3 Marker Regression Analysis

- marker regression in a backcross
- marker regression in a F_2 intercross
- marker regression by linear regression
- LOD scores
- LOD thresholds
- advantages and disadvantages

3.1 marker regression in a backcross

- consider backcross of P1 to F1=P1xP2
  - sample size $n \approx$ 100-500 individuals
  - collection of $m \approx$ 75-300 markers
    - not necessarily arranged as a linkage map
- goal: identify markers linked to a QTL
  - consider each marker individually
  - split individuals into 2 groups by marker genotype
- examine/test for difference between groups
  - plot data
  - hypothesis test of no QLT vs. QTL linked to marker

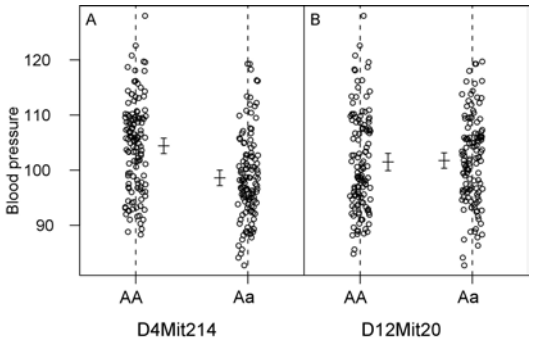
Sugiyama et al. (2001)

- salt-induced hypertension
  - 250 mice (B6 x A) x B6 backcross
  - C57BL/6J (A) and A/J (a) strains
- genotyped at 173 markers
  - 19 mouse chromosomes (autosomes)
  - selective genotyping of 92 mice on most (later)
- hypothesize one QTL in genome
  - consider markers one at a time
  - QTL exactly at marker or just linked?

phenotype split by genotype

- jittered dot plots
- confidence intervals: mean ± 2SE
- D4Mit214: B6 (AA genotype) has more hypertension
- D12Mit20: no apparent difference

considerable spread
environmental?
more QTL?
clinically substantial?



estimating genotype values & SDs

- G_{AA}, G_{Aa} phenotype means for AA, Aa
  - estimated by within-group sample averages
- common standard deviation (SD) of σ
  - weighted average of within-group SDs
- form two-sample *t* test statistic
  - null hypothesis: no QTL, $G_{AA} = G_{Aa}$
  - reject for large values of $|t|$
- cautions/interpretation
  - how to convert to LOD score?
  - how to account for multiple testing across *m* markers?

statistical formula page

$$\hat{G}_{AA} = \text{sum}_i \left(Y_i 1(X_{ij} = AA) \right) / n_{AA}$$

$$s^2_{AA} = \text{sum}_i \left((Y_i - \hat{G}_{AA})^2 1(X_{ij} = Aa) \right) / n_{AA}$$

$$SD = \hat{\sigma}_{pool} = \sqrt{\frac{(n_{AA} - 1)s^2_{AA} + (n_{Aa} - 1)s^2_{Aa}}{n_{AA} + n_{Aa} - 2}}$$

$$t = \frac{\hat{G}_{AA} - \hat{G}_{Aa}}{\hat{\sigma}_{pool} \sqrt{1/n_{AA} + 1/n_{Aa}}}$$

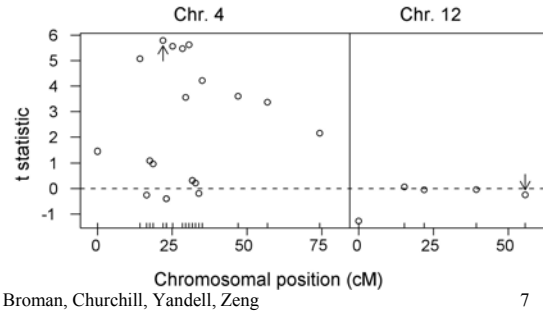
data analysis at two markers

- D4Mit214: n_{AA}=130, n_{Aa}=120
  - G_{AA}=104.4, G_{aa}=98.6, SD = 7.92, t = 5.78
- D12Mit20: n_{AA}=124, n_{Aa}=126
  - G_{AA}=101.5, G_{aa}=101.7, SD = 8.44, t = -0.25

high t statistics near D4Mit214--why?

why do some nearby markers have small t values?

assume only 1 QTL in region …

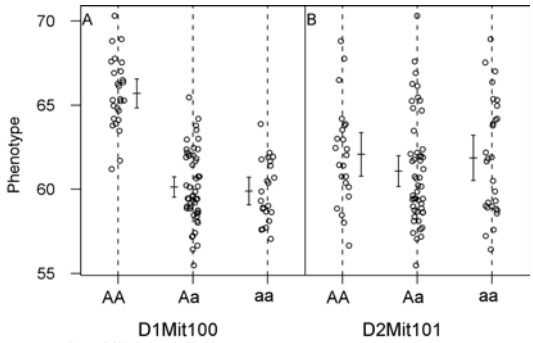


actual vs. apparent QTL effect

- QTL linked to marker
  - recombination r between marker and QTL
  - not all n_{AA} have AA genotype at QTL
- means at marker (G_{AA},G_{Aa}) vs. QTL (μ_{AA},μ_{Aa})
  - $G_{AA} = (1-r)\mu_{AA} + r\mu_{Aa} = \mu_{AA} - r(\mu_{AA} - \mu_{Aa}) = \mu_{AA} - r\Delta$
  - $G_{aa} = (1-r)\mu_{Aa} + r\mu_{AA} = \mu_{Aa} + r(\mu_{AA} - \mu_{Aa}) = \mu_{Aa} + r\Delta$
- apparent effect at marker (attenuated by r)
  - $G_{AA} - G_{Aa} = (\mu_{AA} - r\Delta) - (\mu_{Aa} + r\Delta) = (1-2r)\Delta$
  - $G_{AA} - G_{Aa} = \Delta$ if $r = 0$, $G_{AA} - G_{Aa} = 0$ if $r = 0.5$

3.2 marker regression in F_2 intercross

- 3 genotypes, split individuals into 3 groups
  - D1Mit100 shows higher mean for AA
  - D2Mit101 shows no apparent differences



hypothesis tests for F2

- all means identical at marker
  - null hypothesis: no QTL, $G_{AA} = G_{Aa} = G_{aa}$
  - alternative hypothesis: marker linked to QTL
  - ssume constant variance
- analysis of variance
  - use F statistics in place of t statistics
  - reject for large F in favor of linked QTL

3.3 marker regression by linear regression

- what?
  - recode marker genotype as numeric value(s)
  - set up regression to capture group means
  - test regression slopes = test of group means
- why?
  - always nice to have another perspective
  - can extend idea to multiple QTL
  - can help sort out genetic architecture details
- how?
  - see usual coding on next slide
  - other codings are preferred for multiple QTL (later)

a regression recoding

recode	genotypes			use for
	AA	Aa	aa	
X_{ij}	+1	–1		backcross
A_{ij}	+1	0	–1	F2: additive
D_{ij}	0	+1	0	F2: dominance

$$Y_i = \mu + \beta X_{ij} + e_i \quad \text{backcross}$$

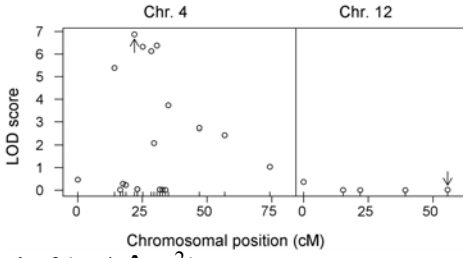
$$Y_i = \mu + \alpha A_{ij} + \delta D_{ij} + e_i \quad \text{F2 intercross}$$

3.4 LOD scores

- LOD scores and F statistics
  - both test null hypothesis of no QTL vs 1 QTL
- F statistics
  - evaluated using F tables (model and error d.f.)
  - based on quadratic forms, linear models
- LOD scores
  - evaluated using chi-square tables (model d.f.)
  - based on large-sample likelihood principle
  - can handle more complicated model forms
  - LOD is approximately proportional to F statistic

LOD score for 1 QTL, F2

- compare null to QTL model
- QTL at marker *j*
- *f* = normal density function



$$L_0(\hat{\mu}, s^2 \mid Y) = \text{prod}_i f(Y_i \mid \hat{\mu}, s^2)$$

$$L(\hat{G}, \hat{\sigma}^2_{\text{pool}} \mid Y, X) = \text{prod}_i f(Y_i \mid \hat{G}_{X_{ij}}, \hat{\sigma}^2_{\text{pool}})$$

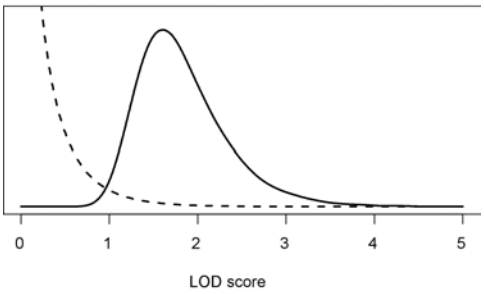
$$LOD = \log_{10}\left(\frac{L(\hat{G}, \hat{\sigma}^2_{\text{pool}} \mid Y, X)}{L_0(\hat{\mu}, s^2 \mid Y)} \right)$$

3.5 LOD thresholds

- how large does a LOD have to be?
  - evaluate LOD under null of no QTL
    - recall chi-square distribution
  - but adjust for many, many tests
- want genome-wide threshold
  - has to be bigger than for a single test
  - depends on genome size, cross, number of markers, missing data, phenotype distribution

genome-wide threshold

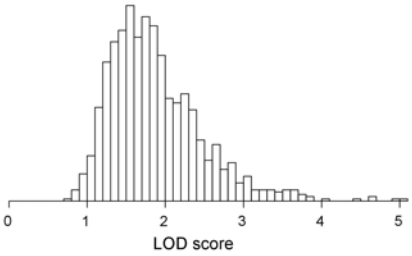
- dashed = 1 marker
- solid = genome-wide
- backcross (idealized)
- often use 95%-ile



- how to evaluate genome-wide threshold?
  - what is maximum LOD over entire genome under null?
  - theory, simulation, or permutation
  - permutation is recommended

genome-wide permutation

- permute phenotypes
  - 1000 times, say
  - random shuffle
  - same genotype data
- compute max LOD
- draw histogram
- find 95%-ile
  - is max LOD from data above this value?



3.6 advantages & disadvantages

- advantages
  - simple: test all markers with *t, F,* or LOD
  - can use standard statistical software
    - easy to incorporate covariates, interactions, design
  - no need for genetic map
- disadvantages
  - discard individuals with missing data at marker
  - cannot inspect positions between markers
  - recombination rate and QTL effect are confounded
  - considers only 1 QTL at a time
    - can use multiple regression on multiple markers (dense map)
    - but missing genotype problem is compounded