Lecture 7: Interaction Analysis

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Lecture Outline

Beyond main SNP effects

- Introduction to Concept of Statistical Interaction
- Standard Gene-Environment Interaction Testing
- Some More Sophisticated GxE Tests
- Even Fancier Methods – High order Interactions

"Interaction"

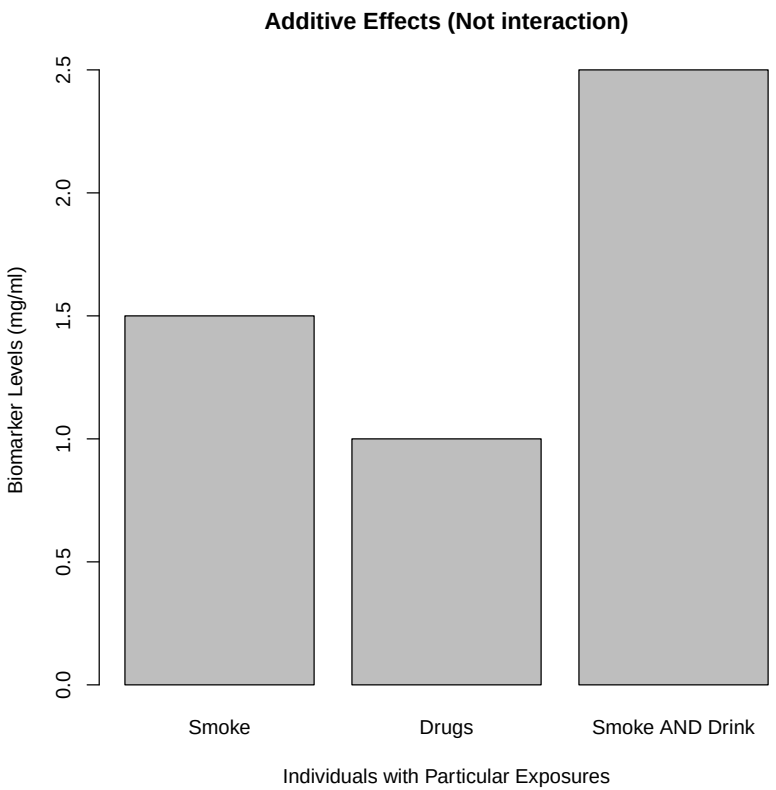
- "Interaction" means different things to different people:
  - Biological
  - Mechanistic
  - Additive
  - Synergism and Antogonisms
  - Statistical (Primarily "Multiplicative")
  - Others — a lot of general vagueness

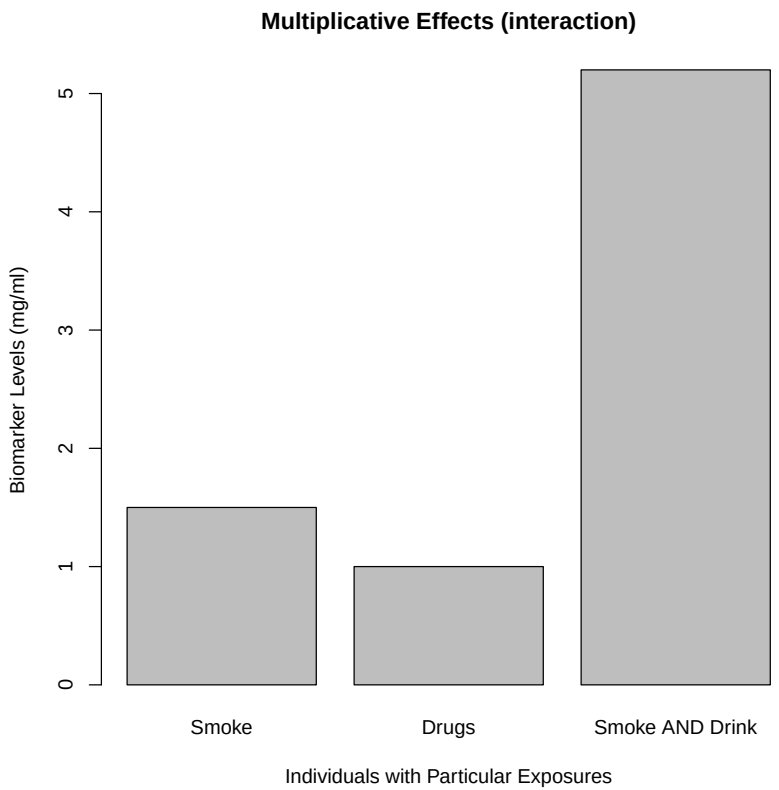
- Statistical (multiplicative) interactions: effect modification (one variable changes the effect of the other on outcome); deviation from additivity

Statistical Interaction

Multiplicative interactions: combined effect exceeds the additive effects of individual variables

Example





Gene-Environment Interactions ($G \times E$)

Complex diseases are caused by interplay of genes & environment.
Identification of $G \times E$ aids in disease prevention.

What is environment (E)?

- "Environment" is just as loaded as "Interaction"
- NIEHS (NIH): Basically, chemical exposures or objective measures (e.g. metabolites) – not primary smoking but second hand is OK
- Anything that is not genetics (G): BMI, race, education, gender, diet, etc.
- Treatment?
- Another SNP (Gene-gene interaction, epistasis): main difference between this and GxE is issue of scale (number of pair-wise tests)
- Operationally: often doesn't matter, but particular scenarios can change assumptions (e.g. independence between E and G)

Marginal Analysis of GxE Interactions

- Idea: Assess statistical interaction between a single exposure of interest and each SNP
- Testing Approaches:
  - Two-way interaction in regression model (standard)
  - Alternative designs
  - Testing joint G and GxE effects
  - Others.
- Multiple comparisons correction: FDR or Bonferroni

Standard 2-way interaction analysis:

Model (quantitative trait):

$$y_i = \beta_0 + \beta_g G_i + \beta_e E_i + \beta_{ix} G_i E_i + \varepsilon_i$$

Then to test for interaction effect:

$$H_0 : \beta_{ix} = 0.$$

If H_0 is true, then G and E can have effects (in the presence of each other), but their effects do not modify each other:

$$G_i = 0 \rightarrow E[y_i] = \beta_0 + \beta_e E_i$$

$$G_i = 1 \rightarrow E[y_i] = \beta_0 + \beta_g 1 + \beta_e E_i$$

If H_0 is false (reject null), then total effect of G and E differs depending on other variable:

$$G_i = 0 \rightarrow E[y_i] = \beta_0 + \beta_e E_i$$

$$G_i = 1 \rightarrow E[y_i] = \beta_0 + \beta_g + (\beta_e + \beta_i x) E_i$$

Standard 2-way interaction analysis:

Operationally

Regress y on G, E and product of G and E. Then can test $H_0 : \beta_i x = 0$ using any 1-df test.

Things to be careful...

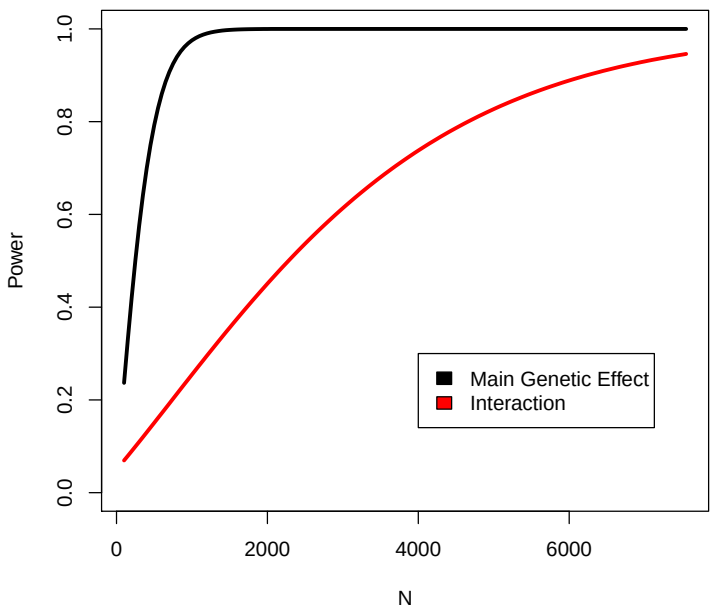
- Scale: particularly for continuous y
- Interaction testing is harder because the null model still has genetics in it. Under H_0

$$y_i = \beta_0 + \beta_g G_i + \beta_e E_i + \varepsilon_i$$

If this model is not correctly specified or captured, then there can be considerable inflation of type I error.

Power of GxE Tests is Low

Power is bad for GxE analysis: Needs many times as many subjects to test for interaction that is equally powerful.



Power as function of sample size: $\alpha = 0.05$ level, disease pop. risk of 0.01%, SNP with MAF of 0.25, environment with prevalence of 20%, both main SNP and interaction effect are 1.25 (OR).

Alternative Strategies?

- Exploit additional assumptions
- Case-only analysis
- Multi-SNP by E Testing (extension of gene/pathway analysis, but harder)
- Intelligently selecting which SNPs to test
- Many more fancy things constantly being developed

Joint Test of G + GxE

Main Idea

Instead of testing just $H_0 : \beta_{ix} = 0$, we test $H_0 : \beta_g = \beta_{ix} = 0$ via 2-df test. Primarily useful for gene discovery: significance does not explicitly inform interaction analysis.

References

- Gauderman and Siegmund (2001) *Hum Herid* **52**:34–46.
- Selinger-Leneman et al. (2003) *Gen Epi* **24**:200–7.
- Kraft et al. (2007) *Hum Herid* **63**:111-9.
- Huang et al. (2011) *Genome Med* **3**:42.

Joint G and GxE Testing: Toy data

Consider the data - a binary response Y, a binary environmental variable E and a binary gene G:

	$Y=1$	
	$G=1$	$G=0$
$E=1$	112	64
$E=0$	112	112

	$Y=0$	
	$G=1$	$G=0$
$E=1$	100	100
$E=0$	100	100

Joint G and GxE Testing

$$\text{logit}(Pr(Y = 1|G, E)) = \alpha_0 + \alpha_1 G + \alpha_2 E$$

P-value for $H_0 : \alpha_1 = 0$ is 0.070. Not significant!

$$\text{logit}(Pr(Y = 1|G, E)) = \beta_0 + \beta_1 G + \beta_2 E + \beta_3 GE$$

P-value for $H_0 : \beta_3 = 0$ is 0.051. Not significant!
But....
P-value for $H_0 : \beta_1 = \beta_3 = 0$ is 0.029. Significant!

Case-Only Analysis

- Suppose we have case-control study.
- *Case-Only Analysis* involves analyzing *only* the cases.
- Key Assumption: Genotype MUST be independent of environment
  - Almost necessarily true for randomized treatment E
  - Often true for traditional exposures (e.g. toxicants, pollution), but can be weird confounding issues
  - Need to be careful for some E like BMI, alcohol use, smoking, etc.
  - Generally: need to consider this situationally and with care
- Assuming the above, then case-only analysis proceeds by looking at the odds-ratio relating environment to genotype.

Case-Only Analysis

	$G=0$		$G=1$	
	$E=0$	$E=1$	$E=0$	$E=1$
$Y=0$	p_{01}	p_{02}	p_{03}	p_{04}
$Y=1$	p_{11}	p_{12}	p_{13}	p_{14}

For multiplicative interaction:

$$logitP(Y=1|G,E) = \beta_0 + \beta_g G + \beta_e E + \beta_{ix} G \times E$$

$$\begin{aligned} \exp(\beta_{ix}) &= \frac{OR_{11}}{OR_{10}OR_{01}} \\ &= \frac{p_{11}p_{14}}{p_{12}p_{13}} \Big/ \frac{p_{01}p_{04}}{p_{02}p_{03}} \\ &= \frac{\text{GxE odds ratio in cases}}{\text{GxE odds ratio in controls}} \end{aligned}$$

GxE odds ratio in controls $= 1$ under G-E independence!!!

Case-Only Analysis

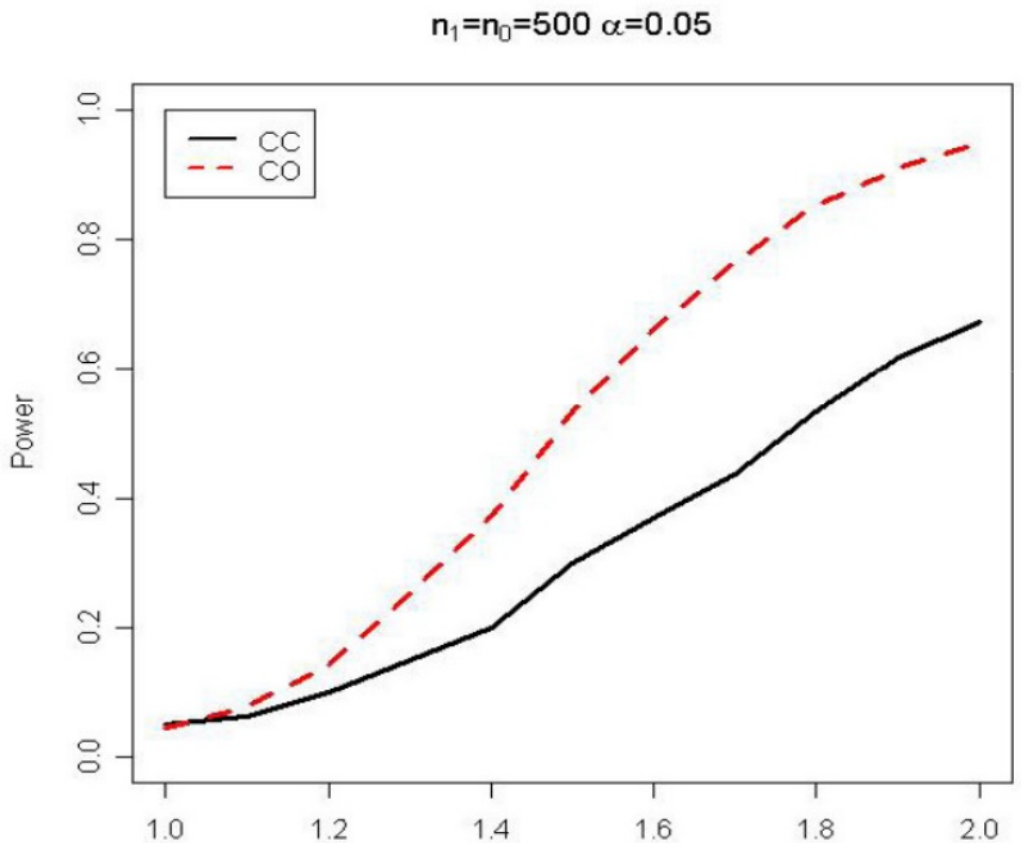
Instead, we model dependency between genotype and environment:

$$
\begin{aligned}
& \frac{P(G=1|E,Y=1)}{P(G=0|E,Y=1)} \\
= \quad & \frac{P(Y=1|G=1,E)/P(Y=0|G=1,E)}{P(Y=1|G=0,E)/P(Y=0|G=0,E)} \frac{P(Y=0,G=1,E)}{P(Y=0,G=0,E)} \\
= \quad & \frac{\exp(\beta_0+\beta_g+\beta_e E+\beta_{ix}E)}{\exp(\beta_0+\beta_e E)} \frac{P(G=1|Y=0,E)}{P(G=0|Y=0,E)} \\
= \quad & \exp(\beta_g+\beta_{ix}E)\frac{P(G=1)}{P(G=0)}
\end{aligned}
$$

with last line holding due to G-E independence (in controls).

Power of Case-Only Analysis

Case-only analysis can lead to improved power, but be careful of assumptions.



Multi-SNP by E Interactions

- Instead of looking at one-SNP at a time, can we again conduct analysis at multi-SNP level?
- Idea:
  1. Group SNPs in gene/pathway/region
  2. Test joint interaction between all SNPs and an environmental variable
- Many approaches for main SNP effects are intuitively applicable, but fail!
  - Interaction term $= G \times E$ is correlated with both E and G; this makes permutation methods more challenging
  - We have to correctly capture null model

Multi-SNP by E Interactions

Consider the following generalized linear model:

$$g(\mu_i) = \mathbf{X}_i^\mathsf{T}\boldsymbol{\alpha}_1 + \alpha_2 E_i + \mathbf{G}_i^\mathsf{T}\boldsymbol{\alpha}_3 + E_i\mathbf{G}_i^\mathsf{T}\boldsymbol{\beta}$$

- Outcome: Y_i, has distribution from exponential family and $\mu_i = E(Y_i|\widetilde{\mathbf{X}_i})$.
- q non-genetic covariates: $\mathbf{X}_i$.
- environmental factor: E_i.
- group of p variants: $\mathbf{G}_i = (G_{i1}, \cdots, G_{ip})^\mathsf{T}$.
- p $G \times E$ interaction terms: $\mathbf{S}_i = (E_i G_{i1}, \cdots, E_i G_{ip})^\mathsf{T}$.

We are interested in testing if there is any $G \times E$:

$$H_0 : \boldsymbol{\beta} = \mathbf{0}.$$

Averaging/Collapsing Tests for Interactions

Idea: let G^* be a (weighted) average of genotypes within a gene/region/pathway.

To test for main effects:

$$H_{1m} : g(\mu_i) = \alpha_1^* + \alpha_2^* E_i + \alpha_3^* G_i^*$$
$$H_{0m} : \alpha_3^* = 0$$

Can we use it to test for interactions?

$$H_{1x} : g(\mu_i) = \alpha_1^* + \alpha_2^* E_i + \alpha_3^* G_i^* + \beta^* E_i G_i^*$$
$$H_{0x} : \beta^* = 0$$

Bias analysis for Collapsing $G \times E$ tests

Intuition

Null model has to be correctly specified for valid inference.
Collapsing $G \times E$ tests may not give valid inference as main effects of the SNVs may not be sufficiently accounted for.

Continuous Outcome: No, even if $\boldsymbol{G} \perp E$.

- $\boldsymbol{G}$ and E are independent:
  Model for mean of Y is valid;
  Model for variance of Y is $\boxed{not}$ valid.
- $\boldsymbol{G}$ and E not independent:
  Model for mean of Y is not valid;
  Model for variance of Y is $\boxed{not}$ valid.

Bias analysis for Collapsing $G \times E$ tests

Binary Outcome: Yes if disease is rare and $\boldsymbol{G} \perp E$.

- $\boldsymbol{G}$ and E are independent:
  Model for mean of Y is valid;
  Model for variance of Y is valid approximately.
- $\boldsymbol{G}$ and E not independent:
  Model for mean of Y is $\boxed{not}$ valid;
  Model for variance of Y is valid approximately.

GESAT: Model

To test if there is any $G \times E$ ($H_0 : \boldsymbol{\beta} = \mathbf{0}$):

$$H_0 : \text{logit}\left[P(Y_i = 1 | E_i, \mathbf{X}_i, \mathbf{G}_i)\right] = \mathbf{X}_i^\intercal \boldsymbol{\alpha}_1 + \alpha_2 E_i + \mathbf{G}_i^\intercal \boldsymbol{\alpha}_3$$

$$H_A : \text{logit}\left[P(Y_i = 1 | E_i, \mathbf{X}_i, \mathbf{G}_i)\right] = \mathbf{X}_i^\intercal \boldsymbol{\alpha}_1 + \mathbf{G}_i^\intercal \left(\boldsymbol{\alpha}_3 + E_i \boldsymbol{\beta}\right) + \alpha_2 E_i$$

In principle, we can do the same thing as with SKAT, but ...

Difficulties

Need to fit null model:

- Need to estimate main effect of variants
- Lots of variants
- LD and rarity make fitting difficult

Modifications are necessary.

GESAT: Extension of SKAT (global test) for GxE

GESAT: Test Statistic

- Assume $(\beta_1, \cdots, \beta_p)^\intercal$ are random and independent with mean zero and common variance τ.
- Testing H_0 reduces to testing $H_0 : \tau = 0$.
- Following Lin (1997), the score test statistic is

$$T = (\boldsymbol{Y} - \widehat{\boldsymbol{\mu}})^\intercal \boldsymbol{S}\boldsymbol{S}^\intercal (\boldsymbol{Y} - \widehat{\boldsymbol{\mu}}) = [\boldsymbol{Y} - \boldsymbol{\mu}(\widehat{\boldsymbol{\alpha}})]^\intercal \boldsymbol{S}\boldsymbol{S}^\intercal [\boldsymbol{Y} - \boldsymbol{\mu}(\widehat{\boldsymbol{\alpha}})].$$

- $\widehat{\boldsymbol{\mu}} = \boldsymbol{\mu}(\widehat{\boldsymbol{\alpha}})$ is estimated under the null model,

$$g(\mu_i | \boldsymbol{X}_i, E_i, \boldsymbol{G}_i) = \boldsymbol{X}_i^\intercal \boldsymbol{\alpha}_1 + \alpha_2 E_i + \boldsymbol{G}_i^\intercal \boldsymbol{\alpha}_3 = \widetilde{\boldsymbol{X}}_i^\intercal \boldsymbol{\alpha}.$$

- Use ridge regression to estimate $\boldsymbol{\alpha}$, impose a penalty only on $\boldsymbol{\alpha}_3$.
- Under H_0, $T \sim \sum_{v=1}^{p} d_v \chi_1^2$ approximately.
- Invert characteristic function to get p-value (Davies, 1980).

Which SNPs to Test?

- Genome-wide analysis: screen association between all SNPs and outcome
- Candidate genes or pathways (functional groups)
- SNPs with significant main effects
- More sophisticated algorithms: data adaptive procedures that use two-stage screening

Which set to use can influence multiple testing adjustments. Not always clear how many tests to adjust for if considering main effects too.

Additional Work

- Already a lot of work assuming independence
- Can model E better: multi-E analysis
  - Not always clear which E to use: smoking can be yes/no, never/ever, pack-years, cotinine etc.
  - Mixtures of toxicants: many toxicants or exposures happen in conjunction
- Monotonicity constraints
- Omnibus strategies
- Weighted hypothesis testing
- Innovative screening strategies

Higher order interactions

Given that 2-order interactions are already hard to fine, why are we interested in higher order interactions?

- power,
- computational, and
- interpretation,

we should only be interested in higher order interactions when we focus attention on a few targeted regions (e.g. genes), selected because of

- studies (carried out on other data sets),
- biology,
- ...

It is not a surprise that...

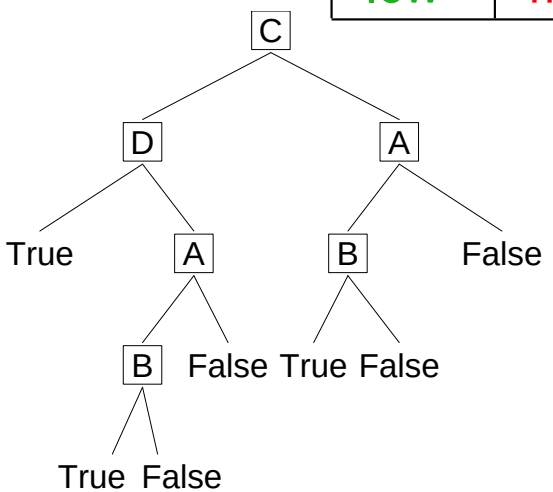
- The power is small.
- As such we may want to see these methods as "hypothesis generating" - i.e. we may identify a limited number of interactions that we can follow up on in new studies.

Models

- SNPs as 3 level categorical variables:

low	low	low
high	low	high
low	high	high

- Decision tree models.



- Boolean rules like:

  You are at increased risk if you have at least one mutant for SNP1 or two mutants for SNP2.

- Classical interaction model

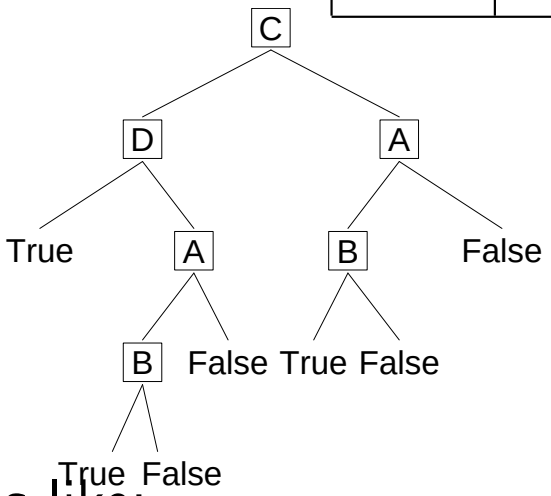
$$g[E(Y|\mathbf{G})] = \beta_0 + \beta_1 G_1 + \beta_2 G_2 + \beta_3 G_3 + \beta_4 G_1 G_2 + \beta_5 G_1 G_3 + \beta_6 G_2 G_3 + \beta_7 G_1 G_2 G_3,$$

Models

MDR SNPs as 3 level categorical variables:

low	low	low
high	low	high
low	high	high

CART Decision tree models.



Logic **Regression** Boolean rules like:

You are at increased risk if you have at least one mutant for SNP1 or two mutants for SNP2.

- Classical interaction model

$$g[E(Y|\mathbf{G})] = \beta_0 + \beta_1 G_1 + \beta_2 G_2 + \beta_3 G_3 + \beta_4 G_1 G_2 + \beta_5 G_1 G_3 + \beta_6 G_2 G_3 + \beta_7 G_1 G_2 G_3$$

Multifactor Dimensionality Reduction

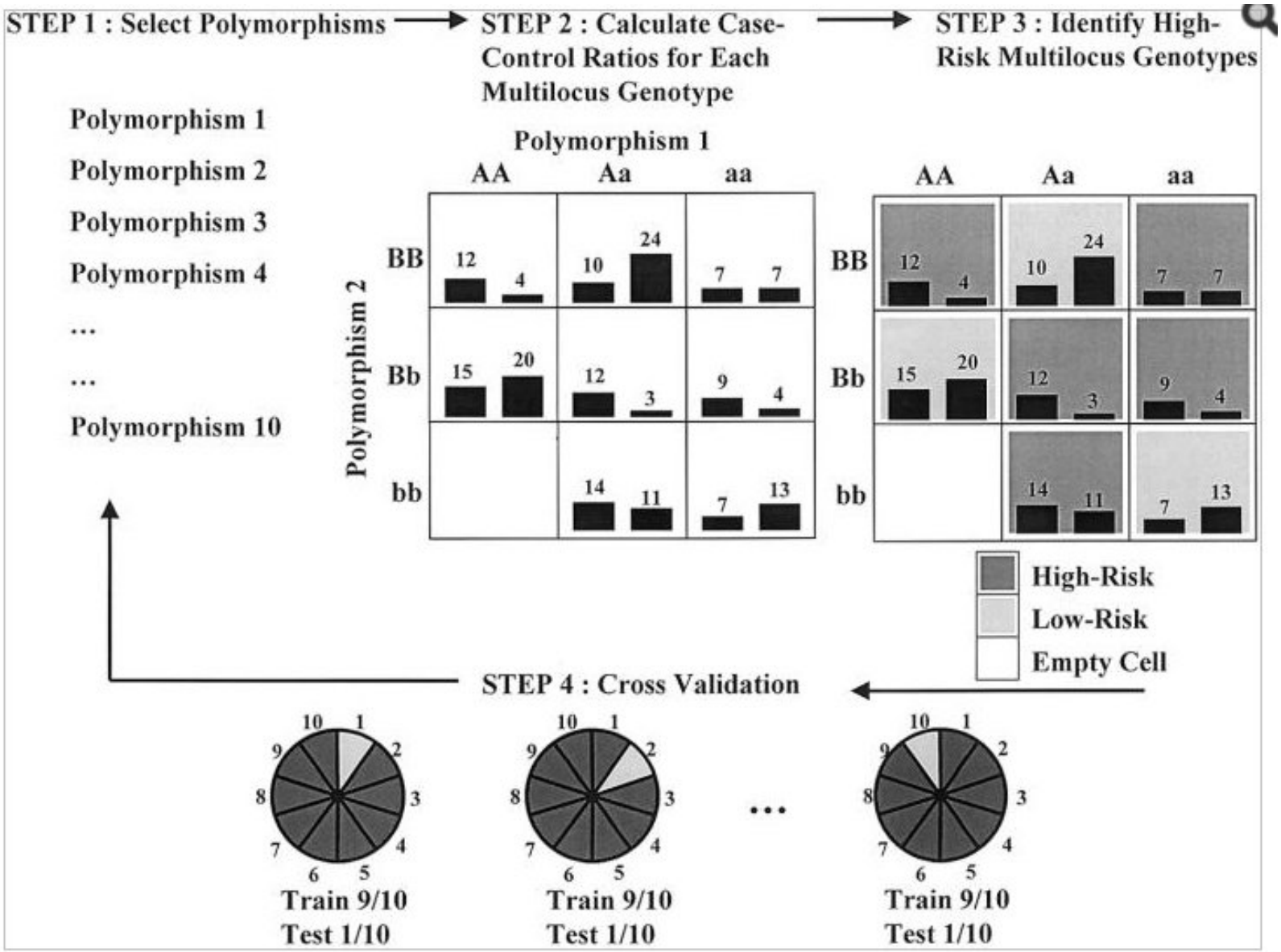
[Ritchie et al. (2001) *Am J Hum Gen* **69**:138–47]
[Hahn et al. (2003) *Bioinformatics* **19**:376–82]
modification of
[Nelson et al. (2001) *Genome Res* **11**:458–70]

- Complex interactions are hard to detect because of sparse data via standard parametric models
- Inaccurate parameter estimates and large standard errors with relatively small sample sizes.
- Reduce the dimensionality and identify SNP combinations that lead to high risk of disease.

Hunting for:

low	low	low
high	low	high
low	high	high

MDR


STEP 1 : Select Polymorphisms
STEP 2 : Calculate Case-Control Ratios for Each Multilocus Genotype
STEP 3 : Identify High-Risk Multilocus Genotypes
Polymorphism 1
Polymorphism 2
Polymorphism 3
Polymorphism 4
...
...
Polymorphism 10
Polymorphism 1
AA
Aa
aa
Polymorphism 2
BB
Bb
bb
12
4
10
24
7
7
15
20
12
3
9
4
14
11
7
13
High-Risk
Low-Risk
Empty Cell
STEP 4 : Cross Validation
Train 9/10
Test 1/10
Train 9/10
Test 1/10
Train 9/10
Test 1/10

MDR

For a particular model with M SNPs (or environmental factors):

- 10-fold Cross-validation
  1. Consider each "cell" (if factors are SNPs, there are 3^M).
  2. On 9/10th of the data decide whether a cell is "high" or "low" risk (for a case-control study the typical cut-off in each cell would be the case/control ratio in the study).
  3. Evaluate the prediction on the remaining 1/10th of the data.
  4. Check how many of the MDR models are the same. Not entirely clear how this is done - if each cell should be consistent, this would work against models that have (m)any cells that are close to 50/50.
- Repeat this a number of times - to achieve stability of the cross-validation. If you have enough computing power, always a good idea.
- Select the model with the lowest prediction error, provided the consistency is better than by chance.

Sporadic breast cancer

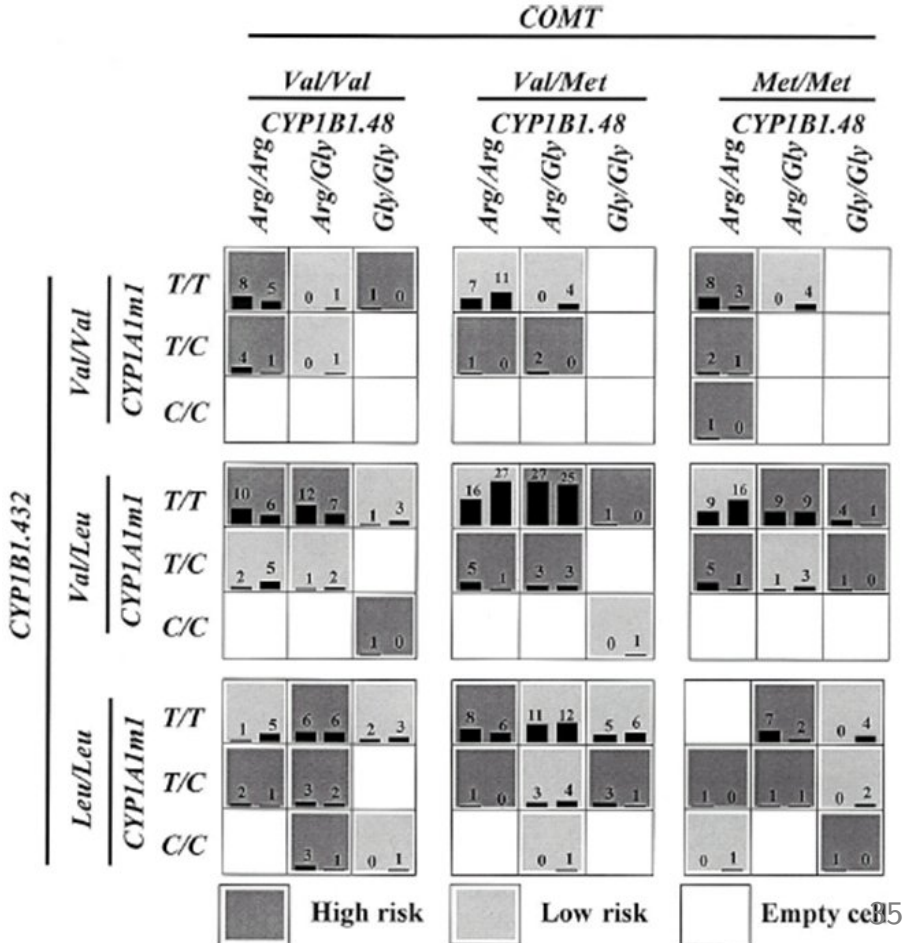
200 women with sporadic primary invasive breast cancer with age-matched hospital based controls, 10 estrogen metabolism SNPs

Summary of Results for Breast Cancer

No. of Loci	Cross-Validation Consistency	Prediction Error
2	7.00	51.06
3	4.17	51.35
4	***9.80****	***46.73***
5	4.71	50.26
6	5.00	48.61
7	8.60	47.15
8	8.20	52.55
9	7.10	53.40

NOTE.—The multilocus model with maximum cross-validation consistency and minimum prediction error is indicated in boldface italic type.

* $P < .001$.



Issues

- While making things binary helps, computation can explode if the number of SNPs in the study is substantial.
- The selected models do not adhere to the usual parsimony that we like in statistics: if a model with, say, 4 factors is ϵ better than a model with 3 factors, MDR will pick 4 factors. Usually we would prefer 3. Conceivably this could be changed fairly easy. The MDR implementation of cross-validation makes this worse, however (next slide).
- The models are very hard to interpret.
- To me, it would make more sense to identify a smaller number of cells with "extreme high" or "extreme low" risk.

Bias in their implementation of Cross Validation

- Consider the number of models with M SNPs out of a total T.

	0	1	2	3	4	5	6	7	8	⋯
10	1	10	45	120	210	252	210	120	45	⋯
25	1	30	435	4060	27405	142506	593775	2035800	5852925	⋯

- Imagine what happens if there is no signal, and every model is equally likely, which size would we most likely end up with...
- The consistency reduces this problem a little, but not by much. Think about the situation where there is one SNP with a strong effect...

Take home message well beyond MDR

When using cross-validation for model selection, if the number of models of size M is different for different M, you can use cross-validation to find the best model of each size, but you cannot use it to find the best size. You need another test dataset for that!

Even more generally: beware of fancy methods, particularly anything for interaction analysis!!

A sobering note

There likely have been more papers written about methods to identify *GxE* and *GxG* interactions, than the number of interactions that have successfully been identified.