EPISTASIS: A Bipolar Short Story

William C. L. Stewart

Collaborators:

Sebastian Zöllner

Nan Xiang

Peng Zhang

Sandra Villafuerte

Melvin McInnis

Margit Burmeister

Outline

- Epistasis: A Slightly Broader Definition
- Bipolar Disorder: A Brief Introduction
- Background Findings: CGs and CG Regions
- Real Study Data Description
- A Rare Mutant Hypothesis
- Evidence for Epistasis
- Conclusions

Epistasis: A General Definition

Literally, the word epistasis means *standing on*.

It was originally used to refer to situations where the genotypic effects on phenotype at one locus could be suppressed by the genotype(s) at another unlinked locus.

For this talk, I'd like to go a little bit further, and consider the two-locus genotypic effects on phenotype at linked loci.

Bipolar Disorder (BP): A Brief Introduction

The Bipolar Disorder (BP) Phenotype is characterized by:

- Severe shifts in mood and energy (prevalence ∼2.6%)
- Increased risk for suicide
- Strong heritable component: (concordance rates ∼70%, ∼23% for MZ and DZ twins resp.)

To date, no one has isolated a functional genetic risk factor; and of course, the disorder also has various subtypes.

Previous studies have found:

- linkage to chr 6q and 8q (McQueen et al. '05)
- suggestive associations at two CGs in the 8q24 region: ADCY8, ST3GAL1 (Zandi et al. '08)

Data Description

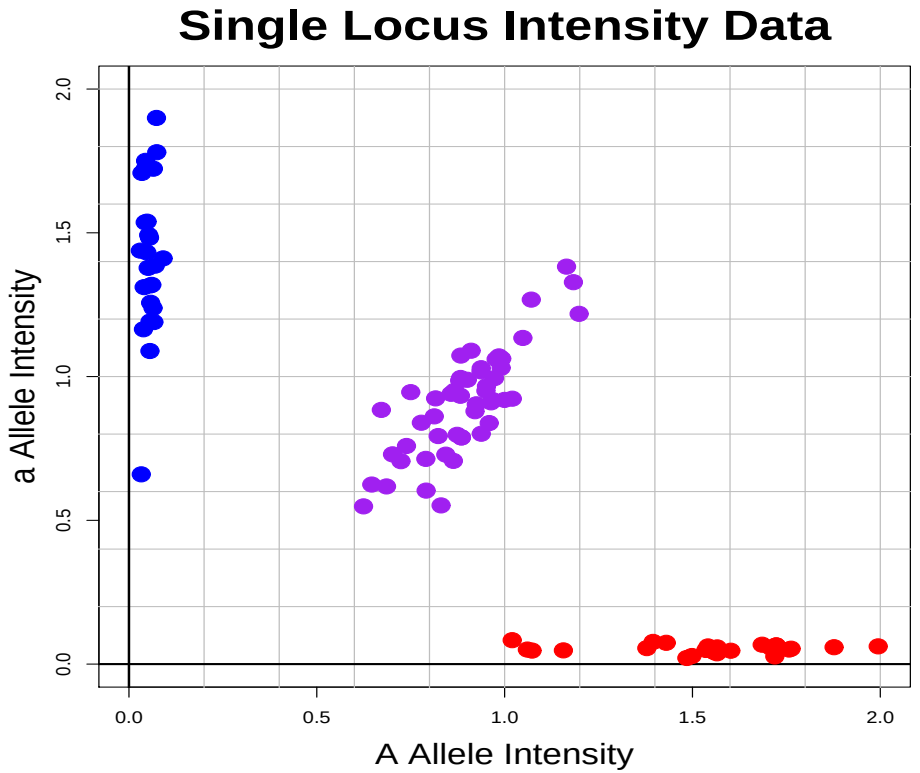
- 737 Bipolar Families of European descent
- Family sizes ranged from 4 - 26
- Genotyped by CIDR using Illumina BeadLab technology
- 1,536 SNPs in a 16 Mb CG region
- Over 2,300 affected individuals
- A total of 4,686 individuals in the study

Data are publicly available through:

http://bioinformoodics.jhmi.edu/chr8project/

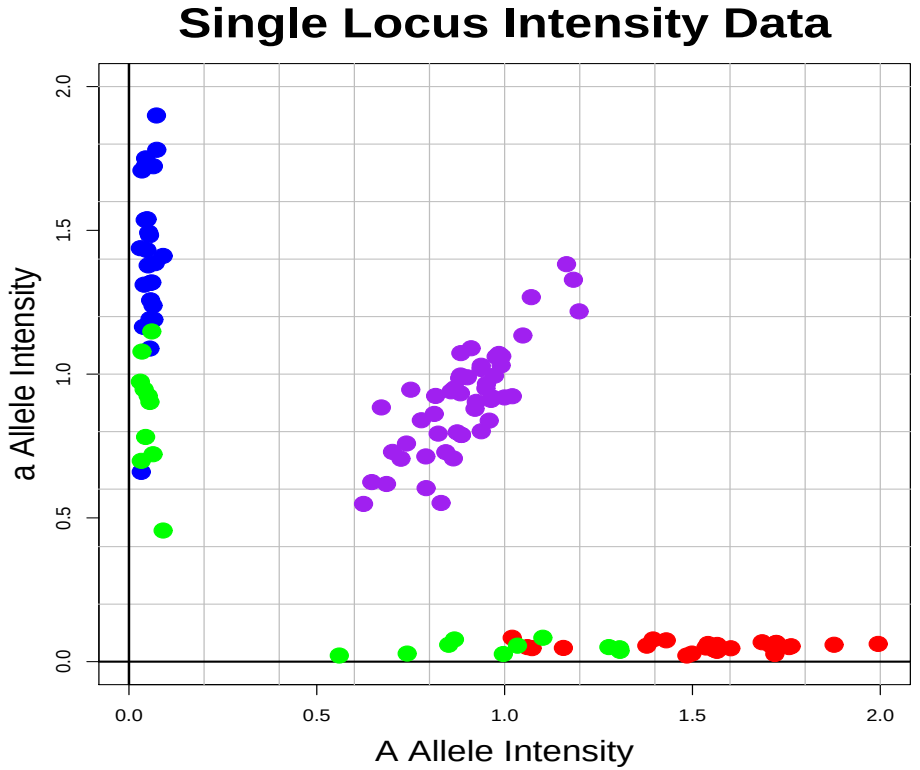
Data Description Continued...

Illumina technology does not measure the discrete levels of each genotype (e.g. (A/A), (A/a), (a/a)). Instead, it measures the allelic intensity, and this bivariate quantitative measurement is then transformed into the three canonical genotypes.



Data Description Continued...

However, cluster-based calls can be misleading in the presence of a deletion.

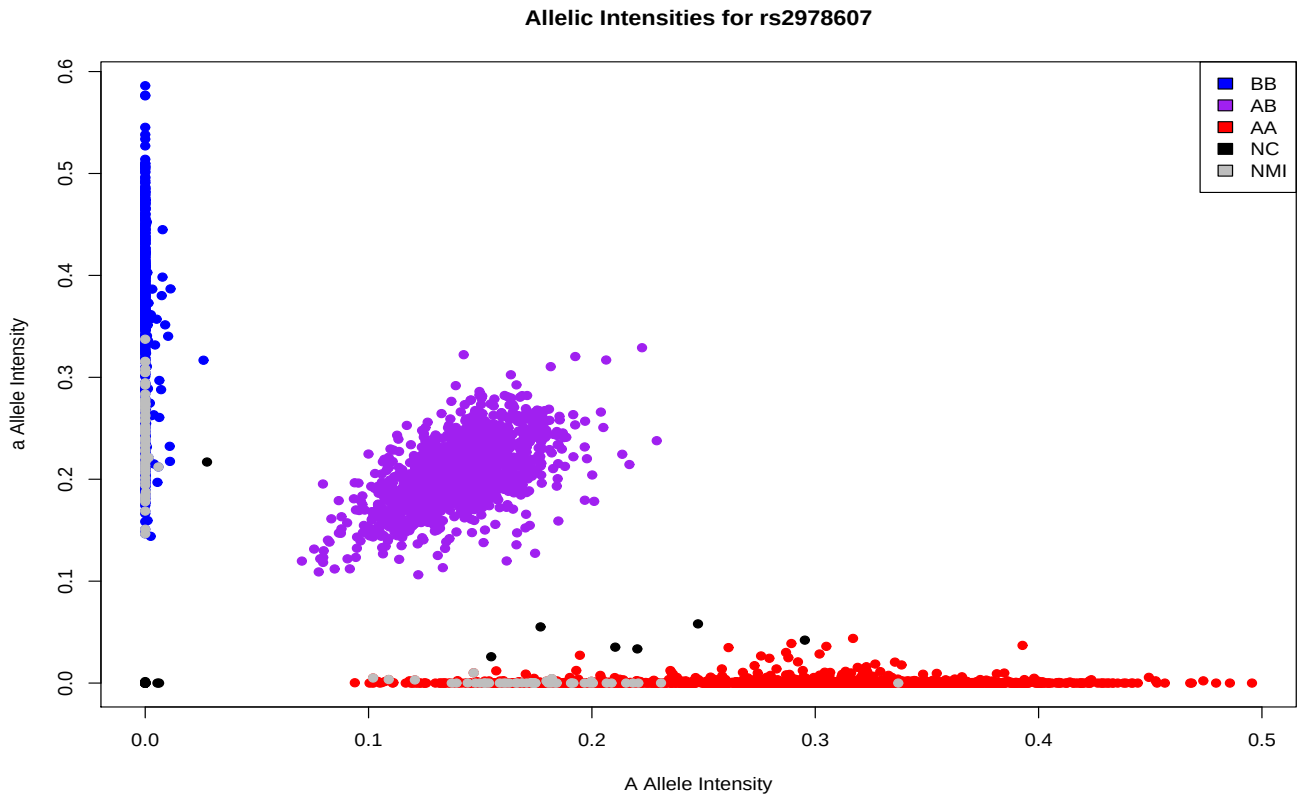


...and this is why I developed the program HEMIZYG.

http://www.columbia.edu/~ws2267/SOFT/soft.html

Data Description Continued...

For SNP rs2978607 the allelic intensities are shown. Grey dots indicate 107 non-Mendelian inheritance (NMI) events, and black dots are unscored genotypes. There was a significant departure from Hardy-Weinberg equilibrium (HWE).



A Rare Mutant Hypothesis

- Pyrosequencing revealed that many of the predicted carriers were diploid, which led us to hypothesize that a novel variant 'b' was segregating at a near by site, and that this variant negatively effects the genotyping.
- Under the assumption of limited haplotypic diversity, we tested this hypothesis by imputing the true genotypes of the 591 individuals flagged by HEMIZYG.

The result:

- the imputed genotypes were consistent with HWE, and there were no NMIs.

A Rare Mutant Hypothesis

- Additional pyrosequencing confirmed that primarily three haplotypes are segregating in these bipolar families: (a-B), (A-B), (a-b), with frequencies 60%, 35%, and 5% respectively, suggesting that the 'b' variant is a rare mutation that arose on and remains on an 'a' bearing haplotype.
- Moreover, the association between carrier status (i.e. carrying the 'b' or 'B' variant) and bipolar is 0.001

Evidence for Two-Locus Epistasis at Linked Loci

Now, given 3 haplotypes, there are 6 possible diplotypes in the population and, due to the ascertainment scheme and the purported association, we were lucky enough to see 5 of the 6 diplotypes in phenotyped samples in our data set:

class 1	class 1^c			
a-b/a-B	a-b/A-B	a-B/a-B	a-B/A-B	A-B/A-B

Interestingly, the a-b/a-B diplotype has an elevated level of risk compared to the a-b/A-B diplotype, and a test for association between BP and diplotype class (1 or 1^c), yields p = 0.0003.

Evidence for Two-Locus Epistasis at Linked Loci

Hence, there is evidence for two-locus epistasis at linked loci since the genotypic effects at locus 2: the novel SNP, depend on the genotype at locus 1: rs2978607.

As a side note, it is also interesting to point out that rs2978607 was not considered in the original fine-mapping study of Zandi et al. due to its:

1. extreme departure from HWE, and
2. unusually large number of NMIs

"*Treasure your exceptions.*" William Bateson

Conclusions

- Though it was originally designed to detect hemizygous individuals, HEMIZYG can also impute carriers of novel variants when individuals are known to be diploid.
- Irrespective of an individuals true ploidy, predicted carrier status can be a useful surrogate for untyped variation when testing for association to disease.
- Two-Locus epistasis is just one of several possibilities in 8q24, and the next step will probably entail sequencing in the region.