Complex Traits Analysis: The New Look in Neurobehavioral Pharmacology

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What is Complex Traits Analysis?

- Complex traits analysis examines brain-behavior functions and pathology
  - As influenced by multiple genes
  - As influenced by the environment
  - As influenced by gene-environment interactions
  - As influenced by gene-gene interactions
  - As influenced by gene-networks
What Are The Methods?

• Quantitative Trait Loci Analysis
  – Multi and univariate analyses
• Genetic correlational analysis
• Gene-gene interaction analysis
• Gene network analysis
What are the Materials?

• Genetically defined animals.
  – Selected lines
  – Inbred Strains
  – Recombinant Inbred Strains
  – Others
  – Heterogeneous animals

• Humans

• Moral of the story: The better the genetic definition, the easier the analysis
Recombinant Inbred Mouse Strains

- Many strains derived from 2 parental strains (in our case, C57BL/6 and DBA)
- Simple two allele model
- Strain mean for any measure is the index
- Can be used for genetic correlational analysis
- If genotyped for polymorphic markers, can be used for quantitative trait loci analysis
Scheme depicting the derivation of BXD recombinant inbred mouse strains

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Our Target Phenotype

- Density of Dopamine D1 receptors in the Caudate-Putamen of female mice
- Measured by $^3$H-SCH 23390 binding to membrane fragments
STRAIN DISTRIBUTION OF BINDING VALUES – A 4-FOLD DIFFERENCE!
Quantitative Trait Loci Analysis

• Based on correlational analysis between our quantitative (continuous) trait, D1 receptors and polymorphisms throughout the chromosomes among the RI strains.
• Remember, there can only be two alleles
• The polymorphisms can be RFLPs, Satellite Repeats and now SNPs or even genes.
• All BXD strains have been genotyped at more than 3000 polymorphic markers throughout the entire genome
## Quantitative Trait Loci Analysis

<table>
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<tr>
<th>Chr</th>
<th>Locus</th>
<th>cM</th>
<th>mb</th>
<th>RI Strain Number</th>
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<td>25.909845</td>
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</table>
QTL analysis of D1 receptor density in Caudate-Putamen in Female BXD mice. Note the two significant areas on chromosome 12 and on chromosome 15. Possible candidate genes include the estrogen beta receptor on chromosome 12 and *Scn8a (sodium channel alpha subunit)*.
Genetic Correlation Analysis

• Correlation between D1 receptors measured in our laboratory and phenotypes measured in other laboratories
• This is possible because the strain means rather than individual animal scores are used as the index of analysis
• Because, the animals are genetic replicates, within strain
<table>
<thead>
<tr>
<th>Phenotype</th>
<th>r*</th>
<th>Investigator (year)</th>
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<tr>
<td>Dopamine transporter caudate-putamen</td>
<td>.75</td>
<td>Jones et al., (1999)</td>
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<tr>
<td>Cocaine-induced stereotypy</td>
<td>.70</td>
<td>Jones et al., (1999)</td>
</tr>
<tr>
<td>Breast cancer tumor growth</td>
<td>.82</td>
<td>Grizzle et al., (2002)</td>
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<tr>
<td>Dopamine D2 receptor density, caudate-putamen</td>
<td>.73</td>
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<td>Ethanol acceptance</td>
<td>-.69</td>
<td>Rodriguez et al., (1995)</td>
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<tr>
<td>Ethanol acceptance</td>
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<td>Fernandez et al., (1999)</td>
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<td>Hippocampal weight</td>
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<td>Lu et al, (2001)</td>
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<tr>
<td>Saccharin preference</td>
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<td>Belknap et al., (1992)</td>
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<tr>
<td>Brain weight</td>
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<td>Williams et al (1998)</td>
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Pairwise Epistasis Analysis

- Are there genes that interact in determining D1 receptor density?
- Do genes at our two big qtl interact?
Whole Genome Scan

• Reveals several possible gene-gene interactions
• What about chromosomes 12 and 15? They are pretty faint on the Whole Genome Scan
• So, let’s blow up the square in the matrix that corresponds to 12X15
The Result…

- It appears that genes near the centromere of 12 and near the centromere of 15 do in fact interact.
- We will conduct marker based, selective breeding to find out the nature of the interaction.
- We will make a F2 population of our two parent strains, genotype for allele types, then breed homozygous *inter se* to make F3 population whose genotypes are known for 12 and 15 chr markers.
- Then we can breed the different 12 and 15 genotypes together
But, Genes Work in Networks

- Network analysis shows the interrelatedness among genes in determining complex phenotypes
- This is the way it really works
- For the following analysis, we used the information available at:
  - [http://www.genenetwork.org](http://www.genenetwork.org)
Gene network (via transcript abundance) related to D1 receptor density (green oval). **Ncam** – neural cell adhesion molecule; **S100**-Ca binding protein a13; **Cte1** – cytosolic acyl-CoA thioesterase 1; **Acbd6** – acyl-Coenzyme A binding domain containing 6; **Stk11** – serine/threonine kinase 11; **Fabp5** – fatty acid binding protein 5, epidermal; **Hsu79266** protein predicted by clone 23627; **Slc39a4** – zinc transporter; **Ftl1**– Ferritin light chain 1; **Gabrb1**– GABA receptor, beta 1; **Nat8**– N-acetyltransferase; **Gria2** – Glutamate receptor – AMPA2; **Ptprd** – protein tyrosine phosphatase; **Prkcb** – protein kinase C beta; **BB129894** beta galacatosyltransferase
This gene cluster tree shows the QTL for the gene transcripts from the previous slide and how the QTLs line up. The detail is too small to read here, but notice that there seems to be a common regulatory area on chromosome 19 – The small orange arrow shows that there is a gene on 19 that is in the network, Hsu, an anonymous gene that may well be involved in regulating the other genes in the network.
QTL Analysis of Cocaine-Related Behaviors and Neurochemistry in BXD/Ty Recombinant Inbred Mice

Why this particular panel??
Cocaine and Locomotor Activity

• C57BL/6 mice vs. DBA/2 mice
  – DBA more highly activated (locomotion)
  – Possible reasons -- greater target tissue sensitivity or difference in pharmacokinetics
Cocaine Protocol

• Behavioral testing
  – Day 1 Saline Day 2 Cocaine 5,15,30,45 mg kg$^{-1}$
  – Behaviors measured for 15 min
    • Distance traveled
    • Nosepokes (exploration)
    • Stereotypy
    • Margin time (thigmotaxis)

• Neurochemical measures, Densities of $D_1$, $D_2$, DA transporter, NAc, CS, VMB, FCx
### Analysis of Variance of Total Distance Difference Scores 
(Cocaine minus Saline) in BXD Mice

**Dose=5 mg kg\(^{-1}\)**

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<th>Source</th>
<th>df</th>
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<th>F</th>
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**Dose= 15 mg kg\(^{-1}\)**

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**Dose=30 mg kg\(^{-1}\)**

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**Dose=45 mg kg\(^{-1}\)**

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QTL map for Cocaine sensitivity in female BXD mice. Dose=15 mg/kg, measure is locomotor activity in an automated activity chamber. There are 3 suggestive QTL on Chr 2, Chr 5 and Chr 12.
QTL genome wide map for density of D1 dopamine receptors in caudate-putamen in BXD female mice. The QTL on Chr 12 overlaps with that for locomotor activity at 15 mg/kg cocaine.
Cocaine Co-localized QTL

Chromosome №

2

12

15

Dist X D₁ CP

Stereo X D₁ CP D₂ CP

Scale, cM

0 20 40 60 80 100

NP X D₁ Fc

Markers

Iapls2-4 D12Mit114 D15Ncvs29
Finally – A QTGene!

• The work of John Crabbe
  – Seizure-resistant/Seizure-susceptible mice

• The follow-up by Kari Buck
  – Nature Neuroscience 7:699-700, 2004 (July)
  – Localization of the Mpdz gene as QTGene for sedative-hypnotic withdrawal seizure susceptibility.

• Extremely important result for those of us using QTL methods to find genes
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WORST CASE SCENARIO OF MAPPING THE HUMAN GENOME...

MC DNA'S
OVER 6 BILLION CLONED

Baby Engineering