Using Population Structure to Map Complex Diseases

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Outline

1. Background ideas

    - Genetic diseases and population structure
    - Problems of current approaches, and
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   - Basics of relatedness and inbreeding coefficient
   - How to estimate relatedness using genetic marker data

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   - Basics of relatedness and inbreeding coefficient
   - How to estimate relatedness using genetic marker data
3. An example

Genetic diseases

A **genetic disease** is a disease caused by abnormalities in an individuals' genetic material (genome).

A **simple disease** (e.g. cystic fibrosis) is caused by a defect in only one gene. By contract, **complex diseases** (e.g. most cancers) are caused by many variations occurred in different genes in the same cell. Different variations may lead to the same symptoms.

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- There are some success in mapping simple disease like cystic fibrosis, but not many.
- Very few genes have been found for complex diseases.
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- Mating is usually regional so traveling (migration) is not easy, especially in the old days.

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- **Migration** is getting easier!

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- Many diseases are recessive. Disease alleles are more likely to be expressed in individuals born to related parents, and in particular to parents who are closely related for the section of their genome in which a susceptibility factor lies. Consequently, **the presence of recessive factors can be inferred wherever affected individuals exhibit unusually elevated levels of relatedness between homologous chromosomes at some place in their genome**. (Illustration: homologous chromosomes)

Basic gene mapping methods

Genes that are close to a disease gene tend to co-segregate with it during meiosis. Therefore, if a gene is over/under-present in the diseased population than in the general population, this gene might be close to one of the disease genes.

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- Collect two groups of people, one with disease (case group) and one without (control group).
- Find out the genotypic information of as many markers as funding allows, and compare the allele frequencies of case and control groups.

If the population is genetically homogeneous and the marker is not linked to a susceptibility factor (disease locus), these allele frequencies should roughly be the same. Any significant difference in allele frequency can then be used to infer an association (linkage) between the marker and a disease locus.

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For example: suppose a sample of cases and controls is drawn from a population containing a number of subpopulations. If the disease of interest is at high frequency in one subpopulation, then we can expect to find that group overrepresented among the cases. Then, any marker allele that is at higher frequency in that subpopulation than in the others will appear to be associated with the disease, regardless of where it is in the genome. In other words, a spurious association will be found.

In the case of complex disease

Population structure is more important when mapping complex diseases because

- Disease susceptibility factors are likely to contribute to some families but not to others;
- Disease gene tends to form clusters among spatially and/or genetically related individuals/families.

If we treat families as independent observations, families for whom the factor is not important or present will contribute background noise that may mask the signal from those families where it does play a role.

Current Fixes to Population Structure

Population admixture has been widely recognized as the major reason for nonreplicability associations [Ardie et al 2002]. To overcome this problem, people either avoid population based case control studies (use TDT tests instead) or

- use markers throughout the genome to adjust for any inflation in test statistics due to substructure (Genomic control [Bacanu et al 2000, Devlin et al 2001])
- infer the details of the subpopulations (Structured Association [Pritchard et al 2000, Thornsberry et al 2001], etc)

However, there are no clear-cut subpopulations in a sample in many cases. Even there are, it is very difficult to estimate the number of subpopulations and classify samples into them. Homogeneity within subpopulations is also hard to prove.

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- **Estimate family relatedness using marker data**. The family relatedness measures are the averaged relatedness coef between all inter-family offspring combinations, which can be estimated by, for example, Queller's Method [Queller 1989, Lynch *et al* 1999]

  $$\hat{r}_{xy} = \frac{\frac{1}{2}(\delta_{ac}+\delta_{ad}+\delta_{bc}+\delta_{bd})-p_a-p_b}{1+\delta_{ab}-p_a-p_b}$$

  where (a,b), (c,d) are genotypes of individual x and y. p_a is the population frequency of allele a.

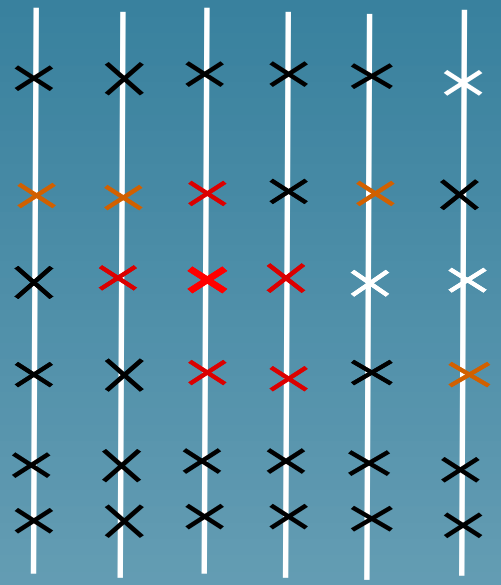
- **Define a weighting system** that falls off with increasing distance away along the chromosome and also with decreasing relatedness across families.

Our Approach (cont.)

- **Measure the inbreeding level of each locus for each family**. The locus-level inbreeding measures are estimated by, for example, Internal Relatedness

$$\hat{r}_x = \frac{2\delta_{ab} - p_a - p_b}{2 - p_a - p_b}$$

- **Average the inbreeding measures using the weighting system**. We infer the presence of recessive factors wherever affected individuals exhibit unusually elevated levels of relatedness between homologous chromosomes at some place in their genome.



- **The significance of high average measures can be tested** by randomizing the relatedness values and the marker locations and asking the extent to which the observed sum is large relative to randomized measures.

Tech details: Inbreeding Coefficient

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- Inbreeding coefficient has other names such as coefficient of coancestry, Consanguinity coefficient, Kinship. They commonly refer to inbreeding between two individuals.

Population Explanation of Inbreeding Coefficient

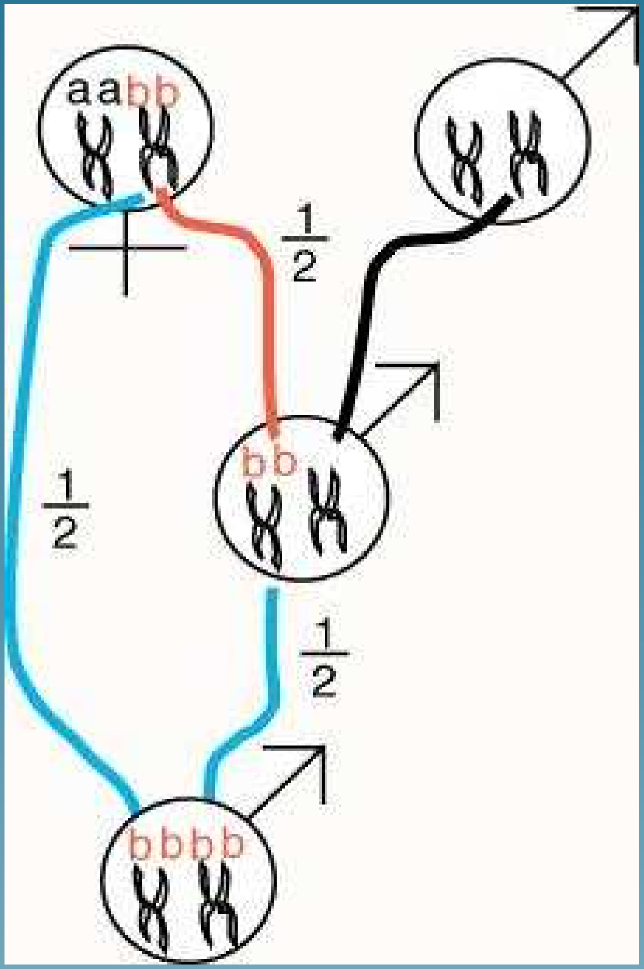
Suppose that p and q are frequency of two alleles at a locus. Under HWE, the frequency of heterozygous genotype should be $2pq$, we call this frequency in general H_0. In case of breeding, this frequency is H_1 and inbreeding coefficient (for this population)F is defined as

$$F = \frac{H_0 - H_1}{H_0}$$

One can deduce formulae of genotype frequencies in a population with inbreeding level F. We can see that inbreeding causes a decrease of heterozygosity.

	genotype frequency wih inbreeding	under Hardy-Weinberg Equilibrium
AA	$p^2 + pqF$	p^2
Aa	$2pq\left(1 - F\right)$	$2pq$
aa	$q^2 + pqF$	q^2

Estimate Inbreeding Coef. from Pedigree Data



Wright's (1922) formula:

$$F_I = \sum_i \left(\frac{1}{2}\right)^{n_i} (1 + F_{A_i})$$

where i is the ith common ancestor, n_i is the number of individuals inside the loop $I \rightarrow A_i \rightarrow I$.

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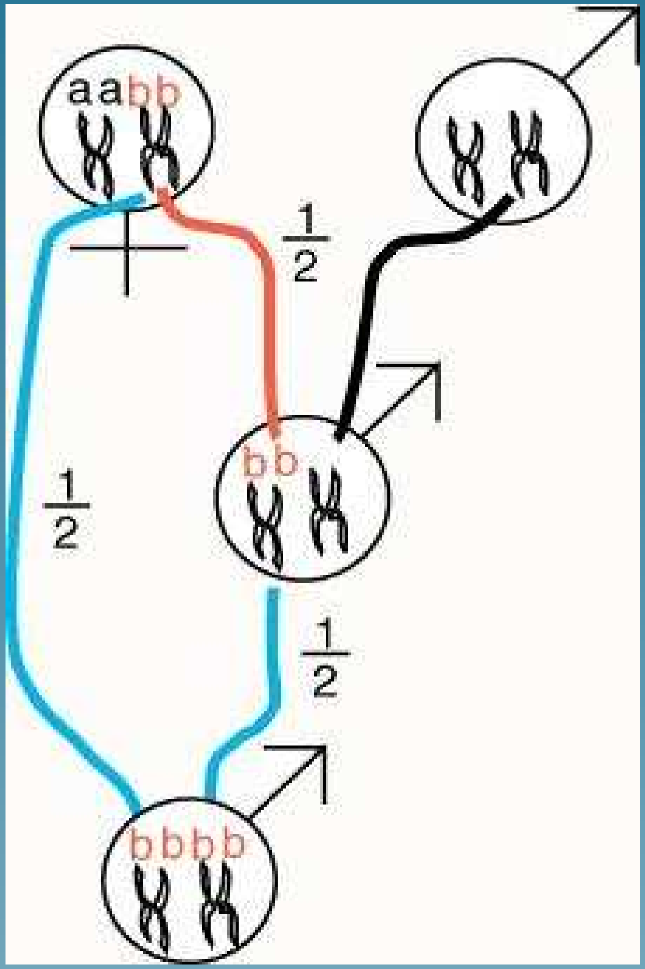

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For the left pedigree, $n_G = 2$,

$$F_I = \left(\frac{1}{2}\right)^2 (1 + F_G) = \frac{1}{4}$$

Family Level Relatedness: Dr. Queller's Method

Pedigree data is usually unavailable between families. (Need huge pedigrees?) Fortunately, progress in the developing of methods of estimating parental relatedness from marker data has been rapid. Suppose that individuals x has genotype (a, b) and individual y has genotype . Suppose that the allele frequencies of these alleles are p_a, p_b, p_c, p_d. The (directional) relatedness between x and y is given by

$$\hat{r}_{xy} = \frac{\frac{1}{2}(\delta_{ac} + \delta_{ad} + \delta_{bc} + \delta_{bd}) - p_a - p_b}{1 + \delta_{ab} - p_a - p_b}$$

where $\delta_{ac} = 1$ if $a = c$ and 0 otherwise. Usually, directional measures are averaged to get a better estimate, the formula becomes

$$\hat{r}_{xy} = \frac{\delta_{ac} + \delta_{ad} + \delta_{bc} + \delta_{bd} - p_a - p_b - p_c - p_d}{2 + \delta_{ab} + \delta_{cd} - p_a - p_b - p_c - p_{dd}}$$

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- Other methods are also available, notable from Lynch 1999.

Locus Level Relatedness Measures

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- **d-squared (d^2)**
  Microsatellite alleles diverge in a way such that the square of the length difference between a pair of alleles may be linearly related to time since their common ancestor. Consequently, the average squared allele length difference across either loci or individuals provides an estimator for overall genomic similarity. Let $Mx\,(h)$ be the number of alleles at locus h, denote the genotype of individual i as (a_{ih}, b_{ih}),

$$d^2 = \text{mean}\left(\frac{a_{ih} - b_{ih}}{Mx\,(h) - 2}\right)^2$$

Locus Level Relatedness Measure (Cont.)

- **Standardized heterozygosity (SH)** and **Standardized Observed Heterozygosity** (SOH)
  SH is heterozygosity but weighted by the expected heterozygosity at each locus scored. SOH is a version of SH where the correction is made using observed heterozygosity, rather than expected heterozygosity. This is a more robust method in comparison with others.

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- **Internal Relatedness**
  Internal relatedness was developed by William Amos in Cambridge and quantifies the degree of heterozygosity weighted by the frequencies of the alleles in each genotype. Using the similar notation as that of d^2 measure

$$IR = \frac{\sum_i \sum_h \left(2\delta_{a_{ih}=b_{ih}} - f_{a_{ih}} - f_{b_{ih}}\right)}{\sum_i \sum_h \left(2 - f_{a_{ih}} - f_{b_{ih}}\right)}$$

Log P-values of Locus Association

Leprosy dataset

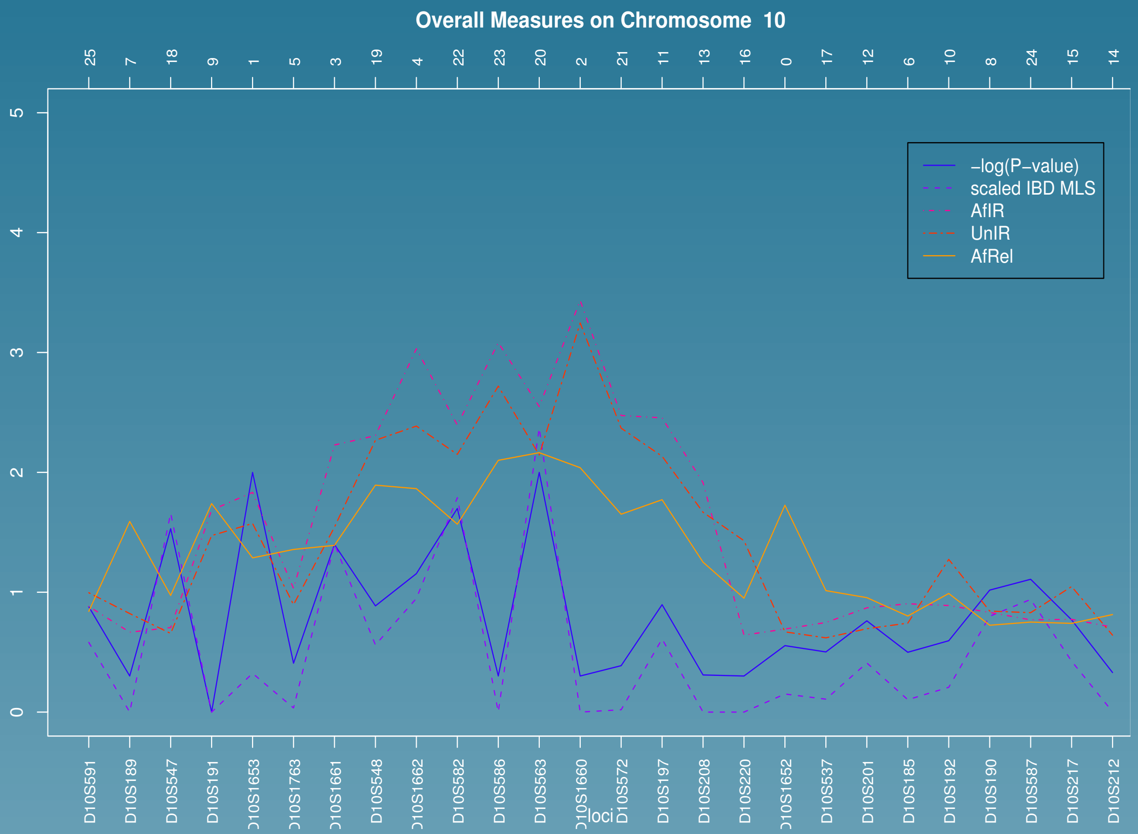
[Siddiqui et al 2001]

394 individuals

96 nuclear families

all offsprings are affected

295 microsatellite markers on 22 autosomes are typed



Our result confirms the reported susceptibility locus on chromosome 10, as well as most of the less-significant ones.

Summary and Future Work

- **What I have done**
  - ⋆ Implement a fast (relative to the extensive computation needed) and flexible algorithm that can perform our method using various family level and locus level relatedness measures, and various randomization methods;
  - ⋆ Test our algorithm on six real datasets; Test the robustness of our algorithm using partial information of the datasets;
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- **Future Work** ... lots of it
  - ⋆ This work is purely empirical right now. Statistical inference is not yet possible.
  - ⋆ Simulate related/unrelated family data and test the strength/variability of our method. Simulation program `EASYPOP` [Balloux 2001] is used.
  - ⋆ Evaluate some new relatedness measures (both family level and locus level).
  - ⋆ Adapt our method to SNP markers for fine mapping purpose.

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