Bipolar DBP Update 2009

Melvin McInnis, MD
Ben Keller, PhD
Rich McEachin, PhD
Bipolar Disorder: Pathological Swings of Mood

• Mania: elevation of energy and mood – Devastating

• Depression: Melancholia, profound slowing of motor, cognitive capacity

• Research emerging that implicates ion channels in Bipolar Disorder
Bipolar Disorder: Pathological Swings of Mood

Comorbidities…The Rule Not the Exception

Bipolar Disorder: Driving Biological Problem

• **Specific Aims**
  • Prioritize genes predisposing to bipolar disorder for further inquiry
  • Perform data mining to identify phenotypic patterns that may predict more homogeneous subgroups of patients
  • Develop an integrated translational research approach to the overlapping behavioral phenotypes

• **Description of current status:**
  • Candidate genes and pathways (calcium and cellular signaling theme)
  • Lithium mediated gene expression changes
  • Deep Phenotyping with measurable neuropsychological testing
  • Integration of substance abuse behavior (tobacco) and bipolar disorder
    • Identification of genes and putative pathways

• **Next Steps: Importance of sequencing**
  • Common-disease / Common-Variant - limitations of methodologies are emerging
  • Rarer variants (CNVs & rare mutations) identified through specific techniques (sequence)
  • Cell systems (Lymphoblastoid cell lines?) to study biology of rare variants
  • Variants within genes within pathways within systems ....
    • Associate with measurable phenotypes
Bipolar Disorder Association Studies in 2008-2009

- Ferreira et al. Nature Genetics, 2008
  - CACNA1C \( (p<10^{-8}) \) and ANK3 \( (p<10^{-9}) \)
- Scott et al. (Pritzker collaboration) PNAS, 2009
  - MCTP1, NEK4 and ITIH1 \( (p<10^{-7}) \)
- Smith et al. (NIMH “G11” Collaboration) Mol. Psychiatry, 2009
  - GAIN - European and African American population
    - AA: DPY19L3, NTRK2;
    - EA: 3q11.2, Xq27.1, NAP5;
    - EA w/o Pritzker: RIN2
Association: Majority of “significance” at $10^{-4}$

- GWAS of common disorders identifies a small number of markers with significant $p$-values that survive multiple-testing correction.

- Multiple genetic effects of small effect?

- Recent NEJM perspectives challenge the common disease-common variant hypothesis; challenges approach of larger samples sizes to solution.
Bio-informatics - Methodologies to prioritize regions & genes: CACNA1C, ANK3 & interactions

KEGG Signaling pathways:
- **Calcium Signaling**
- MAPK Signaling
- Insulin Signaling
Rare Variants – Variable Copy Number

• Are Rare variants driving risk behind Bipolar Disorder?

• 3 Publications in 2008 identified CNVs increased in schizophrenia

• Birdsuite tools (Broad) applied to GAIN (NIMH) BP dataset (“G11” - Zhang et al, Mol Psychiatry 2009)

• Identified evidence for singleton deletions increased in BP (16%) vs controls (12%).

• Two genes: GRM7 and LARGE overlapped with report (Walsh et al, 2008) from schizophrenia findings and contained deletions.
Linkage Analyses of 1,067 BP multiplex pedigrees

Collaborative effort that combined and analyzed primary data from 11 genome-wide studies of BP disorder. (McQueen et al, AJHG, 2005)

Follow-up “fine mapping” of the 8q24 region in 737 multiplex BP families from JHU and the NIMH collaborative sample. 3,072 SNPs in 16 Mb region. (Zöllner et al, in prep, 2009)
Emerging Genes of Interest in BP Disorder

<table>
<thead>
<tr>
<th>Recent Literature</th>
<th>8q24 Findings</th>
<th>CNV report</th>
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<tr>
<td>• CACNA1C</td>
<td>• KCNQ3</td>
<td>• GRM7</td>
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<tr>
<td>• ANK3</td>
<td>• ADCY8</td>
<td>• LARGE</td>
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<td>• DGKH</td>
<td>• ST3GAL1</td>
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<td>• (NEK4)</td>
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<td>• (RIN2)</td>
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Genetic interactions per MiMI
Calmodulin Gene Linkers (top KEGG Pathways)

- CACNA1C
- LARGE
- KCNQ3
- ANK3
- GRM7
- ADCY8
- DGKH
- ST3GAL1

Pathways:
- Calcium signaling pathway
- Glycerolipid metabolism
- Glycosphingolipid biosynthesis - ganglioseries
- Purine metabolism
- No pathway

No pathway
Lithium – the most effective treatment for BP

• Discovered 1949 by John Cade in Australia to be effective in the treatment of Bipolar Disorder.

• Seminal studies emerged from the 1960s supporting the efficacy of Lithium in treatment of mania and depression as well as prevention of recurrence of episodes.

• Only medication currently used for Bipolar Disorder that has been shown to have preventative effect on rate of suicide. More pronounced in age group over 40.

• Generic Medication (< $0.25 per day); little economic incentive from pharmaceutical industry to study mechanism of Li. Study of Li may provide insight to mechanisms behind BP disorder.
Lithium Mediated Expression: Methods

Seed ~ 2x10^6 cells fresh LCL culture into each flask (n=12: 7 BPIs + 5 unaffecteds)

Lithium = 1mM

Sample 5 mL culture at day 4, 8, 16 for microarray analysis

0 mM lithium

Isolate total RNA for microarray expression assays
Lithium Mediated Expression – Key Messages

• Many genes appear to be influenced by culture with lithium as measured by changes in RNA and miRNA levels (correlation between miRNA and RNA levels)

• Variability in biological systems for single genes is models ~20-30%; unclear whether any effect at system level

• Chronological changes in levels of expression for miRNA, RNA in LCLs & mouse embryonic cells
  • Consistent with developmental/dynamic effect on gene expression
  • Several genes show significant “slope” in expression

• Sibling/genetic effect greater than external influence (culturing with lithium)
Two-class paired time course
Plot of genes showing slope changes (FDR <5%)
C8orf33 interacts through HAP1-GPRASP1-GIT1, and the extended gene interaction network
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</table>
These are my pathways….

Calmodulins – linking genes & pathways?
Lithium and GSK3β

- GSK3β was significantly increased by 10% (FDR < 0.001) at day 4 – no change at day 8 or day 16
  - Inhibits activity physiologically

- Inositol Monophosphatase (IMPA2) was significantly decreased by 21% at day 4 – with no significant changes at day 8 or 16

- Mouse brain data (McQuillan) showed decrease of IMPA1 but no change in IMPA2

- Calmodulins showed no significant changes.
Prechter Bipolar Longitudinal Study

April 2009: 232 BPI, 46 BPII, 65 unaffected controls. Deep Phenotyping with longitudinal follow up data: environmental, diagnostic, psychological, biological & DNA.

Measurable State and Trait Phenotypes

**Neuropsychological Testing**

**Personality Testing** NEO-PI

**Measurable State and Trait Phenotypes**
Building Bridges Fellowship

• Rich McEachin and collaborators at Washington University St. Louis

  • Laura Beirut, Nancy Saccone, Scott Saccone

  • Poster on display: Modeling Complex Genetic and Environmental Influences on Comorbid Bipolar Disorder with Tobacco Use Disorder

  • Assess genetic influences on comorbid Bipolar Disorder (BD) with Tobacco Use Disorder (TUD)

  • Integrate NCIBI, WUSTL group, and publicly available bioinformatics resources
Building Bridges: Candidate Genes

- **MIX** (Kitasato Univ.) meta-analysis: risk for TUD among BD patients 3-fold compared to general population.
  - Hypothesize a genetic basis for the comorbidity

- **Gene2MeSH** (NCIBI tool that maps genes to MeSH entries) identifies overlap with TUD and Bipolar Disorder
  - catechol-O-methyltransferase (COMT)
  - dopamine transporter (SLC6A3)
  - serotonin transporter (SLC6A4)

- **PDG-ACE** (Predicting Disease Genes from Analysis of Common Elements) finds significant commonality among these candidates
  - Psychiatric, substance use, attention deficit
  - Gender specific effects in both psychiatric and substance use disorders
Building Bridges: Network Modeling

- Hypothesize network or candidate genes based on interactions with established candidate genes
  - MiMI (NCIBI), STRING (EMBL), and MetaCore (GeneGo Inc.)
  - Protein-protein binding, regulation of expression, activation, etc.
- Test hypothesized networks for over-representation of genes annotated for BD and TUD
  - Candidate network is over-represented for both BD and TUD – GAD (Genetic Association Database, NIH)
- Prioritize network SNPS for follow-up based on GAIN (BD), NicSNP (TUD) and the GIN (Genetic Information Network, WUSTL) algorithm
Bipolar / Tobacco Use Disorder Pathways
Gene-Go Network – TUD / Bipolar
Year 5 Directions - Sequencing

• Networks and Ion Channels
  • Bipolar Disorder emerging as a channelopathy?
    • CACNA1C - associated with cardiac conductivity disorders – Timothy & Brugada Syndromes)
  • KCNQ3 – voltage gated K channel
  • Calcium modulated genes (Calmodulins) interact with most candidates
• Nicotinic receptor (associated with smoking behavior) – ion channels
  • Overlapping genes (BP/TUD) COMT, DAT, SERT ion dependant
• Cellular Models for Lithium and effect on gene expression
  • Insights into cellular mechanisms behind treatments
  • Pathophysiology and genetics
• Neuropsychology and Neurophysiology generate measurable phenotypes
  • Dimensional – beyond categories
  • Executive functioning / motor speed reflective of neurotransmission?
• Deep Sequencing of Linkage region (8q24) to search for rare variants
• Deep Sequencing of Bipolar I cases with Deep Phenotyping, Neurophysiology, Neuropsychology, Biochemistry, Metabolomics, and Immunology.