Whole Exome Sequencing of Affected Sister Pairs with Early Onset Breast Cancer

BIRS Workshop:
New Statistical Methods for Family-based Sequencing Studies

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Background – Breast Cancer (BRCA)

BRCA is a heterogeneous disease, mutations are rare.

Germline mutation identification for rare diseases benefits from starting with a homogeneous population of cases sharing the phenotype

In young women – BRCA more likely due to germline alterations affecting tumor susceptibility genes

Hypothesis:

Focusing on a genetically homogeneous cohort (young sister pairs with non-*BRCA* BC)

- enrich for the presence of rare intermediate-to-high risk variants
- enable discovery of novel variants

Whole Exome Sequencing Pilot Study - Design

Motivating study data:

Whole exome sequencing (WES) at 50X coverage

- affected sister pairs, at least one early-onset (≤45)
- recruited from high-risk families in Ontario Familial Breast Cancer Registry

Total of 21 families

- Family history of breast cancer
- screened negative for known mutations in high-penetrance genes BRCA1, BRCA2 and CHEK2*1100delC.

Objective: Identify novel rare variants for familial breast cancer => further validation studies.

Whole Exome Sequencing – Filtering Results

Preliminary Studies

Whole-exome sequencing of blood samples at 50X Coverage using Agilent SureSelect on HiSeq 2500

↓

Alignment to Human Reference Genome Hg19

↓

Variant Calling using HaplotypeCaller of the Genome Analysis Toolkit (GATK)

↓

Variant Quality Score Recalibration (GATK)

↓

Variant Annotation (wAnnovar)

↓

261,340 Variants Called

↓

256,337 Variants Passing Quality Control Filters
[Phred-scaled Q Score >= 30]
[Read Depth >= 10]

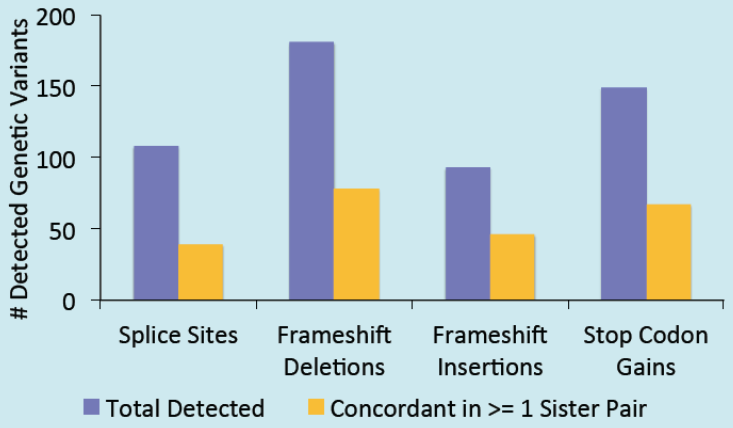
↓

Functional Variants:
1,440 Variants with Expected Functional Consequences (Protein Truncations)
[Stop codon gain mutations, Frameshift insertions or deletions, Splice site mutations]

↓

Rare Variants:
535 Varients Remaining After Removal of Those with Minor Allele Frequencies (MAF) > 0.1% in the Human Population (1000 Genomes Database; ESP6500 Database)

Rare Functional Variants



Concordant Variants of Interest

Gene Functional Category	# Concordant Rare Variants
DNA Repair	7
Cell Proliferation	3
Tumor Suppression	3
Cell Cycle Regulation	4
Other Genes Previously Associated with Cancer	8

Case-control mutation screening in a larger cohort

Statistical Methods to Identify Rare Variants ??

Consider methods with complementary strengths
Allelic and locus heterogeneity are important considerations.

Novel mutations may be family-specific or occurring in few families, with the possibility of extreme heterogeneity.

Methods for RV association analysis in affected sister/relative pairs

- exploit IBD sharing information
- susceptibility variants more often within regions shared IBD by ASPs compared to regions not shared IBD
  - powerful when multiple sibpairs carry shared RVs,
    e.g. multiple different mutations within the same gene
- less effective when families segregate different susceptibility genes

Propose extensions to consider multiple regions
(eg within a shared pathway, such as DNA repair)
more effective when there is locus heterogeneity

Affected Sister Pair Data - Notation

Assume i = 1, 2, … N families each with 2 affected sisters (l=1,2)

A genomic region with j = *1,2, …,R* RV loci (RV = MAF < 0.1%)
filtered on MAF reference information (e.g., 1000 Genomes)
and functional annotation (e.g. ANNOVAR).

Let X_{ilj} be the RV allele count (0,1,2) at locus j in family i, sister l

Q_{ij} is the sum of the RV allele counts (0 - 4) in sibpair i at locus j

Q_i is the sum of Q_{ij} over j = *1,2, …, R* RV loci

Z_i is the # of alleles shared IBD (0,1,2) in the genomic region
(assuming no recombination)

Statistical Inference: Single Region Test

Epstein et al (2015) model the dependence of $Q_i.$ on Z_i

$$E[Q_i.|Z_i] = 4\mu_0 + 2(\mu_1 - \mu_0)Z_i$$

$$Var[Q_i.|Z_i] = 4\sigma_0^2 + 2Z_i(2\sigma_1^2 - \sigma_0^2)$$

Means (μ_0, μ_1) & variances (σ_0^2, σ_1^2) of rare allele counts depend on the IBD sharing

μ_0 - mean of RV sum on parental haplotype NOT IBD

μ_1 – mean of RV sum on parental haplotype inherited IBD

Efficient score test H_0: ($\mu_1 = \mu_0$)

in inverse variance weighted regression

Burden type

Robust to population stratification

Does not require a linkage signal to detect association

More powerful than case-control design

Single Region Regression Test

Simplification:

Assume $\sigma_0^2 = \sigma_1^2$ (true under the null)

$$Q_i.|Z_i = \alpha + \beta Z_i$$

The test of $\beta = 0$ vs. H_a: $\beta \neq 0$ $\quad T_{reg} = \dfrac{\hat{\beta}}{SE(\hat{\beta})} \sim t\,(N-2)$

Weighted version:

$$Q_i.|Z_i = \alpha + \beta Z_i + (4 + 2Z_i)\varepsilon_i$$

where $\varepsilon_i \sim N(0, \sigma^2)$

Allows allele counts in a sibpair to depend on IBD

Multi-Region Regression Test

$$Q_{qi\cdot} = \sum_{j=1,\ldots,R_q} Q_{qij} \qquad q = 1,2,\ldots,p$$

Multi-variate regression: $Q_{qi\cdot}|Z_{qi} = \alpha + \beta Z_{qi}$

A sibpair has a RV allele count for each of p regions:

Count depends on IBD in the region

Assumption of a shared $\beta = \beta_q$

test statistic for $\beta = 0$ vs. $\beta \neq 0$

$$T_{reg} = \frac{\hat{\beta}}{SE(\hat{\beta})} \sim N(0,1)$$

Allelic Parity Test (ignores IBD)

Define $$D_{i\cdot} = \sum_{j=1,..,R} D_{ij}$$

$$D_{ij} = \begin{cases} 0, & Q_{ij} = 0 \\ -1, & Q_{ij} = 1 \\ 2, & Q_{ij} = 2, \end{cases} \quad , \quad \text{for } j = 1,..,R, \quad i = 1,\dots,N$$

$$T_{a.p.} = \frac{\sum_{i=1,..,N} D_{i\cdot}}{SD(D_{i\cdot})\sqrt{N}} \sim Student\ t(df = N-1)$$

Discrete small sample distribution => Synthetic distribution

Multi-Region extension for p regions: $$D_{\cdot i\cdot} = \sum_{q=1,..,p} \sum_{j=1,..,R_q} D_{qij}$$

Simulation Study Design

Heterogeneity Models:

A family potentially segregates one rare mutation that increases susceptibility / reduces age at onset.

The mutation differs between families, but is in the same gene/region * *allelic heterogeneity*

The mutation is in a different gene/region in different families * *locus heterogeneity*

A mutation in any one of several independent regions (eg. that form a functional pathway) can increase risk of disease

Simulation Study Data Generation

Genetic Data:

'sim1000G' R package

- 594 European haplotypes from 1000 Genomes
- 10-20 kb region (one gene) - 100 RV loci with MAF < 0.1%
- 15% are potential "causal" mutations in carrier families
- one RV "carrier" haplotype assigned to each family
- Mendelian segregation to daughters

Age at Onset Data:

'FamEvent' R package

- proportional hazards model under dominant inheritance

$$h(t, X_{ilj}) = h_0(t - t_0)\, exp\, (\beta_j X_{ilj})$$

$$t_0 = 20$$

$$\beta_j = \log(8)$$

Ascertainment:

- Early age at onset criteria for sisters
- One sister age <40, another <50

10,000 datasets: 20, 100, 500 ASPs

Simulation Study Families – Single Region Test

Region A "carriers":

- "causal" mutation at a locus in Region A
- carrier penetrance function
- enriched for early age at onset

Region B "carriers":

- non-carrier in Region A
- "causal" mutation at a locus in Region B
- enriched for early age at onset

Region A&B "non-carriers":

- non-carrier in Regions A & B
- Environmental penetrance
- Sporadic disease with early age at onset

Simulation Study Families – Two Region Test

Three Regions (A, B, C) with "causal" RVs:
Regions A & B included in the test

In Family i, a sister can be one of:

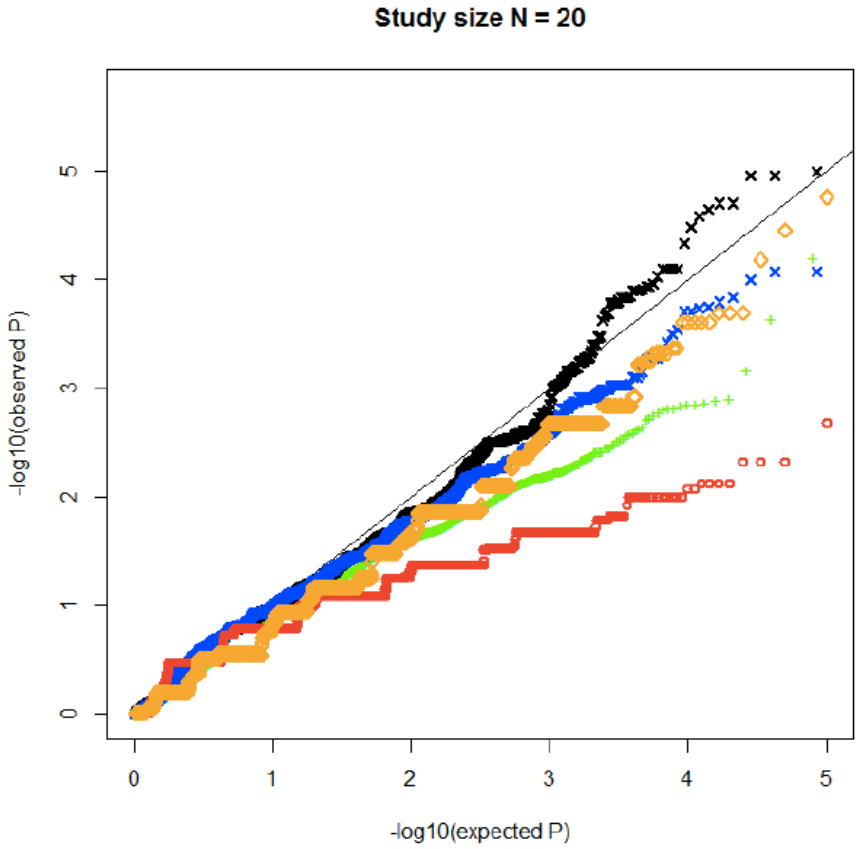
- carrier of a causal mutation in Region A
- carrier of a causal mutation in Region B
- carrier of a causal mutation in Region C
- non-carrier of any causal mutation in any region

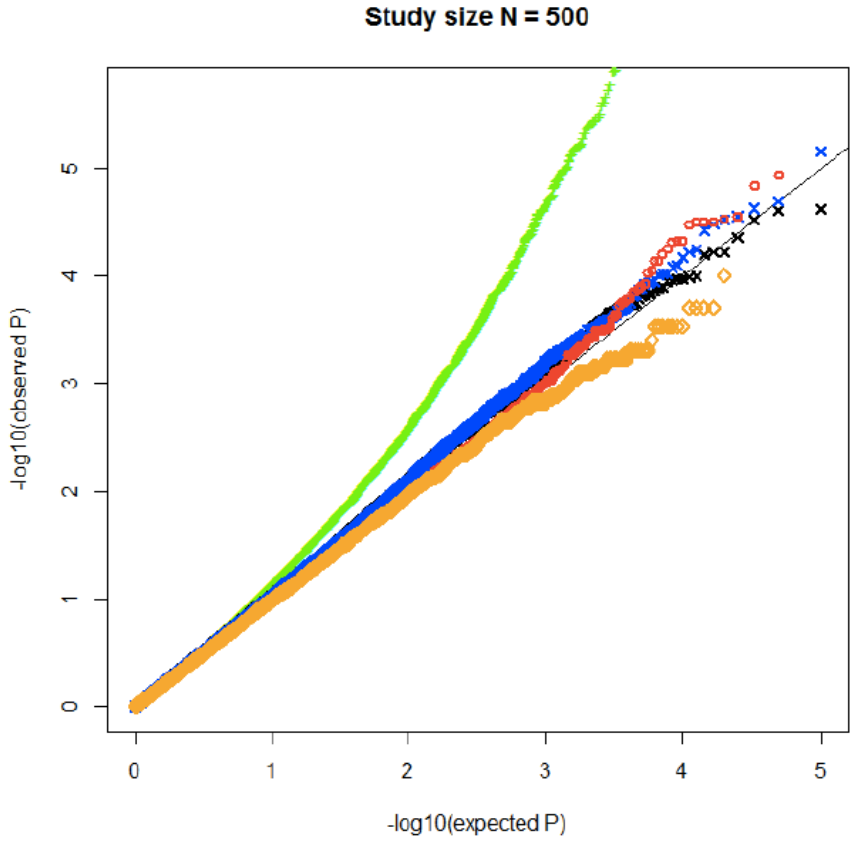
Simulation Study Results - Single Region Test

Type I error

100,000 Datasets

green – Epstein, blue – regression test,
black – weighted regression test,
red – allelic parity test (asymptotic),
orange – allelic parity test (synthetic distribution)





Simulation Study Results - Two Region Test

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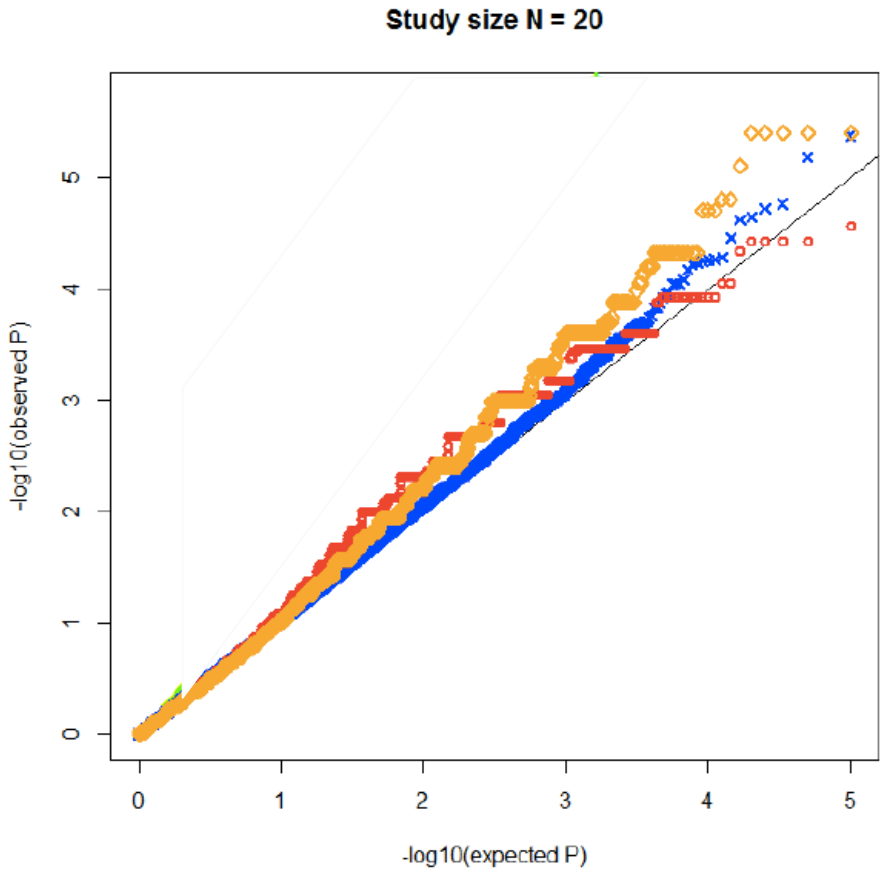

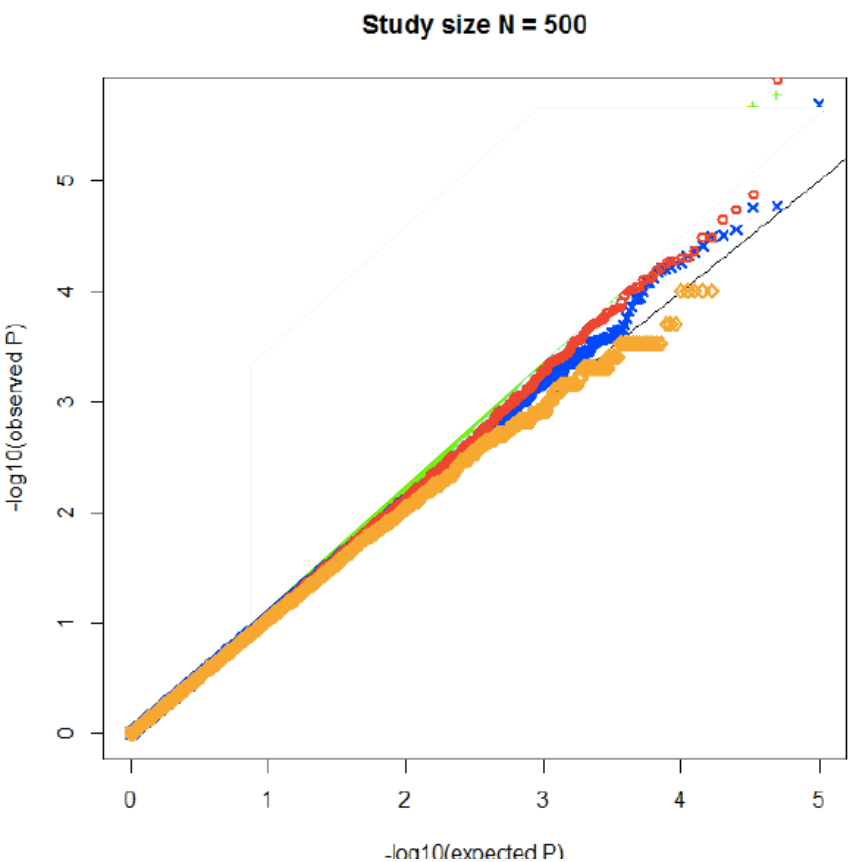

Simulation Study Results - Power

N	20	100	500
	$\alpha = 0.05$		
regression	0.05	0.124	0.346
regresssion (w)	0.048	0.143	0.393
a.p.	0.033	0.229	0.633
a.p.synthetic	0.045	0.285	0.685
Epstein	0.087	0.216	0.419

10,000 Datasets

Single Region

N	20	100	500
	$\alpha = 0.05$		
regression	0.053	0.07	0.152
a.p.	0.06	0.166	0.493
a.p.synthetic	0.082	0.193	0.521

Multi Region

Summary & Discussion

Preliminary results – should be cautious

- Asymptotic assumptions in small samples?
- Robustness to non-normality in simplified linear regression
- Why does Epstein's model lose T1E control in small samples?

Impact of simulation design

- How plausible is the extreme heterogeneity hypothesis?
- Role of background risk due to common variants?

Applications

- How to specify RV sets in a region?
- How to choose regions for multi-region analysis?

Extension to WGS

- How to choose families? How many to re-sequence ?
- How to use pedigree data?

Design for population-based validation/replication?

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Canadian Breast Cancer Foundation

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Ontario Institute for Cancer Research

NSERC CRSNG

CIHR STAGE
Strategic Training for Advanced Genetic Epidemiology

McLAUGHLIN CENTRE
Advancing Genomic Medicine through Research and Education

Thanks

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