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Identifying Genetic Variants Associated with Disease: Case-Control Association Testing with Related Individuals

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Joint work with Catherine Bourgain and Tim Thornton

Case-control association testing: some preliminaries

- We consider a complex trait or disease (e.g. asthma, alcoholism), which we treat as binary (affected/unaffected).
- **Complex** trait: may be influenced by multiple genetic and non-genetic factors
- Goal: identify some of the genetic risk factors related to the trait.

Terminology

- A person who has the disease may be called an **affected** or a **case**.
- **Control** could be someone who does not have the disease or someone whose disease status is unknown (these types are treated differently in the analysis).

Terminology (continued)

- **SNP** (single nucleotide polymorphism) — site in genome with single base-pair change that distinguishes some individuals from others in same population, e.g. A**A**GGCTAA vs. A**T**GGCTAA

- The two different variants at a SNP are called **alleles**.

- **Genotype** is the pair of alleles of an individual at a SNP. Typically observe only number of copies of each allele held by individual, e.g. i copies of allele A, which is equivalent to 2-i copies of allele T, $i = 0, 1, 2$.

Case-control association testing

- For a given marker, compare the allele or genotype distributions of cases and controls.

- Null hypothesis is that there is no difference between case and control allele/genotype distributions at the given marker.

- E.g. consider classical χ^2 test for association with a biallelic marker, equivalent to test for independence in 2×2 table

	Case	Control
Allele 0	C_{00}	C_{01}
Allele 1	C_{10}	C_{11}

- This χ^2 test is valid when alleles are independent within and between individuals under the null hypothesis

Use of related individuals in case-control testing: how it arises, why it can be desirable

- Families sampled for a linkage study may be included in an association study.
  - **Linkage** is co-inheritance of trait with allele or genotype within a family.
  - Linkage analysis of families with multiple affected individuals may be used for coarse mapping of genetic variants.
  - Then population-based association used for finer-scale mapping.
- Sampling individuals from families with multiple affecteds may increase power to detect assocation with complex traits because of enrichment for genetic cases.
  - Complex diseases such as breast cancer and heart disease have both genetic and non-genetic causes.
  - Cases from families with multiple affecteds are more likely to have predisposing genetic variants.

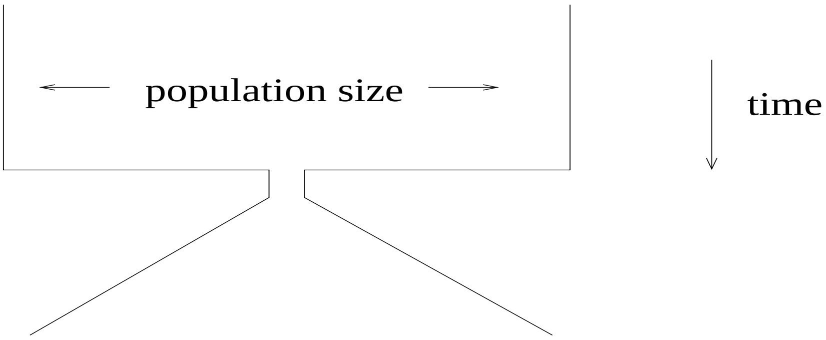
Use of related individuals in case-control testing: how it arises, why it can be desirable (cont.)

- Use of unaffected relatives of cases as controls can provide some measure of protection against potential problems of population substructure.
  - Frequency of disease may differ across sub-populations, frequency of allele or genotype may differ as well.
  - Sub-population may be a hidden covariate contributing to false detection of association.
  - Using related cases and controls makes this problem less likely.

- Founder populations, in which most or all individuals are related, can be particularly valuable for genetic studies.

Complex trait mapping in founder populations

- **Founder population:** a population in which a recent bottleneck has resulted in a large number of individuals all descended from a small number of founders



- Founder populations may be particularly useful for complex trait mapping because of
  - reduced genetic heterogeneity due to small number of founders
  - in some cases, reduced environmental heterogeneity
  - linkage disequilibrium may exist over greater distances ⇒ less dense map required to detect association

Statistical issues that arise with use of related individuals in case-control testing

- When some individuals in the samples are related, it creates dependence among the observations.

- Both case-control status and allele/genotype run in families

- Type I error:

  - application of standard methods can result in a dramatic increase in false detections (Newman et al. 2001; Bourgain et al. 2003).

- Power:

  - information on relatedness can be used to increase power

  - explicitly taking into account the fact that there is an enrichment for predisposing variants in affecteds with affected relatives can also increase power

- We develop quasi-likelihood (QL) methods for case-control association testing of genetic traits with related individuals. Properties include:
  - uses only first and second moments
  - applicable to any sample of related individuals
  - Type I error is corrected for the dependence
  - weights depending on case-control status and relationships of individuals are used to optimize power (maximize non-centrality parameter) within a linear class of statistics
  - allows us to leave unspecified some parts of the model of which we are ignorant $\Longrightarrow$ retains a major part of the appeal of the original case-control association test
  - computationally feasible, even in large complex inbred pedigrees with multiple inbreeding loops

- More generally, QL inference methods can be used to extend other types of classical population genetic inference to founder populations, e.g.
  - allele frequency estimation
  - Armitage test for case-control association
  - Hardy-Weinberg equilibrium test

- Agenda for remainder of talk:
  - General QL approach
  - 3 approaches for case-control association testing in related individuals:
    - correct the variance of the standard χ^2 statistic: $W_{\chi^2_{corr}}$
    - QL approach under a simple model for case-control differences: W_{QLS}
    - Improvement of power by more detailed consideration of properties of a genetic trait: M_{QLS}
  - Simulations and an example

Quasi-likelihood (QL) estimation

- Wedderburn (1974), Godambe (1960), Jarrett (1973), McCullagh and Nelder (1989), Heyde (1997)

- Let $X_{n\times 1}$ be random with $E(X) = \mu_{n\times 1}$ and Var$(X) = V_{n\times n}$, where
  - μ is a known, twice differentiable function of unknown parameter $\theta_{m\times 1}$,
  - V is a known differentiable function of θ (or sometimes known only up to unknown scale factor σ^2 not depending on θ), V invertible.

- Let $U(\theta) = D^T V^{-1}(X - \mu)$, where $D_{ij} = \partial\mu_i/\partial\theta_j$

- $U(\theta)$ is QL score function.

- QL estimator (QLE) $\widehat{\theta}$ of θ is a solution of $U(\theta) = 0$.

- Matrix $i_\theta = D^T V^{-1} D = Cov(U(\theta)) = -E(\partial U/\partial\theta)$ plays similar role to Fisher information.

- Under regularity conditions, $i_\theta^{1/2}(\widehat{\theta} - \theta)$ is asymptotically $N(0, I)$, where$(i_\theta^{1/2})^T i_\theta^{1/2} = i_\theta$.

QL estimation: a few more details

- In special case when both of the following hold:

  1. $\mu = D\theta$, where D is known, i.e. μ is a linear function of θ and
  2. $V = Ks(\theta)$, where K is a known invertible matrix and s is a possibly unknown scalar that may depend on θ,

  then the QL estimator for θ is same as generalized regression estimator $(D^T K^{-1} D)^{-1}(D^T K^{-1} X)$.

- However, for some of the problems we are interested in, both 1 and 2 fail to hold.

- More generally, $\widehat{\theta}$ is not linear in X and can be obtained by Newton-Raphson with Fisher scoring:

  $$\widehat{\theta}_{j+1} = \widehat{\theta}_j + (\widehat{D}_j^T \widehat{V}_j^{-1} \widehat{D}_j)^{-1} \widehat{D}_j^T \widehat{V}_j^{-1} (X - \widehat{\mu}_j),$$

  where $\widehat{D}_j = D|_{\theta=\widehat{\theta}_j}$, $\widehat{V}_j = V|_{\theta=\widehat{\theta}_j}$.

- QL estimating equation is of linear type $H(\theta)^T(X - \mu(\theta)) = 0$, where H and μ do not depend on X. QLE is asymptotically optimal among estimators obtained as solutions of linear estimating equations (under regularity conditions).

QL score test

- Simple null hypothesis
  - Suppose want to test null hypothesis $H_0 : \theta = \theta_0$ vs. alternative $H_A : \theta \neq \theta_0$.
  - Let ρ be the dimension of θ.
  - By analogy with usual likelihood score test, consider

$$W = U(\theta_0)^T i_{\theta_0}^{-1} U(\theta_0)$$

    assuming i_{θ_0} invertible.

  - Compare to a χ^2_ρ distribution asymptotically

QL Score test: composite null hypothesis

- Set $\theta = (r, a)$ and consider testing null hypothesis $H_0 : r = r_0$ vs. alternative $H_A : r \neq r_0$.

- Let ρ be the dimension of r.

- Let
$$U(r,a) = \left(\begin{array}{c} U_r(r,a) \\ U_a(r,a) \end{array} \right) = \left(\begin{array}{c} D_r^T V^{-1}(X-\mu) \\ D_a^T V^{-1}(X-\mu) \end{array} \right),$$
where $D_r = \partial\mu/\partial r$ and $D_a = \partial\mu/\partial a$.

- By analogy with usual likelihood score statistic for composite null, consider
$$W = U_r^T(r_0, \hat{a}_0) i^{rr}(r_0, \hat{a}_0) U_r(r_0, \hat{a}_0),$$
where
  - $i^{rr}(\theta)$ is the (r,r)th entry of i_θ^{-1}
  - $\hat{a}_0$ is the QLE of the nuisance parameter a when $r = r_0$, i.e. $a = \hat{a}_0$ is solution of $U_a(r_0, a) = 0$.

- Compare to a χ^2_ρ distribution asymptotically

Some terminology and notation

- **IBD:** identical by descent; a set of alleles is IBD if the alleles are inherited copies of the same ancestral allele

- **HBD:** homozygous by descent; an individual is said to be HBD at a locus if that individual's two alleles are IBD. This occurs when individuals are inbred.

- **kinship coefficient:** ϕ_{ij} is the probability that a randomly chosen pair of alleles, one each from individuals i and j are IBD at a given locus, conditional on the genealogy connecting i and j

  - For example, the kinship coefficient for siblings or for parent-offspring is .25, for first cousins it is .0625

- **inbreeding coefficient:** h_i is the probability that individual i is HBD at a given locus, conditional on the genealogy connecting i's parents; $h_i = \phi_{mf}$, where m and f are the parents of i

Case-control association testing with relatives Approach 1: Correct the variance of the standard χ^2 statistic

- For simplicity, consider a biallelic locus. All results generalize to multiple alleles.

- Let $X_j = \frac{1}{2}$(the number of alleles (0, 1, or 2) of type 1 held by individual j), $j = 1, \ldots n$

- Let D_r be case indicator vector with $D_{rj} = 1$ if j is a case, 0 if j is a control.

- The standard Pearson's χ^2 statistic for the test of allelic association can be written
$$W_{\chi^2} = \frac{n[\sum_{j \in \text{cases}}(X_j - \bar{X})]^2}{\frac{1}{2}\bar{X}(1-\bar{X})n_{\text{case}}n_{\text{con}}},$$
where $n_{\text{case}} = 1^T D_r$, and $n_{\text{con}} = n - n_{\text{case}}$.

- This statistic has the form $W = S^T[\widehat{\text{Var}_o}(S)]^{-1}S$, where $S = V^T X$ is linear in X.

- Standard χ^2 statistic has the form $W = S^T[\widehat{\text{Var}_o}(S)]^{-1}S$, where $S = V^T X$.

- Thus, $\text{Var}_o(S) = V^T \text{Var}_o(X) V$

- With unrelated outbred individuals, $\text{Var}_o(X) = \frac{1}{2}a(1-a)I_{n \times n}$, where $a = E(X)$ is the allele frequency under the null hypothesis. Can approximate a by $\bar{X}$ under the null.

- With related and possibly inbred individuals, $\text{Var}_o(X) = \frac{1}{2}a(1-a)L$, where $L_{ij} = 1 + h_i$ if $i = j$ and $2\phi_{ij}$ if $i \neq j$, with h_i and ϕ_{ij} denoting inbreeding and kinship coeffs, respectively.

- Then the corrected χ^2 statistic is $W_{\chi^2_{corr}} = W_{\chi^2}\,\gamma$, where

$$\gamma = \frac{n_{case} n_{con}}{n(D_r^T L D_r - 2\frac{n_{case}}{n} 1^T L D_r + (\frac{n_{case}}{n})^2 1^T L 1)}.$$

- Compare to χ^2_1 distribution under the null hypothesis of no association between the locus and the trait.

Case-control association testing with relatives Approach 2: QL approach under simple model

- The corrected χ^2 test works reasonably well, but can we increase power with minimal additional effort?

- Let $\mu = E(X)$, and consider the simple model: $\mu_i = a+r$ if i is a case, a if i is a control (constrain $0 < a < 1$, $0 < a + r < 1$)

- Null hypothesis is $H_0 : r = 0$ vs. alternative $H_A : r \neq 0$

- We have $\text{Var}_o(X) = \frac{1}{2}a(1-a)L$

- $D_r = \partial\mu/\partial r$ has $D_{rj} = 1$ if j is a case and 0 if j is a control

- $D_a = \partial\mu/\partial a = \mathbf{1}_{n\times 1}$

- Then we obtain QL score statistic $W_{QLS} =$

$$\frac{[D_r^T L^{-1}(X - \hat{a}_0\mathbf{1})]^2}{\hat{a}_0(1-\hat{a}_0)[D_r^T L^{-1} D_r - (D_r^T L^{-1}\mathbf{1})^2(\mathbf{1}^T L^{-1}\mathbf{1})^{-1}]}$$

where $\hat{a}_0 = (\mathbf{1}^T L^{-1}\mathbf{1})^{-1}\mathbf{1}^T L^{-1} X$.

Case-control association testing with relatives Approach 2: QL approach under simple model (cont.)

- Both $W_{\chi^2_{corr}}$ and W_{QLS} are of the form $W = S^T[\widehat{\text{Var}_o}(S)]^{-1}S$, where $S = V^T X$ is linear in X. Call this class of statistics $\mathcal{W}$.

- When the assumed model holds, i.e. $\mu_i = a + r$ if i is a case, a if i is a control, then W_{QLS} is optimal in the sense that it maximizes the non-centrality parameter.

- Thus, W_{QLS} should be more powerful than $W_{\chi^2_{corr}}$ under the assumed model.

- E.g., to compare the allele frequency in, say, Swedes vs. Han Chinese, with related individuals in the samples, W_{QLS} is much more powerful.

- Difficulty: our simple model does not take into account the fact that in complex diseases, cases with affected relatives are more likely to have a predisposing genetic variant than are cases without affected relatives.

- As a result, W_{QLS} can do worse than $W_{\chi^2_{corr}}$ for complex trait mapping, because it downweights related cases compared to cases with no relatives in the sample.

Case-control association testing with relatives
Approach 3: QL approach with better model

- Initial model (frequency $a + r$ in cases and a in controls, i.e. $\mu = a + rD_r$) was too simple to work well.

- We obtain slightly less simple model as follows:
  - Consider a two-allele trait model specified by an allele frequency a and penetrance parameters p_0, $p_1 = p_0 + \epsilon_1$, and $p_2 = p_0 + \epsilon_2$, where $p_i =$ P$\{$affected$|$ have i copies of allele$\}$.
  - Let $\nu_j = E(X_j | D_r)$, calculated under the two-allele model.
  - Let $\nu = E(X_j | D_{rj} = 1)$.
  - Let
    $$\tilde{D}_r = \lim_{\epsilon_1, \epsilon_2 \to 0} \frac{\nu_j - a}{\nu - a}.$$
  - Our model is $\mu = a + r\tilde{D}_r$.
  - Intuitively: $r \approx \nu - a$, where ν is frequency in cases (unconditional on affection statuses of relatives) and a is population frequency.

Case-control association testing with relatives Approach 3: QL approach with better model (cont.)

- Model $\mu = a + r\tilde{D}_r$, where $\tilde{D}_r = \lim_{\epsilon_1,\epsilon_2 \to 0} \frac{\nu_j - a}{\nu - a}$.
- $\tilde{D}_r$ turns out to have a simple form: $\tilde{D}_{rj} = L\delta$
- Here, L is the matrix defined before with $L_{ij} = 1 + h_i$ if $i = j$ and $2\phi_{ij}$ if $i \neq j$, with h_i and ϕ_{ij} denoting inbreeding and kinship coeffs, respectively.
- δ is the vector with jth entry = 1 if j is affected, $-K_p/(1 - K_p)$ if j is unaffected, and 0 if j's status is unknown (e.g. population-based control).
- K_p is the population prevalence of the trait; in practice, an estimate of K_p can be used, if available, or an arbitrary number could be used.
- The M_{QLS} is the QL score statistic based on this model: $M_{QLS} =$
$$\frac{[\delta^T(X - \hat{a}_0\mathbf{1})]^2}{\hat{a}_0(1 - \hat{a}_0)[\delta^T L\delta - (\delta^T\mathbf{1})^2(\mathbf{1}^T L^{-1}\mathbf{1})^{-1}]}$$
where $\hat{a}_0 = (\mathbf{1}^T L^{-1}\mathbf{1})^{-1}\mathbf{1}^T L^{-1} X$.

Case-control association testing with relatives
Approach 3: QL approach with better model (cont.)

- We do not believe the simple model.
- Nonetheless, the resulting statistic captures the property that in complex diseases, cases with affected relatives are more likely to carry a genetic variant predisposing to the trait than are cases without affected relatives.
- Misspecification of the population prevalence K_p has no effect on validity of the test.
- We use simulation studies to assess
  - power of the method for multilocus trait models (i.e. 2-allele model does not hold).
  - power when K_p is drastically misspecified

Simulations to assess power and Type I error

- 60 extended outbred pedigrees, each with 16 individuals in 3 generations, ascertained for multiple affecteds.

- 20 pedigrees with 4 affecteds each, 20 with 5, and 20 with 6

- Each individual from pedigree is included in study if at least half of his first-degree relatives are affected

- Control sample includes 200 unrelated unaffecteds in addition to the unaffected relatives in the pedigrees

- Models

  - Model I: 2 unlinked causal SNPs with epistasis; 2 penetrance params.

  - Model II: 2 unlinked causal SNPs with epistasis; 4 penetrance parameters

  - Model III: 3 unlinked causal SNPs with epistasis; 2 penetrance parameters

Power to Detect Association

(at .05 level, based on 5,000 simulated replicates)

	Estimated Power		
Model	$W_{\chi^2_{corr}}$	W_{QLS}	M_{QLS}
I-a	0.57	0.42	0.85
I-b	0.78	0.60	0.98
I-c	0.77	0.66	0.90
II-a	0.65	0.55	0.75
III-a	0.57	0.51	0.77
III-b	0.85	0.74	0.94

- Type I error verified at nominal level for each test.
- In each simulation
  - Use of the M_{QLS} drastically increases power.
  - More than 1 causal locus is involved in true model.
  - Assumptions used to derive M_{QLS} are false.
- M_{QLS} captures property that cases with affected relatives are enriched for predisposing variants.
- M_{QLS} seems to have high power under complex trait models.

Robustness of M_{QLS} to misspecification of K_p

Power of M_{QLS}

Assumed K_p	Multiple of True K_p	Estimated Power (s.e.)
0.039	1/2	0.78 (.006)
0.078	1	0.77 (.006)
0.156	2	0.79 (.006)
0.312	4	0.68 (.007)
0.390	5	0.46 (.007)

- Model has 3 unlinked causal SNPs with epistasis.

- True K_p is .078

- When assumed K_p is within a factor of 2 of true K_p, there is no change in power in this case.

- Power of M_{QLS} appears to be fairly robust to misspecification of K_p.

Example: Testing for Association With Alcoholism, Using Genome Screen Data

- Genetic Analysis Workshop (GAW) 14 data from Collaborative Study for the Genetics of Alcoholism (COGA)

- 143 pedigrees each with at least 3 affecteds

- 506 cases and 202 controls

- 10,081 autosomal SNPs analyzed

- In the M_{QLS}, set $K_p = .05$, an estimate from the National Institute on Alcohol Abuse and Alcoholism (NIAAA).

- Binary phenotype (Affected with ALDX1 or “pure unaffected”).

Most significant SNPs in COGA Data Set

(p-value $<$ 5.0e-5 by at least 1 test)

					p-value		
chr	pos.	n_{ca}	n_{co}	p	$W_{\chi^2_{corr}}$	W_{QLS}	M_{QLS}
16	59.8	383	137	0.85	1.0e-4	1.2e-4	$\underline{\text{2.5e-7}}$
6	153.7	397	144	0.85	1.6e-2	3.3e-1	$\underline{\text{9.2e-6}}$
3	158.2	350	142	0.82	1.1e-2	7.2e-3	$\underline{\text{2.3e-5}}$
7	123.7	419	159	0.84	1.8e-3	$\underline{\text{2.7e-5}}$	3.7e-2
18	104.7	266	111	0.62	3.3e-1	8.3e-1	$\underline{\text{4.1e-5}}$
18	95.8	394	143	0.67	8.7e-3	6.3e-3	$\underline{\text{4.6e-5}}$
1	188.1	477	183	0.92	3.1e-2	3.7e-2	$\underline{\text{4.9e-5}}$

Markers are tsc1750530, tsc1288916, tsc0175005, tsc0043946, tsc0046696, tsc0054146, and tsc0275539, respectively.

- SNP on chromosome 16 is genome-wide significant (p-value .008) after Bonferroni correction for 10,081 SNPs and 3 tests per SNP: 2.5e-7 $\times 10,081 \times 3 =$ 7.6e-3

- The most significant results are generally obtained with M_{QLS}.

Summary

- The QL framework provides a way to extend standard testing and estimation methods to the situation when sampled individuals are related.
- First moments under null and alternative and 2nd moments under null are required for our testing methods.
- Methods are very fast, even in complex inbred pedigrees.
- We currently use QL methods for a number of genetic problems when samples contain related individuals:
  - allele frequency estimation
  - Hardy-Weinberg testing
  - case-control association testing
    - allelic tests (← discussed today)
    - genotypic tests (analogue of Armitage trend test)
- For case-control association testing, use of modified QLS greatly improves power

Extensions

- Analyze quantitative traits
- Incorporate covariates
- Extension to haplotype analysis when complete haplotype information is not available

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