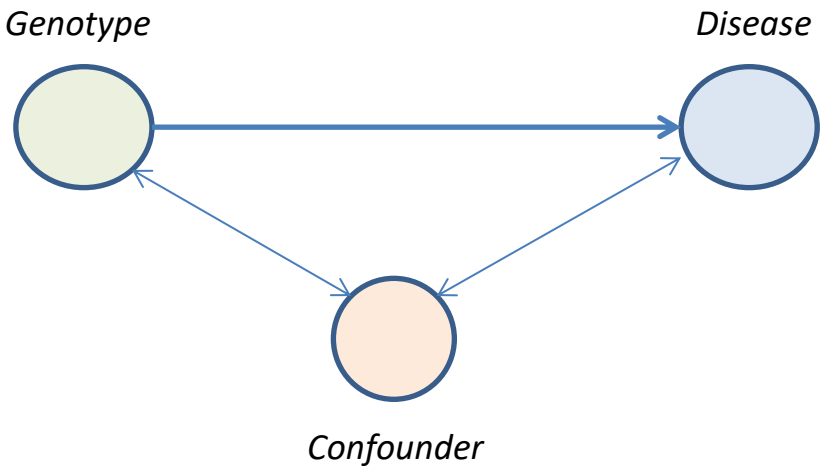
- Need to adjust for confounders*? In the absence of indicators for ancestry-based population stratification: rarely....*

  Other types of confounding difficult to argue



GWAS Analysis

Important steps

- Select genotypic disease models to test (e.g. multiplicative, recessive, dominant). *Multiplicative model most powerful under most disease models - apart from recessive!*
- Need to adjust for covariates*? In the absence of indicators for ancestry-based population stratification: rarely....*
- Adjustment for non-confounding covariates to 'absorb phenotypic noise'?

  See: Pirinen et al., Nature Genetics 2012; 44: 848-850

  – In case-control setting (logistic regression models):
    - Will reduce power when disease prevalence is < 2% (many diseases!)
    - Will only gain power when disease prevalence is > ~20%

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  – Can be of value:
    - In linear regression models of quantitative traits
    - In individual studies prior to meta-analyses, to avoid effect-size heterogeneity
    - Where interaction effects between genetic variants and covariate exist (e.g. gender effects)

    - **Conclusion: think very carefully about adjustments...!**

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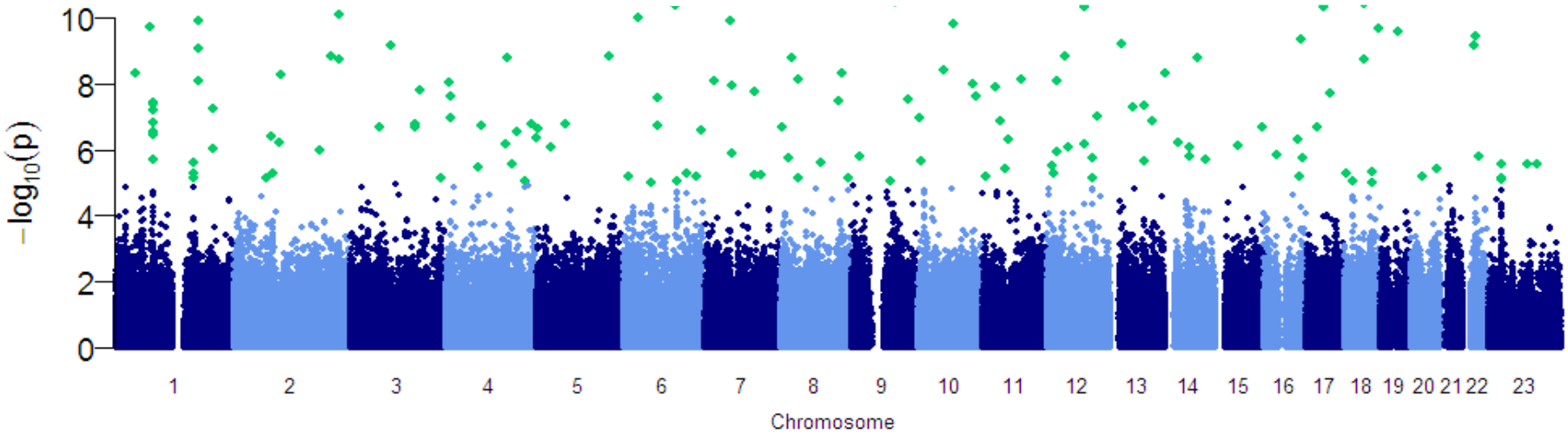
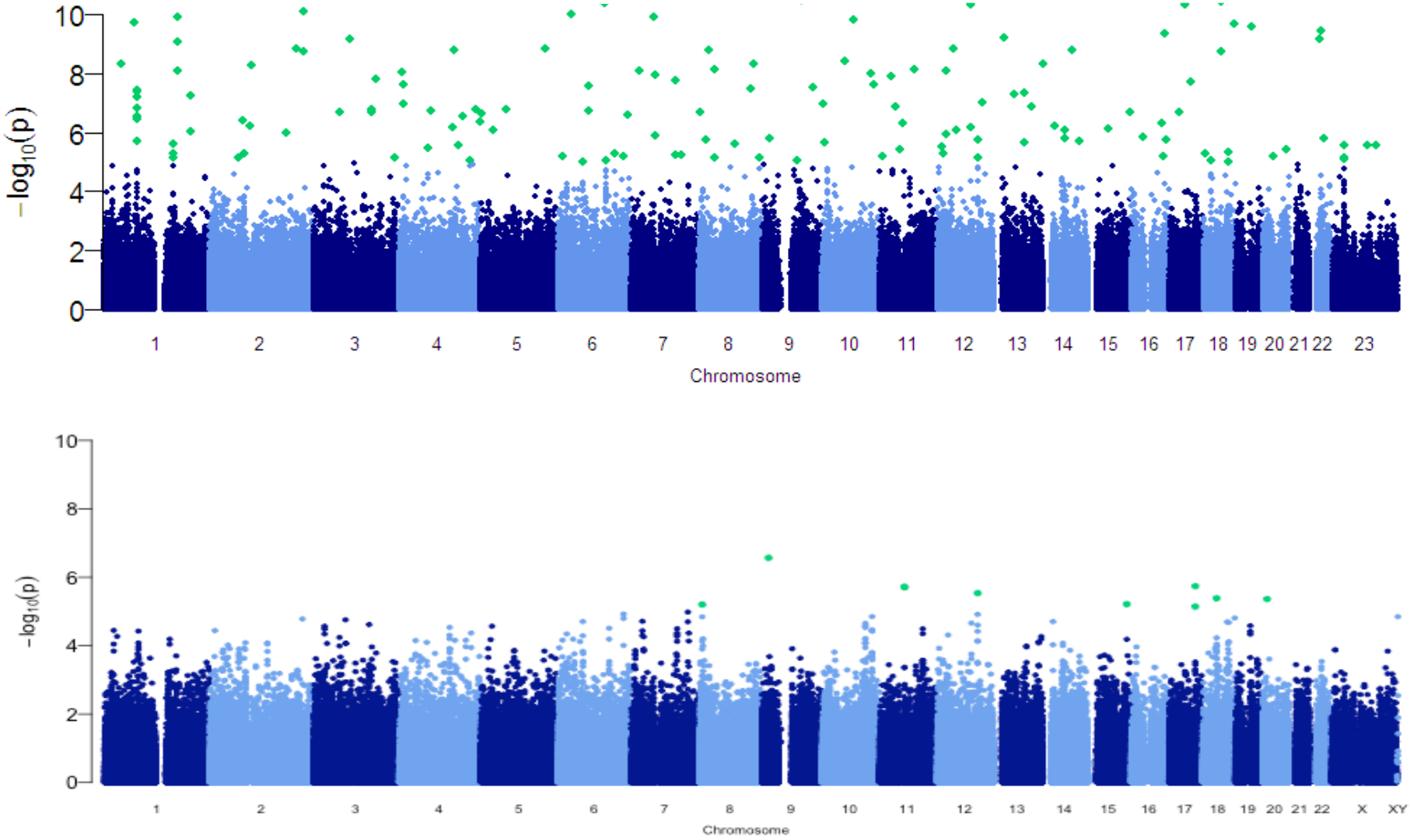
- Visualisation and interpretation of results

Manhattan Plots



Manhattan Plots

Before and after QC….



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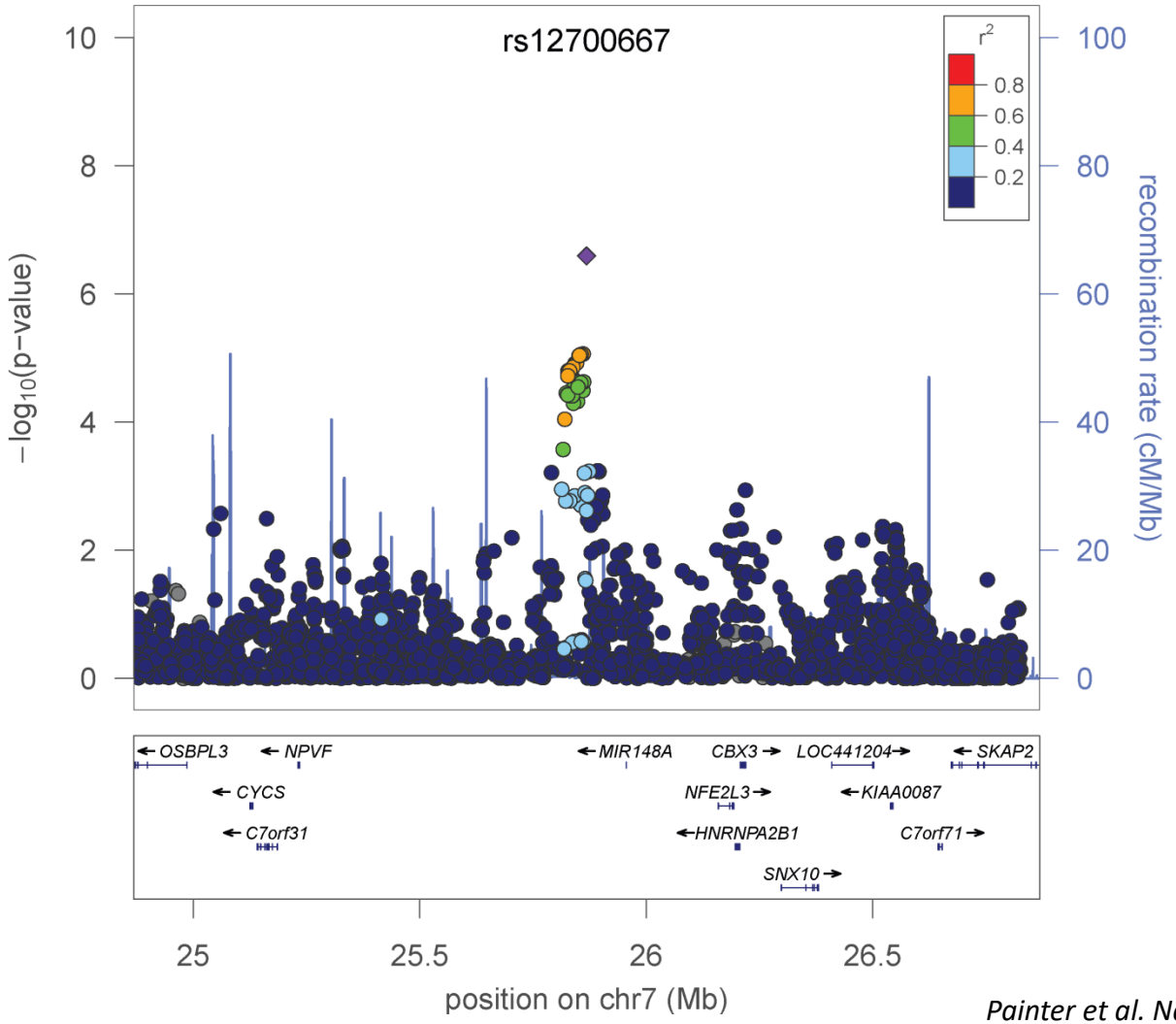
Regional association plots (e.g. Locuszoom)



Painter et al. Nat Genet 2011; 43: 51-4

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- Visualisation and interpretation of results
- What is ‘significant’? ‘Multiple testing’?

GWAS Analysis – reducing false positives

- Adjustment of ‘genome-wide significance’ threshold for # tests conducted?

*

GWAS Analysis – reducing false positives

- Adjustment of 'genome-wide significance' threshold for # tests conducted?
- **Much more complex....** WTCCC paper: factor determining the threshold is not the number of tests performed, but the a-priori probability that there is likely to be a true association at any specified location in the genome ('Bayesian' statistical theory)
- Different significance thresholds proposed, converging on the one most commonly used now: 5×10^{-8} (independent of # SNPs tested)

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- Replication replication replication (and meta-analysis....)

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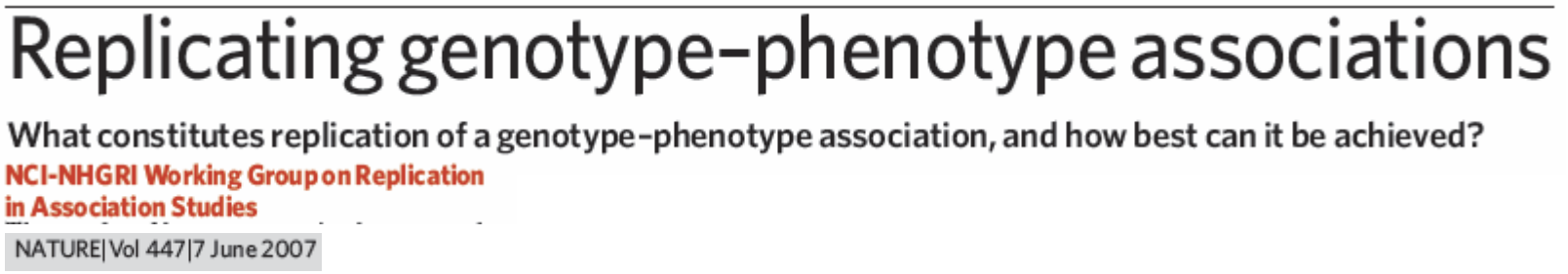
Replication



Replicating genotype–phenotype associations

What constitutes replication of a genotype–phenotype association, and how best can it be achieved?

NCI-NHGRI Working Group on Replication in Association Studies

NATURE|Vol 447|7 June 2007

- Same study population as original finding
- Same/similar case definition
- Same marker (with supporting evidence from others in high LD)
- Good study design practices, including sufficiently large sample size

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  - Central analysis plan shared
  - Often, QC + imputation + analyses performed by the individual centres (to comply with data sharing policies)
  - Association statistics for each SNP calculated and shared:

    (effect size, SE/CIs, allele freqs, sample size, p-values)
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Important steps (beyond GWAS-specific QC):

1. File-level QC (cleaning and checks)
2. Meta-level QC: comparison study-specific results (Identification of analytical issues by SE-N and P-Z plots; allele frequency or strand problems; population stratification through lambda-N plots)
3. Meta-analysis QC (identifying analytical issues)

Fixed-effects Meta-analysis

- Let β_i denote the allelic effect (aligned to a fixed baseline allele) of the *i*th study, with variance denoted v_i.
- Estimate of the allelic effect over all *N* studies is then given by

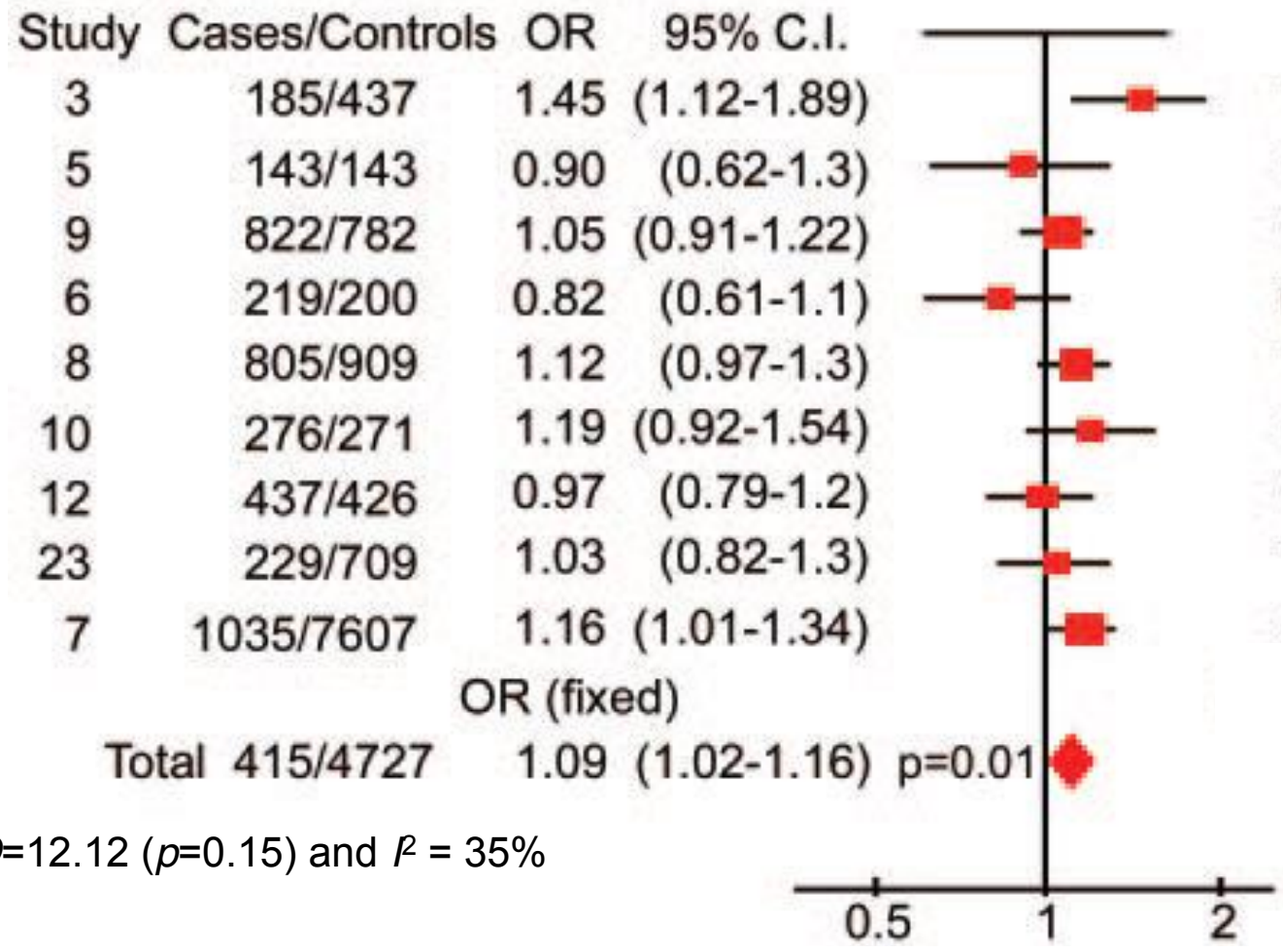
$$B = \frac{\sum_i w_i \beta_i}{\sum_i w_i}$$

  where $w_i = 1/v_i$, with variance given by $V = \left[\sum_i w_i\right]^{-1}$.
- Test for association over all studies given by $X^2 = B^2/V$, having an approximate chi-squared distribution with one degree of freedom.

Assessing heterogeneity

- Fixed effects meta-analysis assumes the same odds ratio (allelic effect) over all studies.
- We can test for heterogeneity between effects using the statistic $Q = \sum_i w_i (B - \beta_i)^2$, which has an approximate chi-squared distribution with *N*-1 degrees of freedom.
- An alternative statistic, I^2=[*Q*-(*N*-1)]/*Q*, quantifies the extent of heterogeneity from a collection of allelic effect sizes.
- Important to investigate source of potential heterogeneity.

Example: sporadic amyotrophic lateral sclerosis



Q=12.12 (p=0.15) and I^2 = 35%

Random-effects Meta-analysis

- Random effect meta-analysis often utilised when a SNP demonstrates significant evidence of heterogeneity in allelic effects between studies.
    - Random-effects meta-analysis: assume distribution of true allelic effects instead of a single underlying true effect size.
    - Random-effects variance component given by

$$\tau^2 = \max\left(0, \frac{Q-(N-1)}{\sum_i w_i - \left(\sum_i w_i^2 / \sum_i w_i\right)}\right)$$

    - Weight assigned to *i*th study then given by

$$w_i^* = (\tau^2 + v_i)^{-1}.$$

A comment on random effects

- Important to investigate the source of heterogeneity between studies: variability may be due to phenotype definition, population background, interaction with exposure to environmental risk factor.

Strand alignment

- Study 1: OR of 1.1 for allele A relative to allele G (aligned to + strand).
- Study 2: OR of 1.3 for allele C relative to allele T (aligned to – strand).
- Effect in study 2 is in opposite direction to study 1 since A is not complementary to C.
- It is straightforward to overcome this issue for non-AT or non-GC SNPs: otherwise rely on correct strand information or matching of allele frequencies (possibly with reference to HapMap or 1000 Genomes data).

Software

- Fixed- and random-effects meta-analysis can be performed for individual SNPs in standard statistical software packages such as R.
- Specialised software for genome-wide association meta-analysis that can handle large numbers of SNPs and studies, and can incorporate checks for strand alignment:
  - METAL: http://genome.sph.umich.edu/wiki/METAL_Program
  - GWAMA: http://www.geenivaramu.ee/en/tools/gwama
  - METASOFT: http://genetics.cs.ucla.edu/meta/

Summary

- Define the case phenotype in adequate detail.
- Select appropriate control group.
- The larger the sample size, the more power to identify variants of smaller effects. Typically at least 2000 cases needed with 1:1 or 1:3 control ratio.
- Quality control is the most important part of GWAS analysis: Sample QC and variant QC .
- Choose appropriate statistical test for association dependent on your phenotype (binary vs. linear, case/control imbalance, related individuals?)
- Post-GWAS: QQ plots and lambda to check for population stratification. Consider adjustment for any additional covariates?
- Replication and meta-analysis for strengthening of evidence for findings.