Post-GWAS characterization of breast cancer-associated SNPs

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Acknowledgments

- Michael Gould
- Michael Newton
- Christina Kendziorski
- Norman Drinkwater
- Shuyun Ye
- Kevin Eng

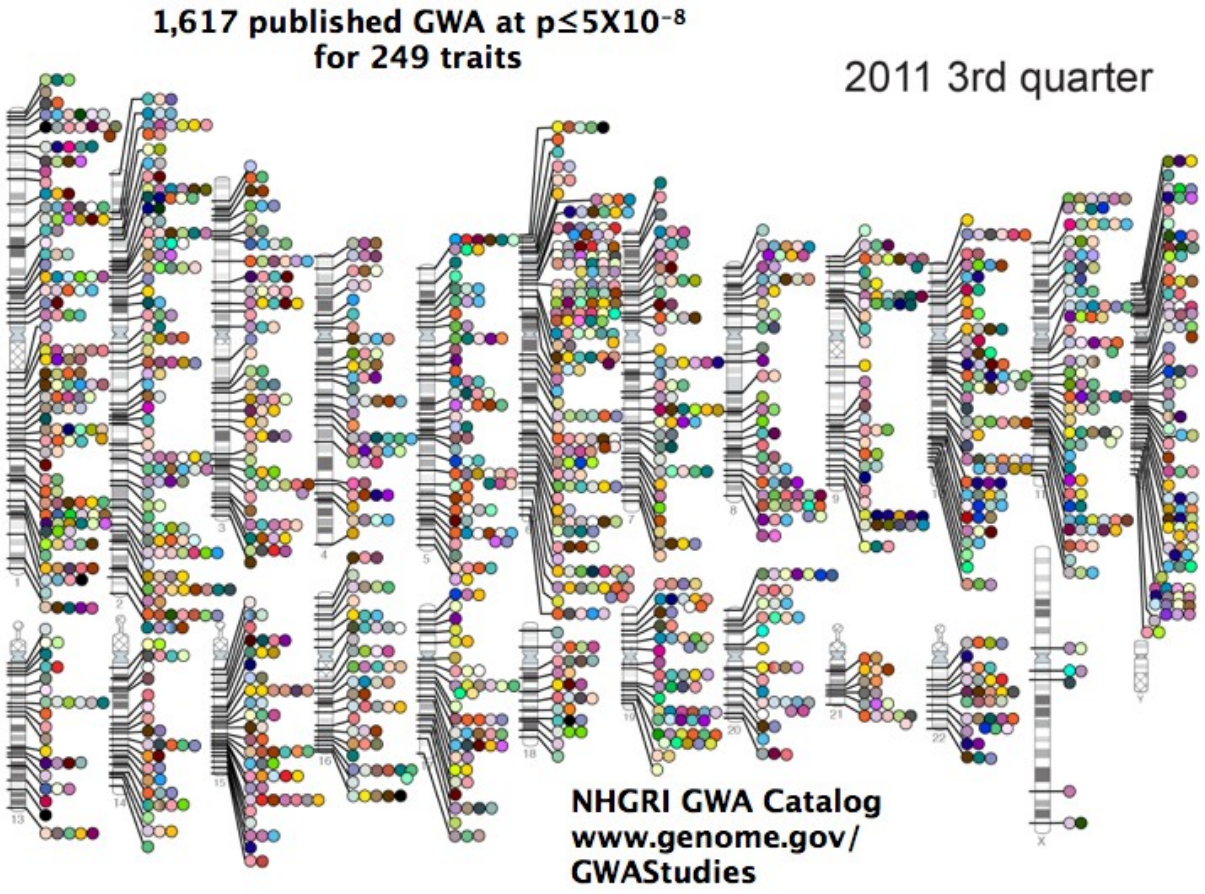
- NIEHS
  - METC Training Grant
  - Gould Lab Grant

Outline

- Motivation
- Methods
- Results
- Conclusions
- Next steps

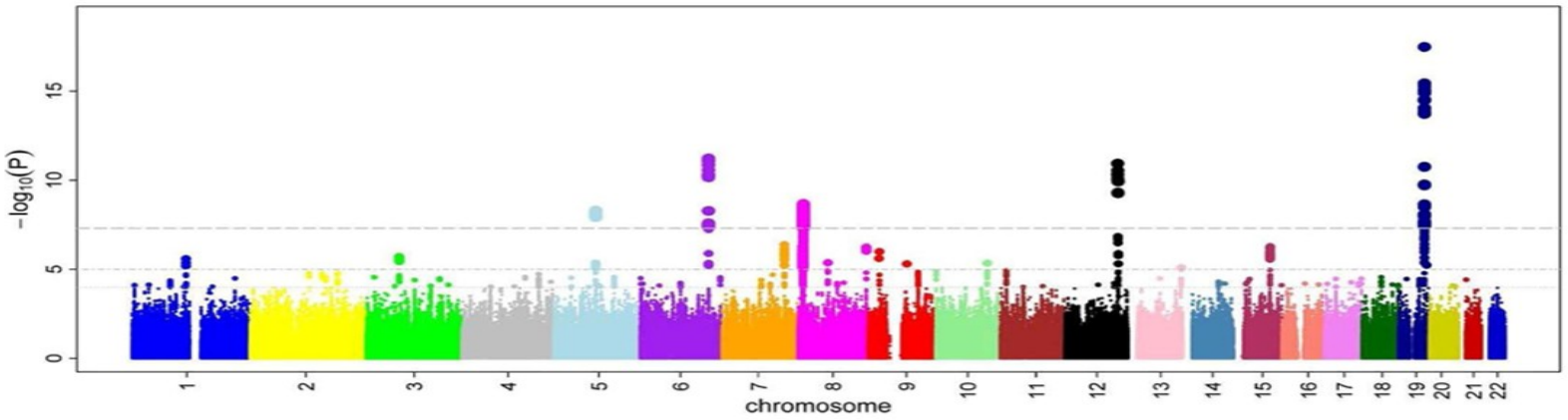
Genome-wide association studies

- Since 2005, genome-wide association studies (GWAS) have identified more than 1000 novel genotype-phenotype associations in humans

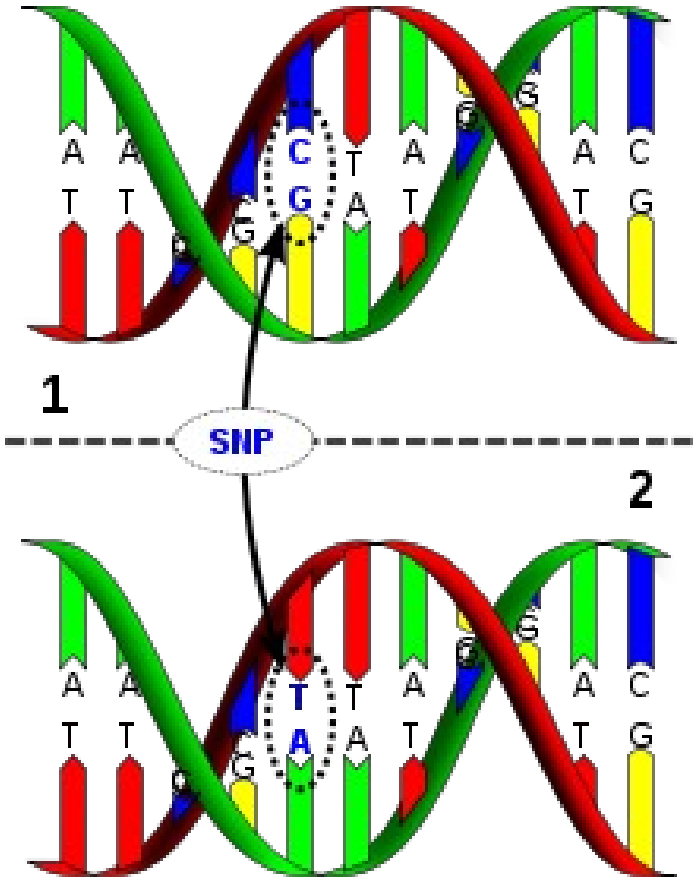


Motivation

- GWA studies identify genotype-phenotype associations
- Don't necessarily inform biology or medicine
- What information might enable translation of GWAS results to aid in biology & medicine?



What is a single nucleotide polymorphism (SNP)?



Question

- Are genotypic differences in breast cancer-associated SNPs associated with differences in expression levels?
  - Might a breast cancer-associated SNP be an eQTL?
  - Perhaps a SNP's genotypic differences are associated with expression level differences for a set of (functionally) related genes

Approach

- Acquire genotypic data & expression data from breast tissue samples
- Develop a pipeline for analysis & characterization of SNP-expression probe associations
- Generate a prioritized list of breast cancer-associated SNPs for subsequent biological & toxicological studies
  - To understand gene-environment interactions

Tissue samples

- Reduction mammoplasty human mammary epithelial cell (HMEC) samples
- 62 female subjects without history of breast cancer
  - 30 from Berkeley, CA
  - 32 from Madison, WI
- Ages & other demographic info unavailable
  - Most are thought to be in their 20s in age
- Clinical data unavailable

SNP genotype data

- Illumina Omni chip data for all 62 subjects
  - ~1 million SNP loci with called genotypes
- Two subjects were genotyped more than once due to low genotype call rates

Affymetrix GeneChip expression data

- Human ST 1.0 chip
- ~25,000 normalized expression levels for all 62 subjects
- Raw data processed with Robust Multi-array Average (RMA) methods

Ghoussaini et al. (2012)

- 72 SNPs
  - Genotyped (ie, not imputed) & breast cancer-associated ($p < 0.0001$) in one or both of two UK breast cancer GWAS
- Meta-analysis of ~ 70,000 cases & 68,000 controls
  - 41 case-control studies & 9 breast cancer GWAS
- Identified 3 SNPs with very small p-values for association with breast cancer

Plan

- Characterize the 72 Ghoussaini SNPs using our newly developed analysis pipeline
  - Calculate a statistic to identify associations
  - Generate heatmaps for the associated traits
  - Perform gene set enrichment analysis for each SNP's associated traits
  - Generate neighborhood plots for our top SNPs

Methods

- Which statistical methods to use to identify associations?
  - Between genotypic variations at a given SNP and expression levels for a single probe
- Standard approach in the scientific literature is to use ANOVA-based methods
- Due to our small sample size (n=62) we don't want to use ANOVA
  - ANOVA assumes constant variance among genotype classes

Strategy #1: `pmax' statistic

- Consider a single expression probe and a single SNP (from among Ghoussaini's 72 SNPs)
- Regress

  expression ~ PC1 + PC2 + siteIndicator
- Save residuals for subsequent analysis

Strategy #1: `pmax' statistic

- Use above residuals to calculate t-test-based p-values for all 3 pairwise comparisons
  - Genotype = 0 v. Genotype = 1
  - Genotype = 0 v. Genotype = 2
  - Genotype = 1 v. Genotype = 2
- without equal variance assumption
- Assign pmax to be the maximum of the three p-values

Strategy #1: `pmax' statistic

- For SNPs with only two genotypes:
  - i.e., when there are no minor allele homozygotes in our sample
  - Assign pmax to be the p-value for comparison of the heterozygotes and major allele homozygotes
- Declare a SNP-gene association when pmax is below an arbitrary threshold
  - i.e., when all 3 p-values are small

Problem with pmax-based strategy

- Preferentially selects SNPs with only two genotypes in our sample
- We need to consider other statistics

Strategy #2: `phom' statistic

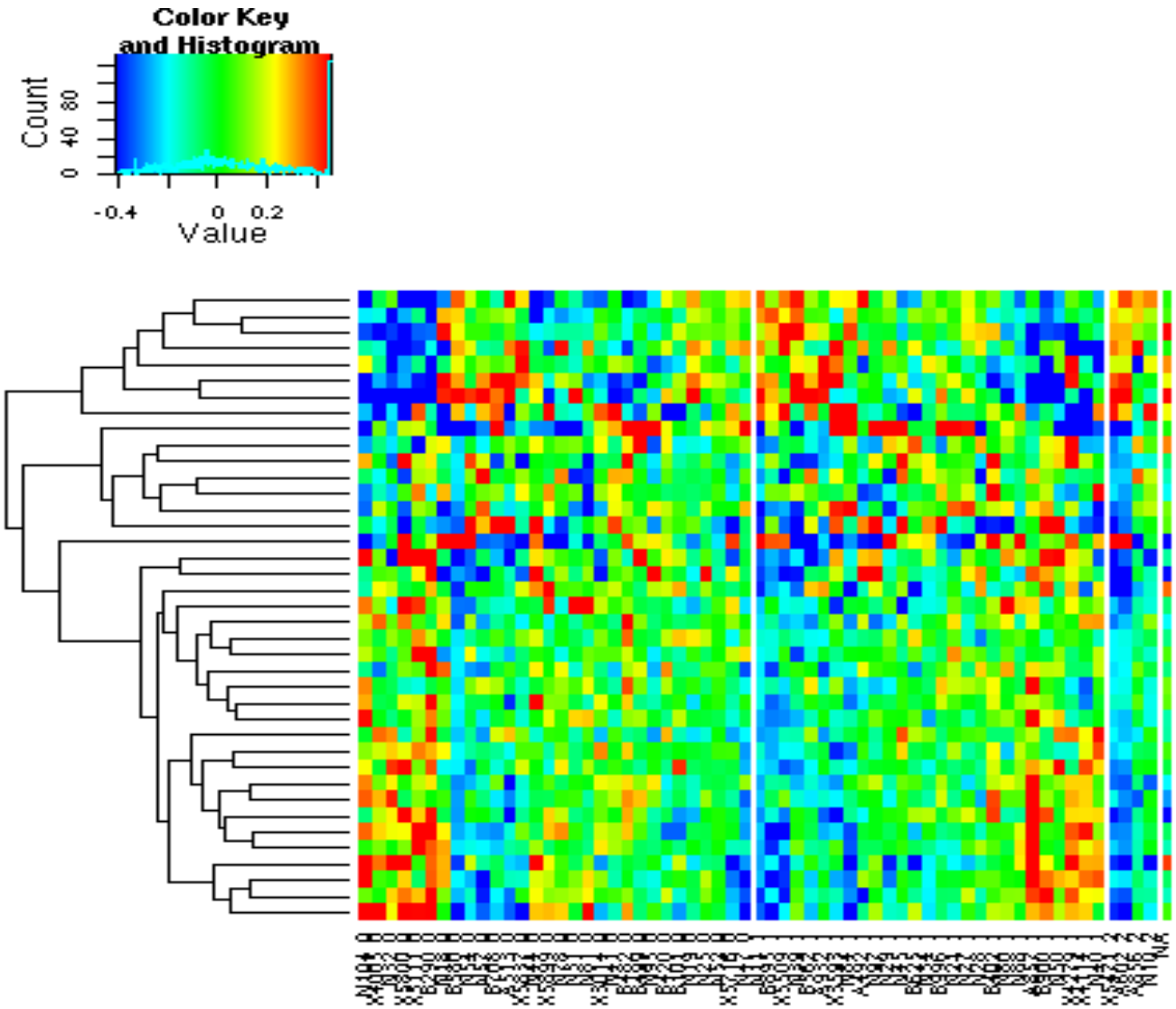
- Calculate the p-value for a t-test between the two homozygote classes
- Fewer genetic model assumptions
  - Since we don't require anything of the heterozygotes
- Rank SNPs by number of traits with phom < 10^-5
- Require that minor homozygote class have at least 4 subjects

Results

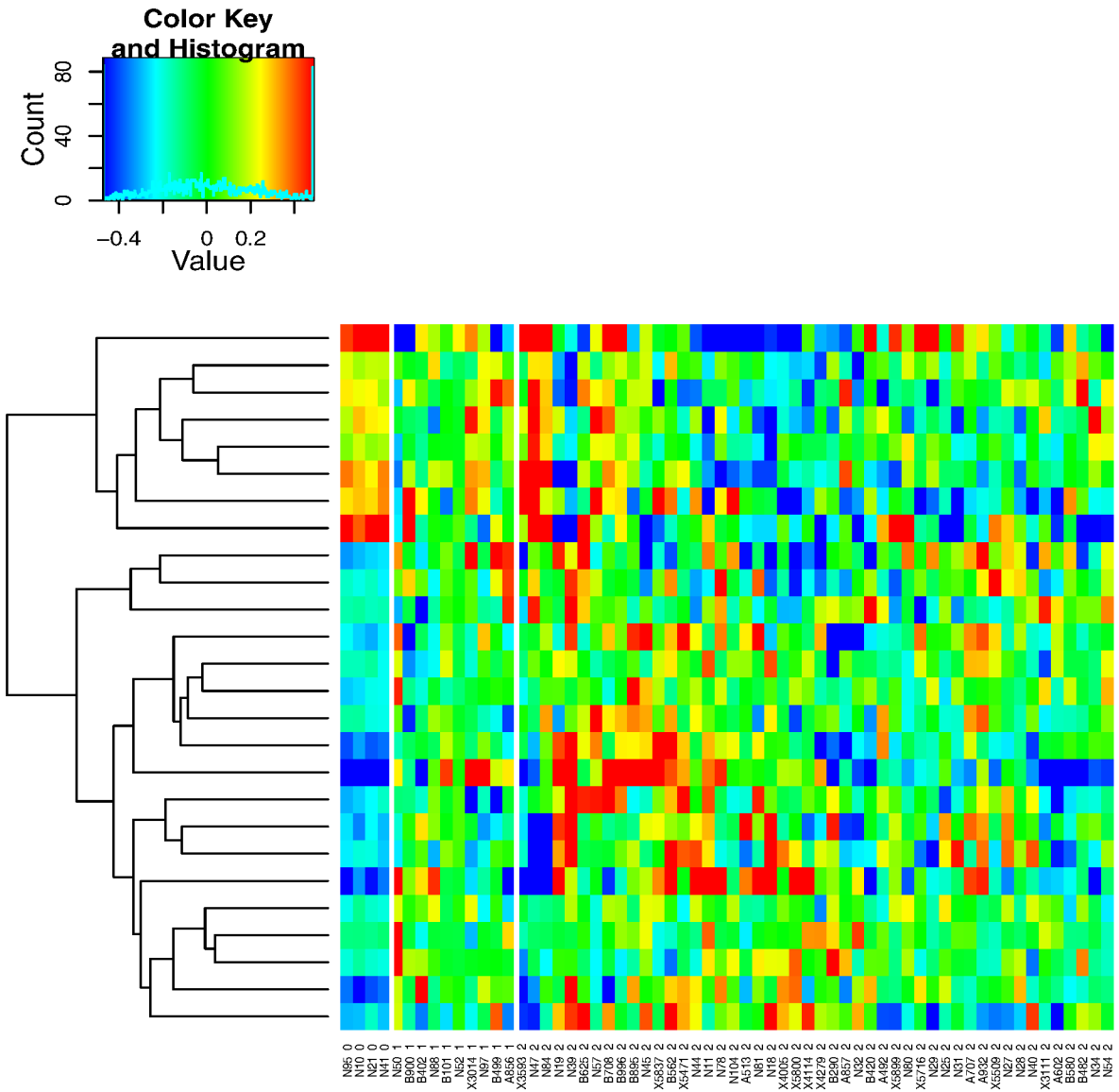
Lead SNP	Chrom	MAF	Proximal Genes	Proxy	R^2	n.AA	n.AB	n.BB	phome-5
rs11241138	5	0.41	NREP; 7SK	rs7718087	0.96	30	27	4	39
rs3101649	15	0.19	OCA2	rs3101649	1	4	10	48	26
rs2284424	12	0.22	GRIN2B	rs2284425	1	4	25	32	11

- NREP: neuronal regeneration related protein homolog
- 7SK: a small nuclear RNA
- OCA2: oculocutaneous albinism II
- GRIN2B: glutamate receptor, ionotropic, N-methyl D-aspartate 2B

Heatmap for rs7718087



Heatmap for rs3101649



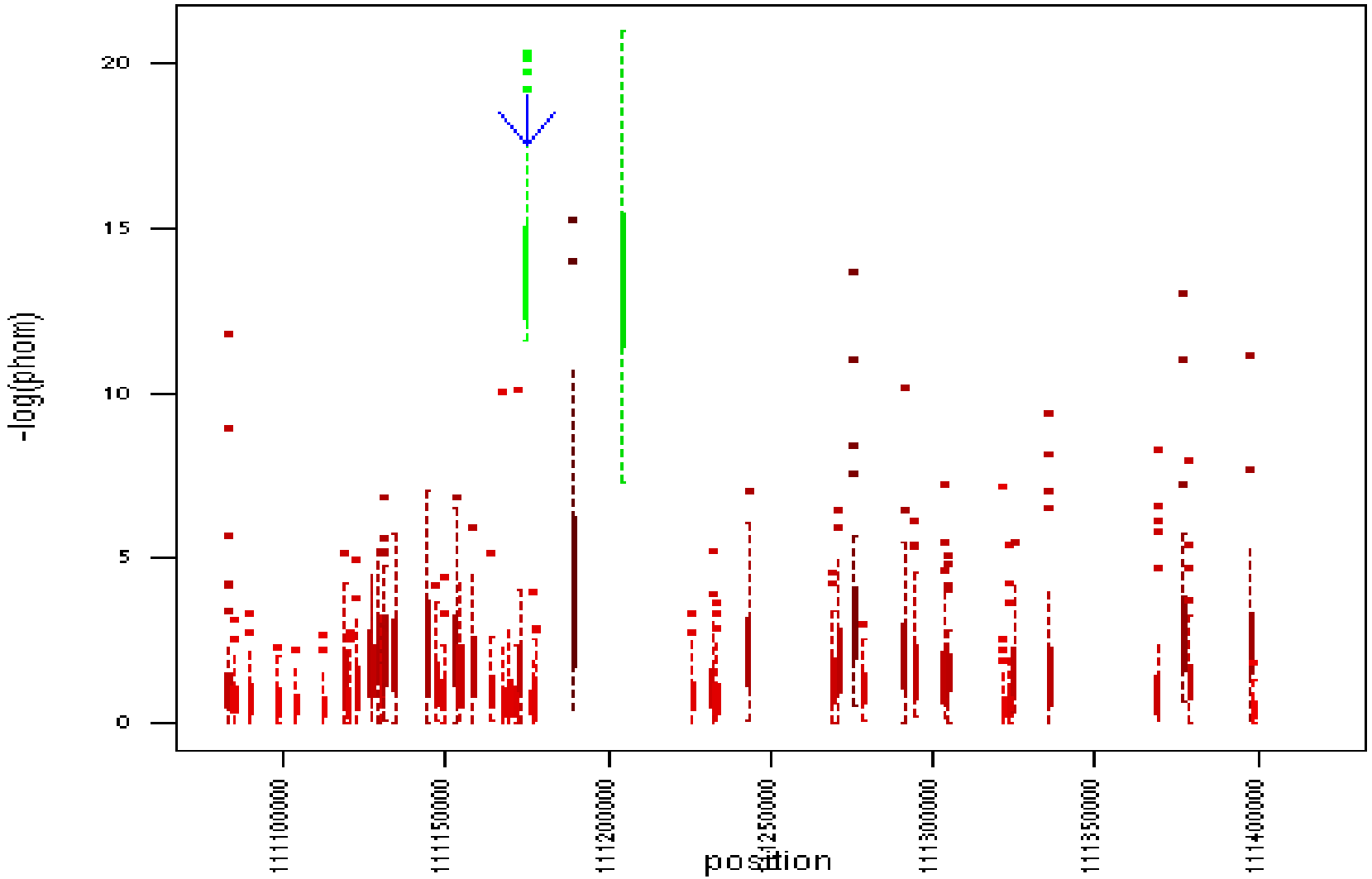
rs3101649 GSEA results

SetID	SetName	Count	NGenes	SetMean	ZScore	LeadGenes
GO:0032206	positive regulation of telomere maintenance	1	5	0.2	15.032	TNKS
GO:0001875	lipopolysaccharide receptor activity	1	5	0.2	15.032	LY96
GO:0051016	barbed-end actin filament capping	1	5	0.2	15.032	CAPG
GO:0004499	flavin-containing monooxygenase activity	1	5	0.2	15.032	FMO5
GO:0004791	thioredoxin-disulfide reductase activity	1	5	0.2	15.032	TXNRD3
GO:0031013	troponin I binding	1	5	0.2	15.032	RCAN3

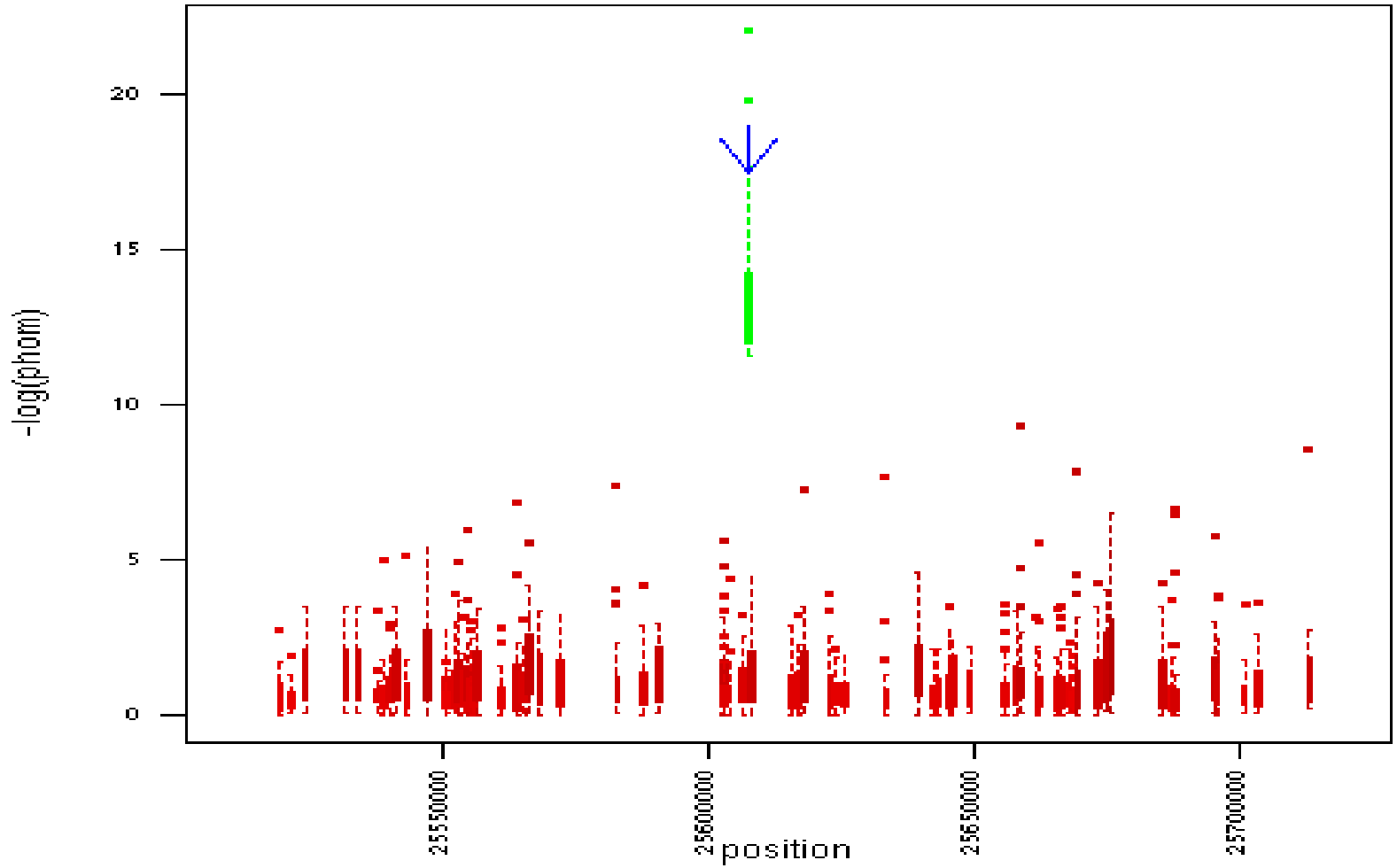
rs7718087 GSEA results

SetID	SetName	Count	NGenes	SetMean	ZScore	LeadGenes
GO:0016199	axon midline choice point recognition	1	5	0.2	10.412	ROBO3
GO:0031702	type 1 angiotensin receptor binding	1	5	0.2	10.412	BDKRB2
GO:0021877	forebrain neuron fate commitment	1	6	0.167	9.488	NKX2-1
GO:0022601	menstrual cycle phase	1	6	0.167	9.488	NKX2-1
GO:0042538	hyperosmotic salinity response	1	6	0.167	9.488	NKX2-1
GO:0006386	termination of RNA polymerase III transcription	2	12	0.167	13.421	POLR2H LZTS1
GO:0006385	transcription elongation from RNA polymerase III promoter	2	12	0.167	13.421	POLR2H LZTS1
GO:0016198	axon choice point recognition	1	6	0.167	9.488	ROBO3
GO:0044224	juxtaparanode region of axon	1	6	0.167	9.488	KCNAB2

Neighborhood plot for rs7718087 (39 traits)



Neighborhood plot for rs3101649 (26 traits)



cis-eQTL

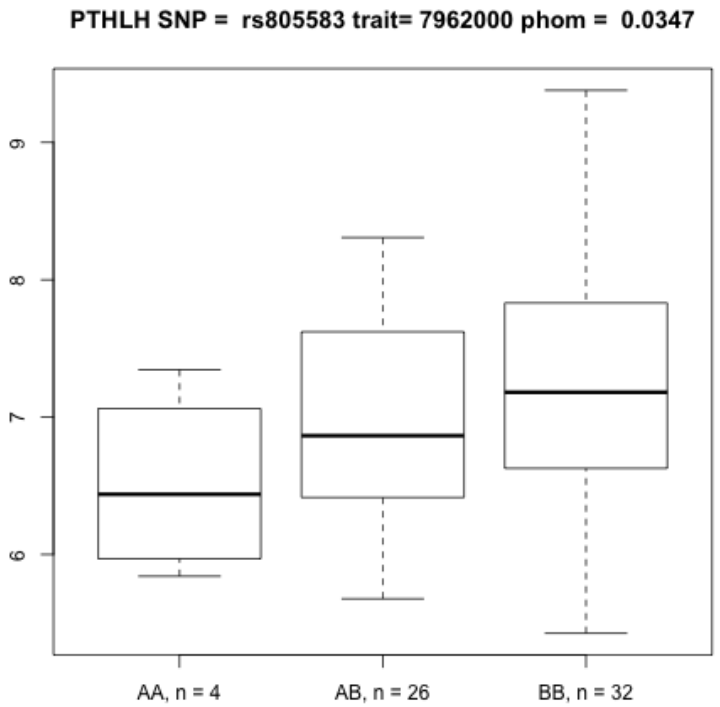
- For each of 494 proxy SNPs
  - Calculate phom for all genes within 300kb
- Rank all SNP-trait pairs by phom values

Cis-eQTL

GeneSymbol	Chromosome	Start	Stop	chromosome	PHOM	rs.id
OR3A3	chr17	3270612	3271577	chr17	3.11E-08	rs16953025
SPINT2	chr19	43447007	43474948	chr19	1.53E-07	rs2960337
GRHL2	chr8	102574162	102750995	chr8	1.38E-06	rs7822657
GRHL2	chr8	102574162	102750995	chr8	2.53E-06	rs1131863
GRHL2	chr8	102574162	102750995	chr8	2.53E-06	rs1131862
SNORA13	chr5	111525081	111525213	chr5	5.47E-05	rs980888

Boxplot of a top cis-eQTL

Box plot of a PTHLH cis-eQTL



Conclusions

- Several breast cancer-associated SNPs exhibit SNP-gene associations
- Low signal to noise ratio in our small sample
  - Similar studies often have n > 1000
  - GSEA results don't suggest extensive involvement of a single known biological pathway

Future directions

- Expand our set of SNPs from the 72 Ghoussaini SNPs to all breast cancer-implicated SNPs (per the NHGRI GWAS Catalog)
- If sample size is the issue, how many subjects might we want to better prioritize our SNPs?
  - Address with simulations?
  - Other approaches?

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