Introduction to association studies

...including (but not limited to) genome-wide association studies (GWAS)

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Overview and Aims

- To provide a reminder of the basic concepts, definitions, terminology etc. in genetics
- Historical perspective on genetic studies (of monogenic diseases)
  - Familial aggregation/segregation analysis
  - Linkage analysis of family (pedigree) data
- Association analysis (for complex genetic diseases)
  - Families or unrelated individuals
  - Candidate genes/candidate variants or genome-wide
    - GWAS using SNP chips
    - Next-generation sequencing studies

Basic Genetics

- Series of molecules (nucleotides, bases) arranged in a double helix structure

A	Adenine
C	Cytosine
G	Guanine
T	Thymine

- For our purposes, we can consider DNA as a long strong of bases

ACCTGTGTGCCCAATGGCGTCCCATACTATCGG

Basic Genetics

- Series of molecules (nucleotides, bases) arranged in a double helix structure
    - A Adenine
    - C Cytosine
    - G Guanine
    - T Thymine
- For our purposes, we can consider DNA as a long strong of bases

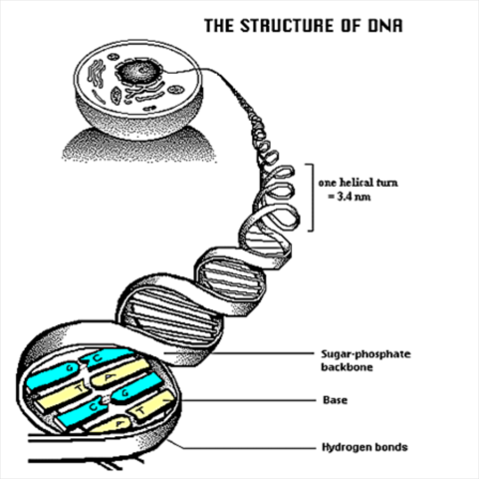
  ACCTGTGTGCCCAATGGCGTCCCATACTATCGG

- Actually, 2 such strings, known as the 'forward' and 'reverse' strands
    - With a redundancy in pairing, such that A always pairs with T, and G with C

      ACCTGTGTGCCCAATGGCGTCCCATACTATCGG
      TGGACACACGGGTTACCGCAGGGTATGATAGCC

    - (This redundancy means we don't need to show both strings)

Structure of DNA



Genetic variation

- The sequences of unrelated humans are 99.9% identical
- Differences are mostly single nucleotide polymorphisms (SNPs) (= single base changes)

Three DNA sequences

ACCTGTGTGCCCAATGGCGTCCCATACTATCGG

ACCTGTGCGCCCAGTGGCGTCCCATACTATCGG

ACCTGTGCGCCCAATGGCGTCCCATAGTATCGG

- Different sequences are said to possess different alleles at these positions

Genetic variation

- As well as SNPs

```
ACCTGTGTGCCCAATGGCGTCCCATACTATCGG
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```

other types of variation include deletions or inversions

```
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```

- Or differences in the number of repeats e.g. copy number variants (CNVs) or short tandem repeats (STRs) (sometimes called microsatellites)

```
ACCTG AGTT AGTT AGTT AGTT AGTT ATACTATCGG
ACCTG AGTT AGTT AGTT ---- ---- ATACTATCGG
```

Alleles and genotypes

- Each person has two alleles at each genetic position (=location, locus)
  - One inherited from their father, one from their mother
- Their genotype at a locus is the combination of alleles they possess

Two individuals

Person 1	ACCTGTGTGCCCAATGGCGTCCCATACTATCGG ACCTGTGCGCCCAATGGCGTCCCATACTATCGG
Person 2	ACCTGTGCGCCCAGTGGCGTCCCATACTATCGG ACCTGTGCGCCCAGTGGCGTCCCATAGTATCGG

- The term haplotype denotes alleles on the same sequence (inherited from the same parent)

Mendelian inheritance

- Within a family, inheritance depends on the physical proximity of the loci on the DNA strands
  - Alleles at loci that are physically closer tend to get transmitted together (i.e. in coupling)
  - Alleles at loci that are sufficiently far apart (or on different chromsomes) are transmitted independently

Parental transmission

Parent	ACCTGTGTGCCCAATGGCGTCCCATACTATCGG
	ACCTGTGCGCCCATTGGCGTCCCATAATATCGG
Child 1	ACCTGTGTGCCCAATGGCGTCCCATACTATCGG
Child 2	ACCTGTGTGCCCAATGGCGTCCCATAATATCGG
Child 3	ACCTGTGTGCCCATTGGCGTCCCATAATATCGG

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Alleles and loci

- A locus or marker = location on a chromosome (on the genome)
- Said to be polymorphic if different forms of genetic material (i.e. different alleles) can exist at that location
    - E.g. at a SNP it might be possible to have an A or a G
    - At a repeat marker it might be possible to have the sequence `AGTT`, or `AGTT AGTT`, or `AGTT AGTT AGTT`
- Often we label the alleles alphabetically (e.g. A,B,C,D; a,b,c,d) or numerically (e.g. 1,2,3,4)
    - An individual with the same alleles at a locus (e.g. AA) is said to be homozygous
    - An individual with the different alleles at a locus (e.g. AB) is said to be heterozygous

Measuring genotypes

- Most genetic epidemiological studies do not measure full genome sequences
    - Too expensive/complicated for routine use in genetic epidemiological studies
    - Starting to be used in small-scale studies
    - Or in large-scale projects such as:
        - The international 1000 Genomes Project
        - The UK Department of Health 100,000 Genomes Project
        - The NHLBI Trans-Omics for Precision Medicine (TOPMed) program

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- Most genetic epidemiological studies involve genotyping a subset of known genetic variants
  - Specific candidate genes (or loci)
  - Or else a set of known polymorphic markers (SNPs, microsatellites) spaced at intervals across the genome
    - Chosen based on surveys of human genetic variation such as HapMap and 1000 Genomes

Disease loci

- The phenotype is the characteristic or trait (e.g. eye colour, height, occurence of a diabetes) that results from having a specific genotype
- Simple Mendelian or monogenic disorders show a close correspondence between genotype (at a single genetic locus) and phenotype
  - In dominant disorders, only one 'disease' allele is required for an individual to get the disease
  - In recessive disorders, two 'disease' alleles are required for an individual to get the disease

Penetrances

- The penetrance is the probability of being diseased, given genotype

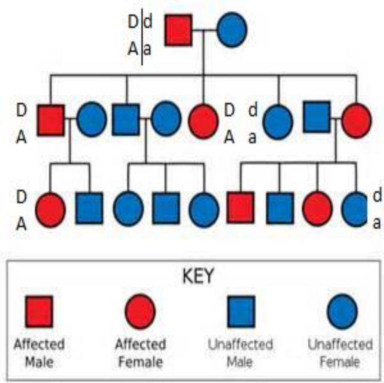
	Dominant	Recessive	Incomplete Penetrance	Genotype Relative Risk (GRR) or odds ratio (OR)
dd	0	0	0.1	1.0
dD	1	0	0.5	5.0
DD	1	1	0.8	8.0

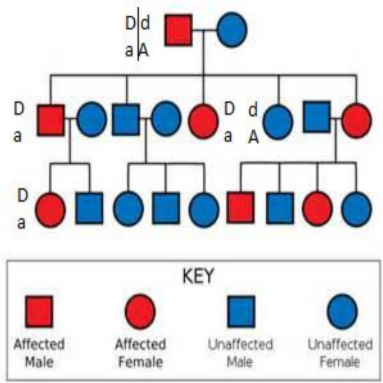
= factor by which your
baseline penetrance
should be multiplied
(for 0,1,2 copies of D)

Parametric linkage Analysis

- Traditionally, genetic determinants of (Mendelian) disease have been identified using parametric linkage analysis
  - Relies on ascertaining (a small set of) large families (pedigrees) each containing a number of affected individuals
  - Idea is to examine co-segregation (co-transmission) of disease phenotype and alleles at one or more genetic marker loci
    - Under the assumption that disease phenotype reflects an underlying disease genotype

Linkage analysis





Parametric linkage analysis

- Calculate likelihood (probability) of observed genotype and phenotype data in (a small set of) large families
    - Under the assumption that the disease is caused by a disease locus situated at recombination fraction θ from a genotyped marker locus
    - Under some assumed mode of inheritance (e.g. dominant, recessive)

- Estimate θ by maximum likelihood techniques

- Test for linkage using likelihood ratio test (LOD score) of the null hypothesis that $\theta = 0.5$
    - "Convincing" evidence for linkage is usually taken as a LOD of 3
        - Corresponds to a likelihood ratio of 1000, i.e. data is 1000 times more likely under the alternative hypothesis than under the null hypothesis

Genetics of common diseases

- Parametric linkage analysis has been a highly successful strategy for identifying (localising) genes involved in rare monogenic (single-gene) disorders
    - e.g. Huntingdon's disease, Cystic Fibrosis
- Less successful for common complex disorders
    - Hard to find large pedigrees showing clear disease segregation
    - Complex modes of inheritance: many interacting genetic and environmental factors

      $\Rightarrow$ No one-to-one correspondence between genotype and phenotype

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    ⇒ No one-to-one correspondence between genotype and phenotype
- Non-parametric linkage analysis (e.g. affected sib pair studies) uses a simpler approach
  - Tries to determine whether members of a family with "similar" trait values (e.g. both affected with disease) tend to inherit genetic material in common from their common ancestors
    - More often than would be expected by chance
- However it also has only proved useful in a few instances...

Success of non-parametric linkage

- Type 1 diabetes: confirmed the roles of HLA and insulin genes (Davies et al. 1994)
- Crohn's disease: *NOD2* / *CARD15* gene implicated (Hugot et al. 2001)
- Age-related macular degeneration:
  - Complement factor H gene identified through a combination of approaches, including follow-up of significant regions from non-parametric linkage scan (Haines et al. 2005)
- All of these findings have subsequently been identified through association studies
- Risch and Merikengas (1996) showed that for common genetic variants of small effect, association analysis has greater power

Association vs linkage

- Linkage studies measure correlation between alleles at the marker (test) locus and disease status within families
  - Caused by a lack of recombination between alleles at the marker locus and the underlying disease locus
  - In linkage studies, we test for this lack of recombination directly

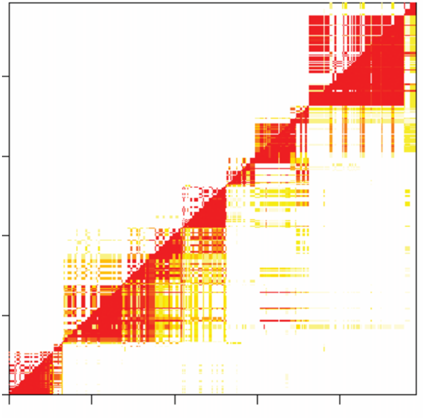
- Association studies measure correlation between alleles at the marker (test) locus and disease status across families
  - Caused by historical lack of recombination between marker and disease alleles over many generations
  - Correlations across families operate even across unrelated individuals (=families of size 1)
    - Motivates the use of population-based association studies of unrelated individuals

Correlation across families?

- Why should the same marker allele be correlated with disease status across many different families?
    - This implies that it tends to occur together with (i.e. on the same haplotype as) the disease allele
- One explanation is that this marker allele is itself, in fact, the causal (disease) allele
- Another explanation arises due to a phenomenon known as linkage disequilibrium (LD)
    - Alleles at loci that are close together tend to show correlation with one another
        - Suppose SNP 1 with alleles A and C, SNP 2 with alleles G and T
        - If SNPs 1 and 2 are in LD, then people who tend to have A allele(s) at SNP 1, also tend to have G allele(s) at SNP 2 (for example)
    - Occurs due to historical lack of recombination over many generations

Visualising LD

- Plot showing LD measures r^2 (upper) and D' (lower):



- LD across the genome shows a block-like pattern, boundaries correspond to 'recombination hotspots'

Definitions of LD

(Skip to slide 29)

- Linkage disequilibrium (LD) corresponds to correlation between alleles at two or more loci, at the population level
  - Suppose 2 dialleleic loci: locus A with alleles A and a, locus B with alleles B and b
  - If loci A and B are in LD, then people who tend to have A allele(s) at locus A, also tend to have B allele(s) at locus B (for example)
- Most easily described by looking at the haplotype frequencies of the 4 possible haplotypes:

$$A-B,\ A-b,\ a-B,\ a-b$$

Linkage equilibrium (LE)

- Two loci are in linkage *equilibrium* when the probability of observing a certain allele at one locus does not depend on which allele is observed at the other locus
- For our diallelic loci, this means

$$p_{AB} = p_A p_B$$

  - p_{AB} is the population frequency (probability) of haplotype $A - B$
  - p_A is the population allele frequency of allele A (at locus A)
  - p_B is the population allele frequency of allele B (at locus B)

Counting haplotypes

- Suppose we could observe a set of haplotypes (e.g. 12 haplotypes from 6 people)

ID	SNP1	SNP2
1a	A	B
1b	A	B
2a	A	B
2b	a	B
3a	a	B
3b	A	B
4a	A	b
4b	A	B
5a	A	B
5b	A	B
6a	a	B
6b	A	b

$$p_A = 9/12, \qquad p_a = 3/12$$
$$p_B = 10/12, \qquad p_b = 2/12$$

$$p_{AB} = 7/12, \quad p_{Ab} = 2/12,$$
$$p_{aB} = 3/12, \qquad p_{ab} = 0$$

Under LE, we expect $p_{AB} = p_A p_B$

Under LD, we expect $p_{AB} \neq p_A p_B$

Deviation from linkage equilibrium

- The *deviation* from linkage equilibrium is the difference

$$D_{AB} = p_{AB} - p_A p_B$$

- In our example, $D_{AB} = 7/12 - (10/12) \times (9/12) = -0.042$
- The sign of D is not usually considered important
  - Indicates whether haplotype $A - B$ is more or less frequent than expected
  - i.e. does allele A tend to go with allele B, or with allele b
- For 2 diallelic loci it turns out that

$$|D_{AB}| = |D_{Ab}| = |D_{aB}| = |D_{ab}| \quad = |D|, \text{ say}$$

D'

- The range of possible values for $|D|$ depends on the allele frequencies
- Therefore a normalised value D' is often used

  $$D' = |D|/D_{\text{max}}$$

  where D_{max} refers to the maximum value $|D|$ could take given the observed allele frequencies
- If $D \geq 0$, D_{max} is given by the smaller of $p_A p_b$ and $p_a p_B$
- If $D < 0$, D_{max} is given by the smaller of $p_A p_B$ and $p_a p_b$

D'

- D' varies between 0 and 1
  - 0 meaning no LD
  - 1 meaning 'complete' LD
- For 2 diallelic loci, if $D' = 1$, it means that at least one of the four haplotypes

  $$\begin{array}{c} A - B \\ A - b \\ a - B \\ a - b \end{array}$$

  does not occur
- Turns out to be a good indicator of historical recombination

r^2

- An arguably more useful measure of the correlation between alleles at two loci is the (squared) correlation coefficient
    - Denote the allele at locus A as a random variable X ($= 1$ or 0 according to whether allele is A or a)
    - Denote the allele at locus B as a random variable Y ($= 1$ or 0 according to whether allele is B or b)
    - Then the correlation coefficient

$$r = \frac{E(XY) - E(X)E(Y)}{\sqrt{\mathrm{Var}(X)\mathrm{Var}(Y)}}$$

and r^2 is the square of this

Formula for r^2

- It turns out that

$$r^2 = \frac{(p_{AB} - p_A p_B)^2}{p_A p_a p_B p_b}$$

- Estimate in our example:

$$r^2 = \frac{(7/12 - 90/144)^2}{(9/12) \times (3/12) \times (10/12) \times (2/12)} = 0.067$$

(correlation coefficient $r = \sqrt{0.067} = 0.258$)

r^2

(Resume here)

- r^2 ranges between 0 (no correlation) and 1 (perfect correlation)
- If $r^2 = 1$, only two of the four possible haplotypes occur
- r^2 has a useful interpretation in terms of power
  - Suppose a sample size of n (e.g. people/chromosomes) is required to detect an association at the causal locus A
  - Then a sample size of n/r^2 is required to detect an association if locus B is typed instead of A.
  - E.g. if 500 cases and controls were required to detect association at A, then $500/0.067 = 7500$ would be required to detect this association via genotyping locus B

Decay of LD

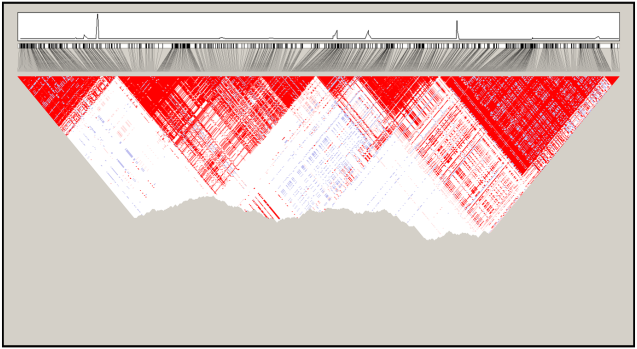
- Since LD is caused by a lack of recombination, and the probability of recombination θ increases with distance, the strength of LD between loci is expected to decline with distance.
- It can be shown that, if in one generation the LD between two loci is represented by D, then in the next generation this measure takes value $(1-\theta)D$
- So after n generations

$$D_n = (1-\theta)^n D_0$$

- LD will thus decay over time (and will tend to 0)
- However, the closer the loci, the smaller the rate of decay

Visualising linkage disequilibrium (LD)

- We often visualise LD through a plot, showing the 'LD blocks':



Tagging SNPs

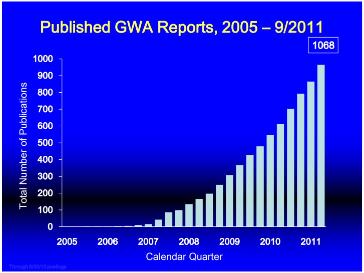
- If two SNPs are in strong LD, then the genotype at SNP2 will be well-predicted by the genotype at SNP1
- If genotype at SNP1 is correlated with a disease phenotype, then genotype at SNP2 will also be correlated with disease phenotype
    - ⇒ don't need to genotype both SNPs in order to detect the association with disease
- Using a sample of haplotypes from a reference sample (e.g. HapMap, 1000 Genomes), we can pick a reduced set of SNPs to genotype that provide us with (almost) as much information as the full set

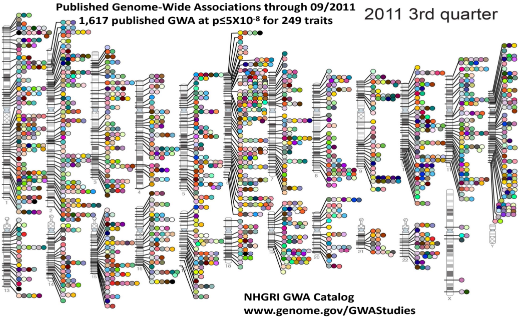
Tagging SNPs

- Often used in candidate gene studies to reduce costs
  - Given a candidate gene or region of interest, need to pick tagging SNPs
    - E.g. using Haploview software
    - http://www.broadinstitute.org/haploview/haploview
- Some (early) genome-wide platforms chose to focus on tagging SNPs
  - Improves efficiency (given a fixed number of SNPs)
  - At the expense of reduced redundency
- Nowadays we have very dense genome-wide platforms containing millions of SNPs
  - **But**, there has been a resurgence of interest in developing cheaper SNP-chips with fewer (carefully chosen) SNPs
    - e.g. UK Biobank Axiom Array used in the UK Biobank project
    - Through imputation, we can infer the genotypes at SNPs that were not actually genotyped

Success of GWAS

- Over the last ∼18 years, there have been a slew of high-profile GWAS, in a variety of different diseases
- Highly successful: many hundreds of associations (between genotype and phenotype) detected




Published Genome-Wide Associations through 09/2011
1,617 published GWA at p≤5X10-8 for 249 traits
2011 3rd quarter
NHGRI GWA Catalog
www.genome.gov/GWAStudies

Testing for association

- Most methods produce a test statistic and a p value indicating how significant the association/correlation between a given SNP and phenotype is
  - i.e. how likely it was to have occurred by chance
- In GWAS, we require stringent significance levels (e.g. $p = 5 \times 10^{-8}$) to overcome the multiple testing problem incurred when we test many SNPs throughout the genome
  - Or, in a Bayesian framework, stringent Bayes Factors to account for the low prior probability that any particular SNP is associated
  - If testing 1 million SNPs using $p = 0.05$, we would obtain 50,000 ‘significant’ results just by chance!
    - We therefore need to use large sample sizes (1000s of individuals) to have sufficient power

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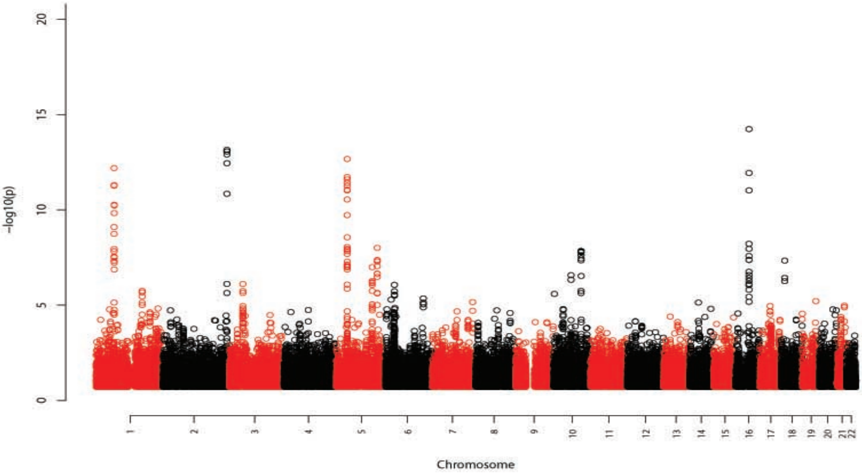
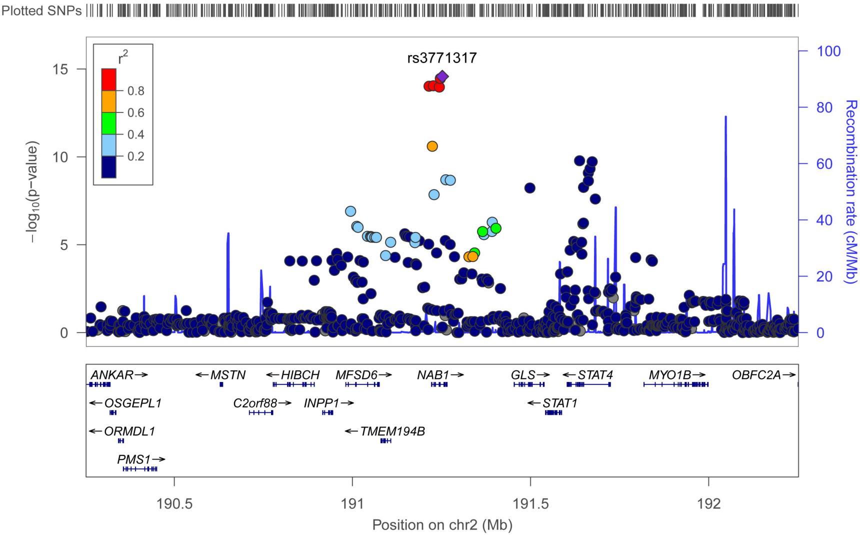
        - We therefore need to use large sample sizes (1000s of individuals) to have sufficient power
- At any location showing 'significant' association, we expect several SNPs in the same region to show association with phenotype
    - Due to correlation (LD) between neighbouring SNPs

Manhattan Plots



Close-up of hit region



Disappointment with GWAS

- GWAS point us to genomic regions (loci) highly likely to harbour disease genes
    - We still don't know the functional (causal) variant, in most cases
    - Indeed, the causal variant may well not even have been genotyped (but SNPs that are correlated with it have been genotyped)
- SNPs identified through GWAS generally have small ORs (< 1.5), suggesting their effects are not very 'important'
    - As we increase sample size, we detect more and more 'significant' SNPs with smaller and smaller effect sizes (ORs)
    - But the SNPs identified do not have strong predictive value (e.g. for predicting disease status)

Disappointment with GWAS

The case of the missing heritability

- Problem of ‘missing heritability’
  - SNPs identified through GWAS do not fully account for observed correlations in phenotype between close relatives
  - Suggesting there are additional genetic factors to be found...

Is this disappointment warranted?

- GWAS are best considered as a hypothesis generating exercise
  - Identifying 'candidate' genomic regions for further investigation
    - Possibly via different types of experiment
  - And potentially pointing us to new biology
    - Ankylosing spondylitis (IL-23 pathway)
    - Schizophrenia (calcium signalling)
    - Inflammatory bowl disease
      (IL-23 pathway, autophagy pathway, innate immunity)

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- See discussion in
  - Visscher et al. (2012) AJHG 90:7-24 “Five Years of GWAS Discovery”
  - Visscher et al. (2017) AJHG 101:5-22 “10 Years of GWAS Discovery: Biology, Function, and Translation”
  - Abdellaoui et al. (2023) AJHG 110:179-194 “15 Years of GWAS Discovery: Realizing the promise”