
Complex Trait Genomic Analysis
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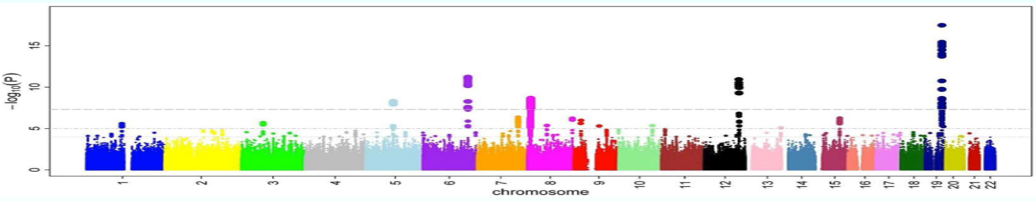
Complex Trait Genomic Analysis
Genome Wide Association Studies



Manhattan plot

Genome Wide Association Studies (GWAS)

1. Why disequilibrium?
2. What could go wrong? (almost everything)
3. Testing: False Discovery Rate (FDR)
4. Population Structure
5. Mixed Models

The Future of Genetic Studies of Complex Human Diseases

Neil Risch and Kathleen Merikangas

Geneticists have made substantial progress in identifying the genetic basis of many human diseases, at least those with conspicuous determinants. These successes include Huntington's disease, Alzheimer's disease, and some forms of breast cancer. However, the detection of genetic factors for complex diseases—such as schizophrenia, bipolar disorder, and diabetes—has been far more complicated. There have been numerous reports of genes or loci that might underlie these disorders, but few of these findings have been replicated. The modest nature of the gene effects for these disorders likely explains the contradictory and inconclusive claims about their identification. Despite the small effects of such genes, the magnitude of their attributable risk (the proportion of people affected due to them) may be large because they are quite frequent in the population, making them of public health significance.

age analysis we have chosen for this argument is a popular current paradigm in which pairs of siblings, both with the disease, are examined for sharing of alleles at multiple sites in the genome defined by genetic markers. The more often the affected siblings share the same allele at a particular site, the more likely the site is close to the disease gene. Using the formulas in (*1*), we calculate the expected proportion Y of alleles shared by a pair of affected siblings for the best possible case—that is, a closely linked marker locus (recombination fraction $\theta = 0$) that is fully informative (heterozygosity = 1) (*2*)—as

$$Y = \frac{1+w}{2+w} \text{ where } w = \frac{pq(\gamma-1)^2}{(p\gamma+q)^2}$$

If there is no linkage of a marker at a particular site to the disease, the siblings

linkage analysis for loci conferring GRR of about 2 or less will never allow identification because the number of families required (more than ~2500) is not practically achievable.

Although tests of linkage for genes of modest effect are of low power, as shown by the above example, direct tests of association with a disease locus itself can still be quite strong. To illustrate this point, we use the transmission/disequilibrium test of Spielman *et al.* (*3*). In this test, transmission of a particular allele at a locus from heterozygous parents to their affected offspring is examined. Under Mendelian inheritance, all alleles should have a 50% chance of being transmitted to the next generation. In contrast, if one of the alleles is associated with disease risk, it will be transmitted more often than 50% of the time.

For this approach, we do not need families with multiple affected siblings, but can focus just on single affected individuals and their parents. For the same model given above, we can calculate the proportion of heterozygous parents as $pq(\gamma + 1)/(p\gamma + q)(4)$. Similarly, the probability for a heterozygote parent to transmit the high risk A allele is just $\gamma/(1 + \gamma)$. Association tests can also be performed for pairs of affected siblings. When the locus is associated with disease, the transmission excess

Science, 1996. Cited by 5700

Locus 1	Locus 2	
	B	b
A	p_{AB}	p_{Ab}
a	p_{aB}	p_{ab}

haplotype frequencies

Remember:

$E(p_{AB}) = p_A \cdot p_B$

$p_{AB} = p_A \cdot p_B - D$

disequilibrium

$D = p_{AB} - p_A \cdot p_B$

allelic frequencies

Disequilibrium measures (i): D'

$$D' = D / D_{MAX} = (p_{AB} - p_A p_B) / D_{MAX}$$

$$D_{MAX} = \min(p_A p_b , p_B p_a) \text{ if } D>0$$

$$D_{MAX} = \max(p_A p_B , p_a p_b) \text{ if } D<0$$

- Lewontin (1964).
- Tells whether recombination has occurred.
- D'=1 indicates lack of recombination, intermediate values are less easy to interpret.
- Very sensitive to rare alleles.

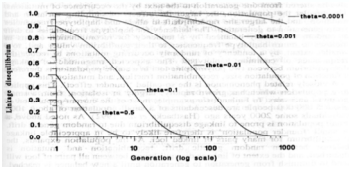
Disequilibrium measures (ii): r^2

$$r^2 = D^2 / p_A p_B p_a p_b$$

- The squared correlation between alleles at both haplotypes.
- It is 1 iff both alleles at the two loci are identical in all haplotypes.
- nr^2 is the power in a Chi-2 test to detect disequilibrium.
- More related to distance than D'.

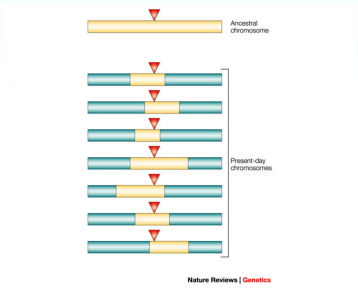
Why disequilibrium?

- Linkage analysis maps the loci with very poor resolution because the number of recombinations analyzed is very low.
- LD considers implicitly all recombinations since the original mutation.
- A coalescence view: the amount of history shared between two loci is inversely proportional to distance.
- Disequilibrium is rapidly eroded by recombination



Ergo

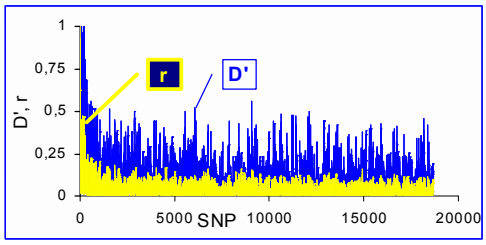
- Two loci that are in strong disequilibrium should be close physically.
- PRINCIPLE OF LD MAPPING: The locus in strongest LD with a given trait should be the causative locus or the closest to the causative locus.



Ardlie et al. 2002

What can go wrong?

- Disequilibrum pattern is highly variable.
- Depends on metrics and on allele frequencies
- LD is affected by all evolutionary forces, including, and most frequently, admixture.



Important remarks

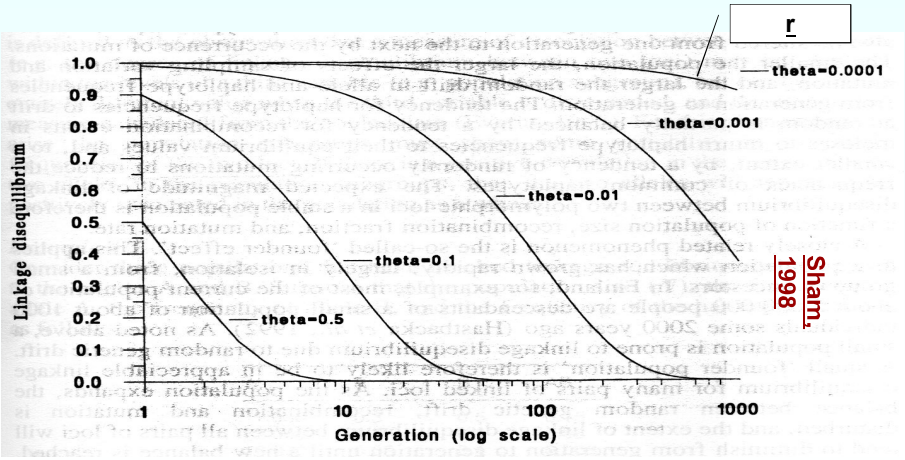
- Obviously, it is the existence of recombination that makes it linkage disequilibrium mapping possible. LD mapping cannot be applied to mtDNA or Y chromosome.
- THERE EXISTS A LARGE STOCHASTIC SAMPLING IN THE AMOUNT OF LINKAGE DISEQUILIBRIUM.

Forces that affect disequilibrium

- Any evolutive force influences LD.
  - Recombination
  - Drift
  - Mutation
  - Admixture
  - Selection
- Mathematical models can be developed for each factor but is difficult to model all interactions between them.

Forces that affect disequilibrium (i): Recombination

$$D_{t+1} = (1 - r) D_t$$



Forces that affect disequilibrium

- Random drift: Under population expansion, LD tends to be conserved and it is eroded less rapidly than Ne increases.
- Mutation: It is the main force on which we are interested. Under the simplest model, a unique mutation causing a disease has appeared in a single haplotype and, by definition, the new allele is in complete LD with the alleles at the other loci.
- Mixture: Two loci that are in equilibrium within each subpopulation are not in equilibrium when the whole population is considered.
- Selection: It also generates disequilibrium because of hitchhiking effect.

Mixture

Pop.	N	p(A)	p(B)	p(AB)	D
A	100	0.5	0.25	0.125	0
B	100	0.10	0.10	0.01	0
Mixed	200	0.30	0.175	0.0675	0.015
A	10	0.5	0.25	0.125	0
B	100	0.10	0.10	0.01	0
Mixed	110	0.136	0.113	0.02	0.005

Panmixia accelerates decay of LD, assortative mating retards it.

Measuring association

- Qualitative traits (disease)
  - Chi-2 tests
  - Cochran-Armitage test
  - Logistic regression
- Quantitative traits
  - Linear models

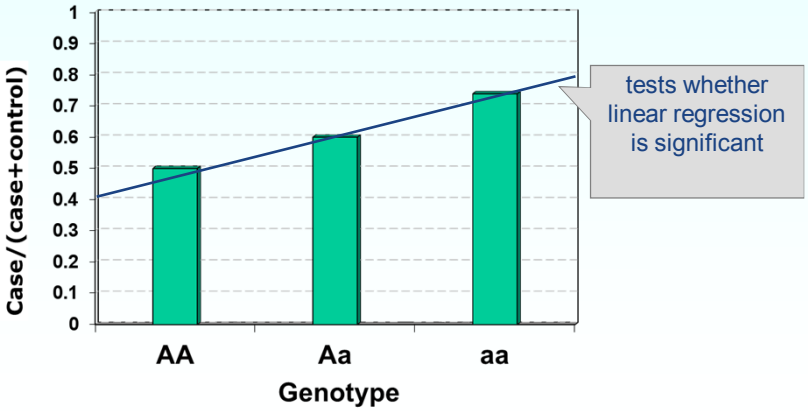
Measuring association (i)
Chi-2 test

	AA	Aa	aa	Sum
Case	n_{+AA}	n_{+Aa}	n_{+aa}	n_{+}
Control	n_{-AA}	n_{-Aa}	n_{-aa}	n_{-}
Sum	n_{AA}	n_{Aa}	n_{aa}	N

$$\chi^2 = \sum_{i,j} \frac{(n_{ij} - n_i n_j / N)^2}{n_i n_j / N}$$

1 d.f.

Measuring association (ii) Cochran-Armitage trend test



Measuring association (iii): logistic regression

- Usual regression assumes the variable of interest is normally distributed, thus continuous.
- Logistic regression is a way of analyzing count data (e.g., binary).
- Is a class of the so called generalized linear methods.
- The threshold (probit) model is a very similar method to the logistic.

Typical regression	Logistic regression
y = Xb + e	P = exp(Xb) / [1-exp(Xb)]

Measuring association (iii): logistic regression

y	SNP	P(y=Y)
1	AA	$1 / [1+\exp(\beta_{AA})]$
1	AA	$1 / [1+\exp(\beta_{AA})]$
0	aa	$\exp(\beta_{aa}) / [1+\exp(\beta_{aa})]$
0	Aa	$\exp(\beta_{Aa}) / [1+\exp(\beta_{Aa})]$
1	aa	$1 / [1+\exp(\beta_{aa})]$
0	Aa	$\exp(\beta_{Aa}) / [1+\exp(\beta_{Aa})]$

$L = \Pi P(y_i=Y)$

exp(Xb) is the odds ratio of the effect, or increase in risk, e.g., $\exp(\beta_{AA}-\beta_{aa})$ is the increased risk of AA vs. aa genotypes

Typical regression	Logistic regression
$y = Xb + e$	$P = 1 / [1 + \exp(Xb)]$

Measuring association (iv): Linear models

$$y = Xb + e$$

- Each SNP genotype can be coded as class, dominance and additive effects are estimated simultaneously, 2 d.f..
- A regression can be carried out on the number of, say, A alleles (0, 1, 2) assuming additivity, 1 d.f.

Advantages

- ✓ Easy to implement.
- ✓ No strong assumption about the mode of inheritance.
- ✓ It can be stratified (say by race).
- ✓ Logistic regression allows for a very flexible modeling strategy.

Disadvantages

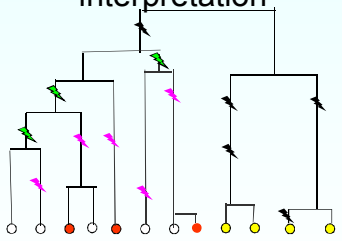
- † Sensitive to a number of uncontrolled factors (admixture, random drift). Leads to spurious association.
- † Loss of power is very rapid when the no. of alleles increase.
- † Chi-2 uses very little information (only one marker at a time, no family structure accounted for).

Whole genome association studies (WGAS)

- RATIONALE: Using a sufficiently dense genotyping should allow us to identify markers that are in strong disequilibrium with the causative mutation(s) AND that are not false positives.
- The recent increase in marker genotyping throughput and decreasing costs have made feasible these studies.
- Currently, arrays allow genotyping millions of SNPs with additional probes to characterize thousands of copy number variations (CNV) in humans.
- A catalogue of GWAS: http://www.genome.gov/gwastudies/
- http://www.ncbi.nlm.nih.gov/gap

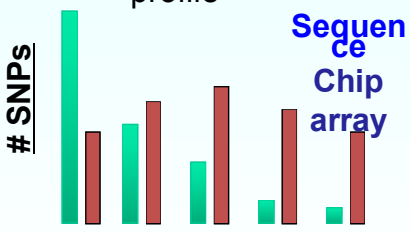
SNPs in arrays are subject to ascertainment bias

Coalescence interpretation



SNPs in panel
singletons in panel
undiscovered SNPs

Effect on SNP frequency profile

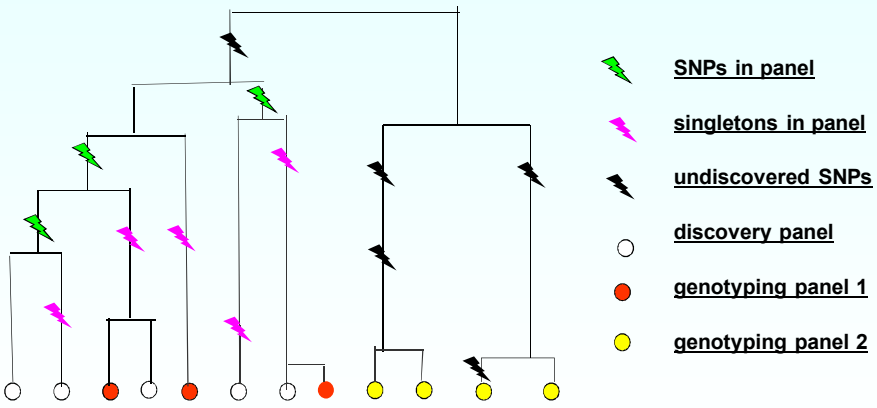


SNP derived allele count

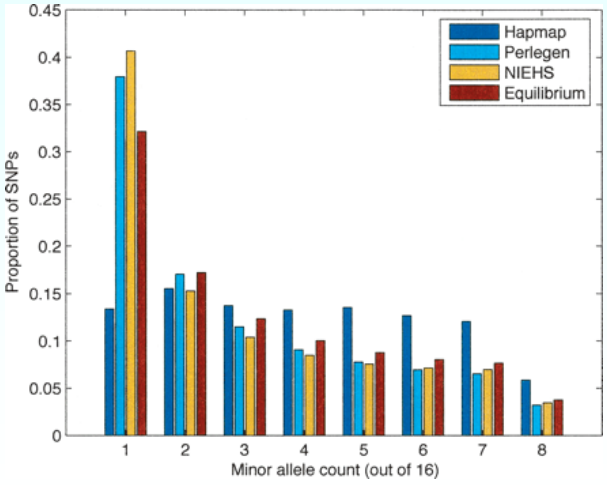
SNP ascertainment bias

- It arises because the SNP discovery panel is different from the panel genotyped (where the association study is carried out).
- SNPs on a chip are chosen for technical reasons and to be as 'informative' as possible across breeds; they are used to genotype additional individuals from same or other breeds.
- There is a strong trend to discard rare SNPs from the SNP panel.
- This is not so serious if common diseases are caused by common variants.
- SNP choice will bias population genetic estimates.

SNP ascertainment bias (ii)



SNP ascertainment bias (iii): Human population frequency spectra



Clark et al. (2005) Genom Res 15:1496

How about sequence?

- It avoids SNP ascertainment bias
- The QTNs are in the data
- Usually, used with imputed data
- Main issue are rare variants. Quality control remain a main issue here.

Am J Hum Genet. 2014 Jul 3; 95(1): 5–23. PMCID: PMC4085641
doi: 10.1016/j.ajhg.2014.06.009

Rare-Variant Association Analysis: Study Designs and Statistical Tests

Seunggeung Lee,[1] Gonçalo R. Abecasis,[1] Michael Boehnke,[1] and Xihong Lin[2,*]

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Abstract

Go to:

Despite the extensive discovery of trait- and disease-associated common variants, much of the genetic contribution to complex traits remains unexplained. Rare variants can explain additional disease risk or trait variability. An increasing number of studies are underway to identify trait- and disease-associated rare variants. In this review, we provide an overview of statistical issues in rare-variant association studies with a focus on study designs and statistical tests. We present the design and analysis pipeline of rare-variant studies and review cost-effective sequencing designs and genotyping platforms. We compare various gene- or region-based association tests, including burden tests, variance-component tests, and combined omnibus tests, in terms of their assumptions and performance. Also discussed are the related topics of meta-analysis, population-stratification adjustment, genotype imputation, follow-up studies, and heritability due to rare variants. We provide guidelines for analysis and discuss some of the challenges inherent in these studies and future research directions.

Summary of Statistical Methods for Rare-Variant Association Testing

	Description	Methods	Advantage	Disadvantage	Software Packages[a]
Burden tests	collapse rare variants into genetic scores	ARIEL test,[50] CAST,[51] CMC method,[52] MZ test,[53] WSS[54]	are powerful when a large proportion of variants are causal and effects are in the same direction	lose power in the presence of both trait-increasing and trait-decreasing variants or a small fraction of causal variants	EPACTS, GRANVIL, PLINK/SEQ, Rvtests, SCORE-Seq, SKAT, VAT
Adaptive burden tests	use data-adaptive weights or thresholds	aSum,[55] Step-up,[56] EREC test,[57] VT,[58] KBAC method,[59] RBT[60]	are more robust than burden tests using fixed weights or thresholds; some tests can improve result interpretation	are often computationally intensive; VT requires the same assumptions as burden tests	EPACTS, KBAC, PLINK/SEQ, Rvtests, SCORE-Seq, VAT
Variance-component tests	test variance of genetic effects	SKAT,[61] SSU test,[62] C-alpha test[63]	are powerful in the presence of both trait-increasing and trait-decreasing variants or a small fraction of causal variants	are less powerful than burden tests when most variants are causal and effects are in the same direction	EPACTS, PLINK/SEQ, SCORE-Seq, SKAT, VAT
Combined tests	combine burden and variance-component tests	SKAT-O,[64] Fisher method,[65] MiST[66]	are more robust with respect to the percentage of causal variants and the presence of both trait-increasing and trait-decreasing variants	can be slightly less powerful than burden or variance-component tests if their assumptions are largely held; some methods (e.g., the Fisher method) are computationally intensive	EPACTS, PLINK/SEQ, MiST, SKAT
EC test	exponentially combines score statistics	EC test[67]	is powerful when a very small proportion of variants are causal	is computationally intensive; is less powerful when a moderate or large proportion of variants are causal	no software is available yet

Common software for GWAS analyses

- Plink: managing data, simple statistics

https://www.cog-genomics.org/plink2

- GCTA: compute marker relationship matrix

http://www.complextraitgenomics.com/software/gcta/download.html

- SNPassoc: R for association

http://cran.r-project.org/web/packages/SNPassoc/index.html

- GenABEL: complex, mixed model modeling

http://www.genabel.org/packages/GenABEL

The Wellcome Trust Case Control Consortium (WTCCC)

- 3000 control in one group of blood donors and a control group.
- 2000 cases in 7 common diseases: Biolar disorder, Coronary artery disease, Chron's disease, Hypertension, Rheumatoid arthritis, Type I diabetes, Type II diabetes.
- UK wide caucasian origin.
- 50 research groups.
- 500k SNP chip genotyped.
- Data publicly available under some restrictions.

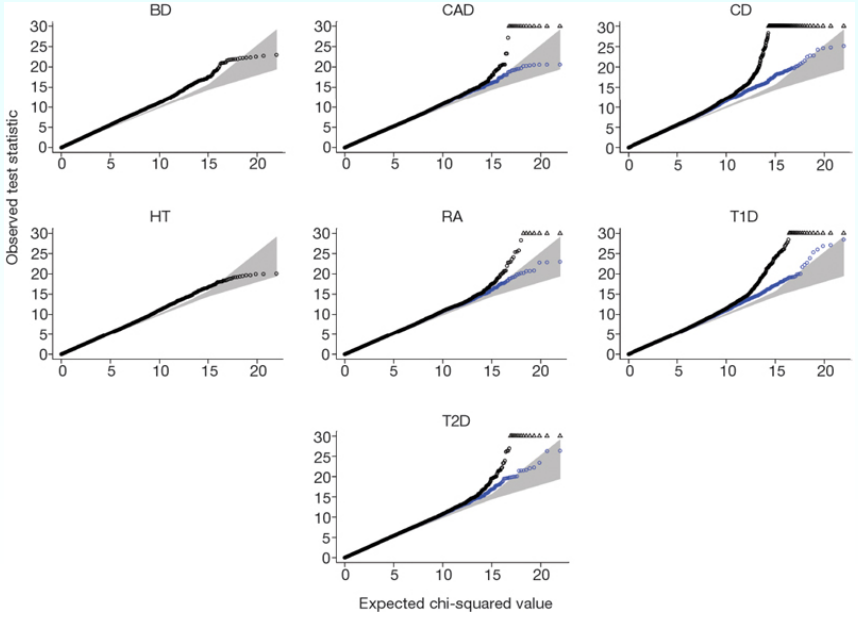
http://www.wtccc.org.uk/

Superseded by larger initiatives such as biobank

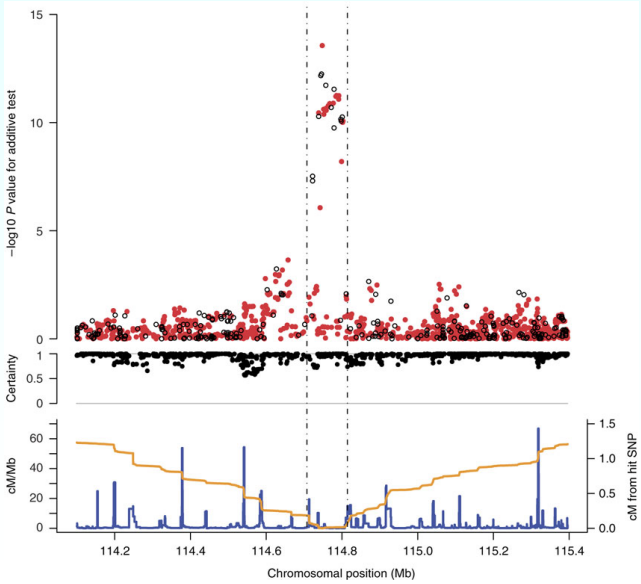
WTCCC: Structure

- Population structure (admixture) can confound association and origin, thus inflating the rate of false positives.
- Principal components and Chi-2 association test between SNP frequency and geographic region.
- The most clear geographic pattern was NW/SE axis.
- The most likely cause for these marked geographical differences is natural selection, most plausibly in populations ancestral to those now in the UK.
- Some known genes that can be involved in adaptation (lactase, toll-like receptor, etc).
- Overall, allowing for structure had little importance on final results.

WTCCC (ii): QQ plots



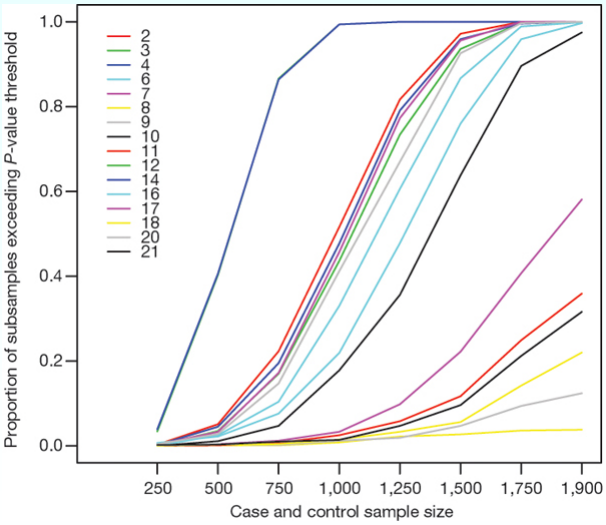
WTCCC (iii): How the results look like



WTCCC: General results

- The increased associated risk is rather small (odds-ratio), in the order 1.2 - 1.5
- 25 regions with $P < 10^{-7}$
  - 12 previously known
  - 10 have been confirmed
  - 1 gave equivocal results
  - 5 more have been replicated

WTCCC: Influence of sample size

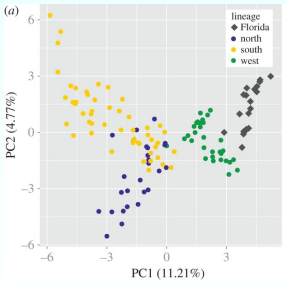


each color is a different associated SNP

For the 16 SNPs in Table 3 with *P* values below 5 x 10^{-7}, we randomly generated 1,000 subsets of our full data set corresponding to case-control studies with different numbers of cases, and the same number of controls (*x* axis). The *y* axis gives the proportion of subsamples in which that SNP achieved a *P* value below 5 x 10^{-7}. SNPs are numbered according to the row in which they occur in Table 3

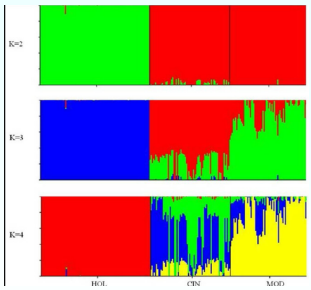
Detecting structure

Principal component analyses



- Compute % of variance explained by each eigenvalue
- PCA is highly sensitive to uneven sampling / population

STRUCTURE software



- Estimate optimum number of clusters

Correcting structure

- Genomic control
- Principal components as cofactors
- Mixed models with genomic relationship

Genomic control

- It address the issue that structure inflates the test statistic distribution.
- It corrects this via an inflation factor (usually called λ), defined as the median of the observed distribution divided by the expected median (0.456 in a Chisq with 1df).
- It assumes that most markers ~ null distribution
- It simply corrects for the excess of FDR, but does not reorder or deals with structuring effects on P-value order

Principal Components

Since PCA reflects structure, the idea is to include the first eigenvectors as fixed effects in the model.

$$y = \mathbf{X}\beta + \Sigma\mathbf{q}'\mathbf{m} + \lambda\mathbf{g} + \mathrm{e}$$

$$\mathbf{G} = \mathbf{M}\mathbf{M}';\ \mathbf{G} = \mathbf{Q}\Lambda\mathbf{Q}^{-1}$$

Price, A. L., Patterson, N. J., Plenge, R. M., Weinblatt, M. E., Shadick, N. A., & Reich, D. (2006). Principal components analysis corrects for stratification in genome-wide association studies. Nature Genetics, 38, 904–909.

Mixed Models

It is a generalization of the previous approach, where all PC are considered. G is the genomic relationship matrix.

$$y = \mathbf{X}\beta + \mathbf{Z}\mathbf{u} + \lambda\mathbf{g} + \mathrm{e}$$

$$\mathbf{u} \sim N(0, \mathbf{G})$$

$$\mathbf{G} = \mathbf{Q}\Lambda\mathbf{Q}^{-1}$$